Fundamentals of
BIOCHEMISTRY

[with MULTIPLE CHOICE Ques.-Ans.]

Dr. A. C. Deb

Fundamentals of

BIOCHEMISTRY

[For Medical Science, General Science, Veterinary Science, Home Science, Life Science, Agricultural and Homeopathy Courses etc.]

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NEW CENTRAL BOOK AGENCY
8/1 Chintamoni Das Lane, Calcutta - 700 009 (INDIA)

Fundamentals of BIOCHEMISTRY

First Edition — 1983
Second Edition — 1986
Third Edition — 1988
Reprint — 1989

Fourth Edition (Revised & Enlarged) — April, 1990

Published by—

A Sen
New Central Book Agency
8/1 Chintamoni Das Lane
Calcutta-700 009

Printed by—

J N Sen (Managing Director)
Books & Allied (P) Ltd (Offset Printing Division)
8 Chintamoni Das Lane, Calcutta-700 009

PRICE : Rs.140 00

ISBN 81-90120

DEDICATED

TO THE MEMORY

OF MY LATE PARENTS

PREFACE TO THE FOURTH EDITION

Fundamentals of Biochemistry deals with the comprehensive treatment of the principles of biochemistry and molecular biology By inserting highlights into the pathologic applications of biochemistry, it provides immense facilities to medical students as regards to the clinical disorders arising from biochemical derangements

Alterations in the fourth edition

The author while preparing this new edition had nourished two main objects to focus the latest advances in biochemistry that are significant to medicine and to offer a book that would be more beneficial to medical students to study with much interest and aim. The following features bring forth the objectives

- A new chapter entitled "Overview of Intermediary metabolism" (Chapter 20) gives assembling of all of the chapters on metabolism.
- A new chapter entitled "Recombinant DNA Technology" (Chapter 23) starts the basic concepts and technique of this topic and illustrates its strong application on biology and medicine.
- A special chapter entitled "Cancer, Oncogenes, and Growth Factors" (Chapter 41) invites the attention of the students to the problem of cancer and shows how biochemical information gathered from the study of oncogenes and growth factors is enlightening its fundamental nature.
- An appendix includes objective type of questions and their answers. This intends to assist students in their achievements derived from keen study

In addition, applied nutrition in detail is added to Principles of nutrition (Chapter 32) for students studying nutrition and the revised gastrointestinal hormones is also added to Chapter 15 to provide recent information.

Acknowledgements

The author is extremely delighted to extend his gratitude to professional colleagues and friends who have conveyed suggestions for improvement and corrections to him for placing the book in the lofty position. He expresses his well wishes to them to encourage their continued effort and interest. Comments from students of 1st MBBS (1st professional MBBS) of Indian as well as foreign universities, the graduate and master course students of general science streams as well as veterinary, agricultural, and home science students, the students of diploma as well as post graduate of medical biochemistry and nutrition are always cordially welcome

The author is highly gratified by the wide acceptance and support this book has received from all corners of learners He also thanks his wife Mrs. Bela Deb and his son Pradipta Kumar Deb who helped him in every affair without which this new edition would not come to light so soon. A nice environment had also been prevailed to work with the publisher, Mr A. Sen and his co-workers for their continued assistance

Calcutta,
April 1990

Dr A C. Deb.

PREFACE TO THE THIRD EDITION

I feel extreme pleasure in bringing out the third edition of the book to the enthusiastic readers within a short period. This edition has come to light with the main following features :

1. The thorough and critical revision of its predecessors.
2. The addition of several new articles.
3. The addition of several schematic representation.

I have the high longings that this edition will signify more beneficial to the learners for their clear recognition. The criticism of the readers is the best guide for the development of the book; hence, suggestions from time to time are highly appreciated for the subsequent edition of the title.

More schematic representations in different chapters have been added in this edition to have an enlightened concepts of the correlations between these chapters.

A new final chapter has been included. This briefly deals with the history and advancement of clinical biochemistry with an account of SI Units.

I higbly desire to acknowledge the great patience and many social sacrifices made by my wife during the year in which this edition was being prepared. Thanks are also due to the publisher for his unfailing co-operation.

Calcutta
December, 1987

Dr. A. C. Deb

PREFACE TO THE SECOND EDITION

The second edition of my book needs revision by the addition of more chapters chiefly dealt with the molecular biology associated with the day to day rapid advancement of biochemistry and hence the number of chapters in this edition has been increased with a view to develop the organization and the presentation of the material of the book. Although much information of each new chapter has been available, I have devoted much care to express the material in a concise and simple manner for the easy and smooth understanding of the enthusiastic learners.

I am too highly glad to avail the opportunity of offering thanks from the core of my heart to those who have extended aid in all respects throughout the second edition of the book to represent it in an accomplished way. Further suggestions are cordially invited from the readers in the subsequent edition of this book.

Calcutta
May, 1986

Dr. A. C. Deb

PREFACE TO THE FIRST EDITION

Recently, adequate advances have taken place in the field of Biochemistry. A limited number of text books has been found suitable for students. To avoid confusion in understanding each topic of the entire subject this text book on Biochemistry has been written in a systematic manner in a very simple approach for the students. Deep attention has been adopted to cover all the necessary aspects of Biochemistry.

The book, although in concise form, can provide sufficient uptodate materials to the learners of medicine, home sciences and life sciences etc. The subcellular and molecular concepts have also been introduced in this book. It is greatly hoped that this book will serve the interests of students and practitioners in the health sciences related to medicine providing them knowledge in basic principles of molecular chemistry and biology. An exercise containing questions of different university examinations is given at the end of each chapter for the benefit of the students to prepare answers thoroughly for their examinations to give the best satisfaction of the examiners. This sort of presentation also will minimise the exhaustive work of the students for finding out questions of each chapter for examinations. The practical portion in detail is also added to make the book a complete one for the facility of learners.

I am thankful to my wife Mrs. Bela Deb, M. Sc. (Botany), B. ED. who had given me valuable suggestions and inspiration for the rapid and nice get up of the book. I am also indebted to my father-in-law Dr. N. C. Bhadra, and my respected professor Dr. P. K. Banerjee for their inspirative suggestion. At last, I am highly thankful to the publisher Sri J. N. Sen, and the artist Sri Biren Das for their continuous unfailing efforts and help throughout the preparation of this book.

Any suggestion from the teachers and students will be highly appreciated so that further improvements of the title can be made in the subsequent edition in the light of the same.

Calcutta
October, 1983

Dr. A. C. Deb

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CHAPTER I

INTRODUCTION

Biochemistry absolutely deals with the chemistry of living organisms—both plant and animal Protoplasm is the basis of all forms of life Although the protoplasm of each different kind of cell in each kind of animal or plant is different in characteristic yet the chemical composition, organization, and chemical processes in these many different forms of protoplasm are similar in many respects Animal biochemistry is significantly developed by the assistance of investigations of the chemical processes of plants and microorganisms and vice-versa The observations on various chemical reactions first made in microorganisms or plants have been finally brought to light in higher animals and the reverse is followed in the true sense

Biochemistry can be divided broadly into four branches (i) Medical biochemistry, (ii) Animal biochemistry, (iii) plant biochemistry, (iv) Biochemistry of microorganisms

Medical biochemistry deals with the following with which the medical students are mainly concerned

The chemistry of tissues and foods.

Since the foods are largely derived from animals or plant tissue the study of the chemistry of one is identical with the other The components of tissues and foods are carbohydrates, fats, proteins, and nucleic acids Carbohydrates provide a greater part of the energy needs of the body Improper utilization of glucose leads to the development of the disease diabetes mellitus Lipids provides a part of the energy needs of the body Essential fatty acids are required for maintaining normal health These essential fatty acids are also the precursors of prostaglandins Complex lipids occur as integral parts of nervous tissue Proteins are necessary for growth and maintenance of positive nitrogen balance Nucleic acids (DNA & RNA) of nucleus and cytoplasm respectively are involved in regulation of gene

The chemistry of digestion and absorption

The food is chiefly composed of large molecules of starch, proteins, and fats These large molecules are digested into smaller molecules like glucose, amino acids, and fatty acids for absorption by the organic catalysts (the enzymes) present in the digestive juice Along with their absorption, the entry of water, minerals, vitamins, and other diffusible molecules of the food also takes place The absence of one of the digestive enzymes leads to serious disorders

The chemistry of respiration.

During respiration, in man, oxygen is taken into the lungs and diffuses into the blood across the membranes It combines with the hemoglobin of red cells and in this form, it is carried to the tissues where it is released for the oxidation of food to liberate energy The carbon dioxide formed equal in volume to that of oxygen passes from the tissues to the blood stream and is exhaled from the lungs being reacted by chemical combination

The chemistry of blood

The blood carries foods to the tissues and waste products from them to the excretory organ The hormones produced by various glands pass into the blood and through its circulation these hormones reach the specific tissues The blood distributes heat from one part of the body to another and exerts cooling effect

The blood also distributes water and salts properly and maintains acid base balance of the body It contains substances that combat infection by micro-organisms The composition of blood is normally constant but it is dynamic. One of the substances present in it increased or decreased causes pathological condition

The chemistry of cell membrane and physical chemistry

Hormones foods waste products, and other necessary substances required for cells pass through the cell membrane. Some substances easily pass through the membranes but some other substances pass by other mechanisms Normal permeability of cell membranes maintains normal physiological processes and abnormal permeability is associated with pathological conditions

The colloidal substances which do not diffuse through membranes exhibit donnan membrane equilibrium resulting in secretion and excretion of substances The buffering system is most essential for maintaining p^H of blood slightly alkaline for normal functioning of the body The electrical potentials across cell membranes is of much significance The ion exchange across the cell membrane maintains the cellular integrity The p^H of blood if altered from the normal value leads to complicated disorders

The chemistry of tissue metabolism.

The oxidation of food stuff in tissues occurs by chemical processes with the liberation of energy and water This is a very complicated process in biochemistry Several diseases occur in the disorder of the metabolism of these food stuffs

The chemistry of glands of internal secretion.

The control through glands of internal secretion is barely dependent on the activities of hormones and nervous system The hormone of one gland regulates the activity of another gland Biological achievement is recorded by the biochemistry of the glands of internal secretion which is one of the most significant chapters The improvement of this chapter has influenced the effective agents of treating diseases Overproduction of hormones also leads to serious disorders

The chemistry of excretion

The excretory organs—kidneys, lungs, intestine, and skin—remove decomposition products of tissues and foods in order to make the composition of the body fluids constant The most important decomposition products are urea uric acid, and creatinine formed from proteins carbon dioxide and water formed from carbohydrates fats and proteins The particular level of these decomposition products in compatible with health but much excess may lead to dysfunction and illness The kidneys and the lungs chiefly perform the excretory function of the body The medical students must be quite aware of the concentration of the excretory products which may indicate certain diseases In severe diabetes mellitus, sugar is excreted to the urine as a result of high blood sugar level and along with this sugar the kidney is to excrete more water also Consequently, there develops both hunger and thirst This symptom gives a hint to the disease

Bio-chemical disorder in diseases

The knowledge of biochemical disorder in various diseases aids in the correct diagnosis and treatment of disease Blood glucose level increased and presence of sugar in the urine indicates diabetes mellitus The severity of the disease is assessed by the concentration of blood sugar and urine sugar So treatment is instituted by antidiabetic drugs Jaundice is accompanied by high level of bile pigments in blood and tissues In kidney failure urea, uric acid etc in blood are

highly increased leading to uremia. The functions of kidneys, liver, pancreas, small intestine etc are determined by biochemical tests.

From the above accounts, it is quite evident that the knowledge of biochemistry is most essential for understanding the functioning of human body in health and disease. From the time of Aristotle, students of biology and medicine have endeavoured to correlate structure and function.

Importance of biochemistry to medicine.

Biochemistry is important to physiology, the other medical subject. Both physiology and biochemistry overlap and merge. Pathological conditions in the body are caused by deranged chemical composition and functioning of tissues and *many of the problems of pathology occur from the chemical view point The*

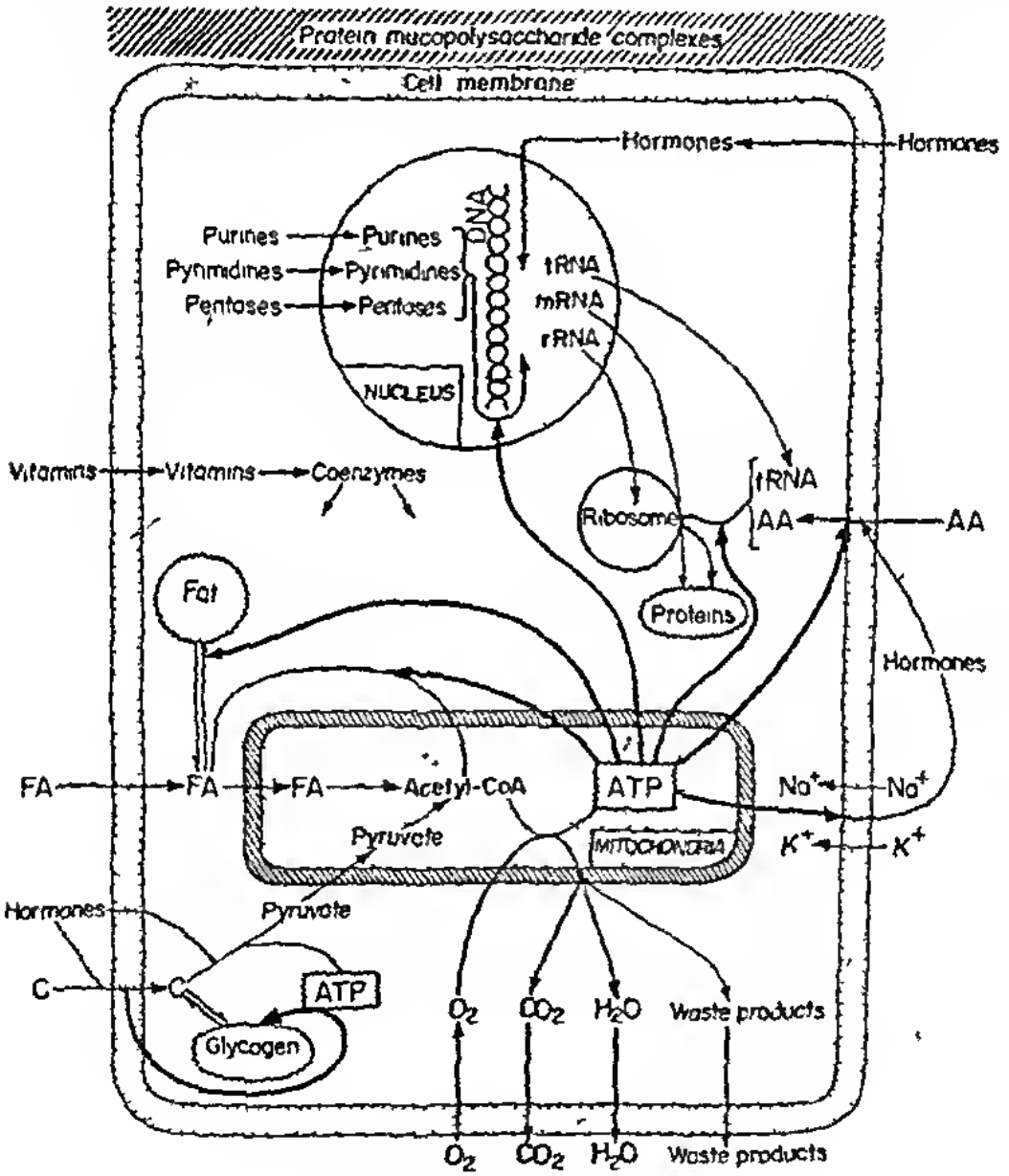


Fig. 1.1. Schematic representation of Biochemistry in brief.

bacteriologist is also concerned with the chemical changes caused by bacteria in tissues resulting in various diseased conditions He is to have ideas of vaccines, serum, and antitoxins The pharmacologist must know the chemical aspects of the body since the action of drugs always involves some alterations in the biochemical events occurring in the body The physicians too have to acquire knowledge of biochemical changes of different food stuffs, hormones, and vitamins etc to diagnose a disease properly for its cure They have also to depend on the large number of biochemical tests for treating diseases

STRUCTURE AND FUNCTIONS OF CELL CONSTITUENTS

The cell is the basic structural and functional unit of all living organisms Very recent studies have revealed the use of electron microscope and differential centrifugation to find out the presence of various structural units of the cell with a view to have an idea of their functions too The structure of an animal cell is shown below in figure 1 2

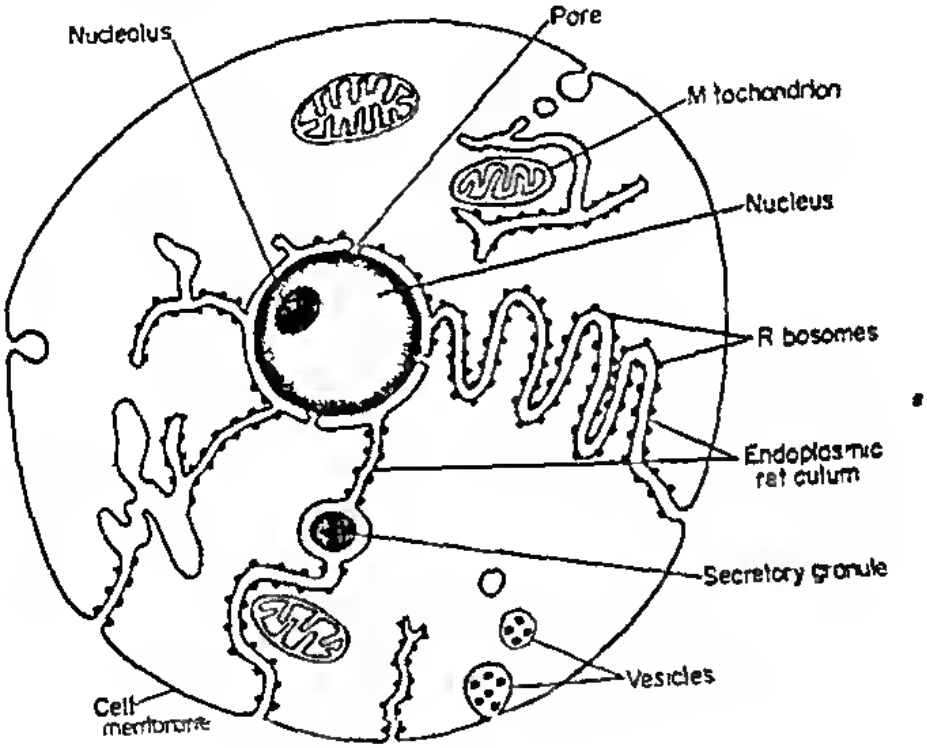


Fig 1 2 Structure of animal cell

Cell Constituents

1 Cell membrane (plasma membrane or unit membrane)
2 Nucleus
3 Mitochondria
4 Endoplasmic reticulum
5 Ribosomes or microsomes
6 Centrioles
7 Lysosomes
8 Cytosol

1. Cell Membrane:

(i) The boundary of every cell has a thin membrane of thickness 75Å units (although the thickness varies from 70Å to 100Å) This is also known as unit membrane or plasma membrane which is formed by the ingredients of the plasma (cytoplasm)

(ii) This membrane consists of three layers (a) Inner layer (20Å) of proteins, (b) Middle layer (35Å) consisting of phospholipids and cholesterol and (c) The outer layer (20Å) consisting of protein and polysaccharide These are arranged continuously to form pores of about 8Å

The phospholipid molecules in the light band are arranged in two rows that the phosphate containing ends (the polar or hydrophobic ends) point to the outside while the non polar or hydrophobic ends point to the inside

(iii) The orientation and nature of the lipid protein components in the cell membrane determines the charge in the membrane and movement of molecules across the membrane

(iv) The surface of the cell membrane bears pores, of internal diameter 3Å and length about 75Å, which lead from cytoplasm to the exterior

(v) The most important function of biologic membranes is to restrict the exchange of substances among various compartments Thus different types of body fluids are separated from each other by different membranes e g R B C membrane separates plasma from R B C This is performed by the selective permeability of the various membranes for which certain substances are passed through different rates namely high, moderate or low, while others are prevented from passing through

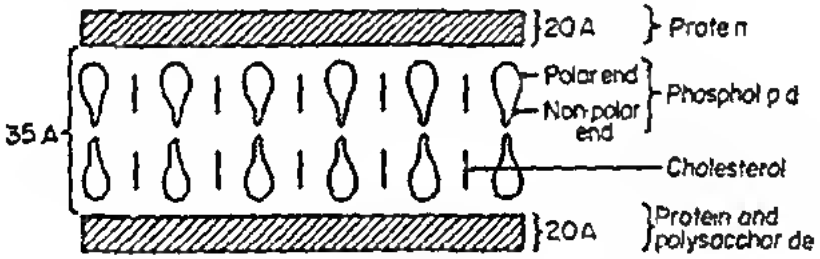


Fig 1 5 A cross section of cell membrane

2 Nucleus:

(i) Nucleus is the heaviest component of the cell and is separated from the cytoplasm by a double membrane The outer membrane is continuous with the endoplasmic reticulum The two membranes are separated from each other by the perinuclear cisterns (150Å) and join at intervals to form pores which allow the passage of materials from the cytoplasm to the nucleus and vice versa

(ii) The pores on nuclear membranes are of higher diameter than those on the cell walls and allow the passage of larger molecules such as protein, nucleic acids etc

(iii) The nucleolus within the nucleus contains ribonucleic acid (RNA) in granular form

(iv) **"Chromatin"**, the regular clums of thread-like material, distributed throughout the nucleus, is the most important component of the nucleus This consists of deoxyribonucleic acid (DNA)

(v) The chromatin, immediately before cell division, organises into simple small thread-like structures known as **"chromosomes"**, which consist of nucleo

protein molecules Most of the DNA present in the nucleus occur exclusively in chromosomes

(vi) The **gene or cistron**, the unit of genetic information, form part of the chromosomes and is a segment of the DNA molecule (about 600 bases) The genes are, therefore, arranged in an orderly manner along the length of the DNA molecule in the chromosomes

(vii) The DNA molecules form the template for the synthesis of RNA which is later on transported into the cytoplasm and the ribosomes The RNA molecules, then synthesize different types of proteins (hormones and enzymes) Since the enzymes regulate the metabolic processes of the body the nucleus is, therefore, said to be governing the metabolic processes in the true sense

(viii) In summary, the nucleus contain *proteins* (about 15 per cent of the cell), *RNA* (about 30 per cent of the cell of which 20 per cent in nucleolus and 10 per cent in chromosomes), and *DNA*

3 Mitochondria

(i) Mitochondria are the largest components in the cytoplasm

(ii) They contain about 35 per cent of the total protein of the liver In addition to proteins, they also contain lipids (25 per cent) most of which (about two-third) are phospholipids, and a small amount of nucleic acids

(iii) They are the "power house of the cell" and each cell may contain from 50 to 2500 mitochondria depending on the respiratory activity of the cell The cells of skeletal muscle, kidney, and liver contain large number of mitochondria while those of heart muscle contain less number

(iv) They vary in shape (spherical, filamental or sausage shaped) and in size (0 5 to 3μ long and 0 1 to 0 6μ wide)

(v) They have two membranes—the outer membrane is smooth while the inner membrane is folded to form *ridges* or *cristae* which extend into the matrix of the mitochondrion Two spaces—the intracristae space and the matrix space are thereby developed The matrix space is rich in enzymes, while the intracristae space contains substances of low molecular weight in solution The outer surface of the outer membrane and inner surface of the inner membrane possess indefinite protuberances or saucer like structures which are rich in proteins, most of which are the enzymes in biologic oxidation namely NAD—and NADP—dependent dehydrogenases, flavin linked enzymes, and cytochromes

(vi) Mitochondria swell extensively in a hypotonic medium and contract again in a hypertonic medium, faster with the addition of ATP Thus mechanism facilitates the exchange of metabolites

(viii) The mitochondria contain a large number of enzyme systems known as 'cyclophorase' which are involved in (i) oxidation of pyruvic acid, acetyl—CoA through Kreb's cycle, fatty acids, amino acids, (b) electron transport and oxidative phosphorylation, (c) synthesis of fatty acids They also contain enzymes concerned with the biosynthesis of amino acids, porphyrin, phosphatides, heme, hippuric acid, and urea They also contain transhydrogenases, adenylate kinase, glutaminase, and the enzymes of phosphorylation of nucleoside diphosphatase and carboxylations

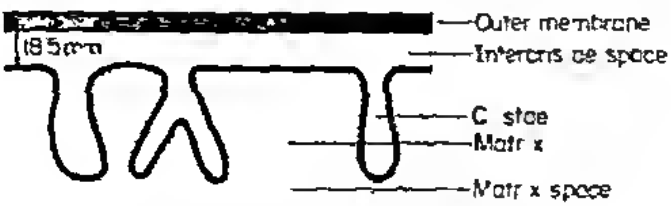


Fig 1 4 Structure of Mitochondria.

4. Endoplasmic reticulum:

(i) This is the complicated and organised system of membranes in the cytoplasm of the cell This membrane is constituted of protein lipid double layers and is very well developed in tissues with active protein synthesis

(ii) The *Golgi bodies* serve as a means of producing and maintaining the internal membrane The interior of this membrane is connected with perinuclear space and sometimes also with the extracellular space The cisternae (enclosed spaces) of this endoplasmic reticulum play a role in the exchange of material between the cell and the extracellular fluid This exchange of material takes place by the processes of diffusion, active transport and pinocytosis

(iii) This and the surrounding matrix are not only meant for specific metabolic activity, but also provide the means of communication between the nucleus, mitochondria and ribosomes

(iv) The important functions of this membrane and cytoplasm matrix are (a) Transport of nutrients and metabolites from in and out of the cell, (b) Regulation of protein synthesis, (c) Glycolysis, (d) Glycogenesis, (e) Pentose phosphate pathway, (f) Fatty acid synthesis

5 Ribosomes:

(i) Ribosomes or microsomes are the small granules on the outsides of the membranes of the endoplasmic reticulum

(ii) The size of the ribosomes ranges from 15 to 20 millimicrons (10^{-6} mm) and the diameter being 150Å

(iii) They are composed of ribonucleic acid (RNA) protein complex They also contain lipids mainly phospholipids

(iv) They occur separately or in a polysomal cluster They consist mainly of particles of two sizes—one with a sedimentation constant of 50S (S=Svedberg unit) and the other with 30S The combination of the two particles is 70S They are the main site of protein synthesis

(v) They also possess steroid reductase (responsible for metabolism of cholesterol and steroid hormones), phosphatases, hydrolases, hydroxylases, glucuronyl transferase, and ATPase

(vi) The Golgi apparatus acts as the store house of various hormones and secretory enzymes which are released at the time of requirement by the process of *emetocytosis*

6 Centrioles:

(i) Centrioles are two short cylindrical structures known to exist on either side of the nucleus at right angles to each other

(ii) They are not bound by any membrane

(iii) They help in the equal division of the chromosomes by taking them apart and they are, thereby, responsible for equal distribution of the characters in the offsprings

7 Lysosomes:

(i) The size (mean diameter 0·4μ) of the lysosomes is in between that of microsomes and mitochondria They are surrounded by a lipoprotein membrane

(ii) Since they contain hydrolytic enzymes they are named lysosomes

(iii) They contain digestive enzyme, such as cathepsin, acid phosphatase, ribonuclease, deoxyribonuclease, catalase, collagenase, α glucosidase, β galactosidase, α mannosidase, Phosphoprotein phosphatase etc

(iv) Unless the lipoprotein membrane of the lysosomes are ruptured, the enzymes are not released into the cytoplasm Hydrolases in lysosomes are separated from their substrates by means of lipoprotein membrane and only when this membrane is ruptured, the hydrolases enter the cytoplasm and destroy the bacteria or foreign particles when the latter enter the cells The rupture of the lipoprotein membrane takes place under conditions of cytolysis which include fat solvents, detergents, protease, lecithinase, acid pH, and high temperature

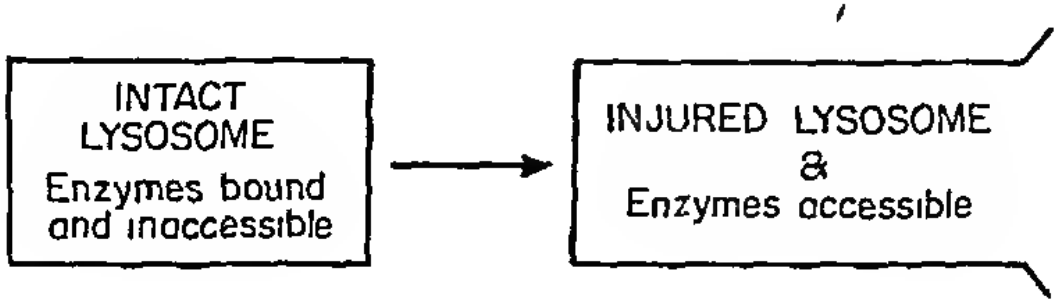


Fig 1 5 The lysosome

8 Cytosol:

(i) Lardy introduced the term 'cytosol' for the cell sap It is the unstructured "soluble" portion of the cell It is the 'supernatant' that remains after centrifugation of mitochondria, microsomes, lysosomes, and nuclei The supernatant as a complex mixture of proteins (35 40 per cent of the total cell), RNA, and other organic or inorganic compounds of low molecular weight

(ii) It mainly contains the enzyme systems involved in (a) glycolysis, (b) fatty acid synthesis, (c) pentose phosphate pathway, (d) the activation of amino acids in protein synthesis, and (e) catabolism of purines and pyrimidines

Separation of structural units of the cell.

(i) The cells are disintegrated in the isotonic media by differential centrifugation to separate the structural units of the cell

(ii) Potter Elvehjem homogenizer or similar equipment is used to prepare the tissue homogenates with the help of 0 25 M sucrose solution at 0°C

(iii) The homogenates are then separated by differential centrifugation below 4°C The results of separation are given below

Centrifugal field	Time in minutes	Structural units
700 g	10	Nuclei, cell membrane, and erythrocytes.
5,000 g	10	Mitochondria and Lysosomes
57,000 g	60	Microsomes and Lysosomes
1,50,000 g	30	Ribosomes
Unsedimented		Cytosol

Fig 1 6 Seperation of structural units of the cell

CHAPTER 2

BIOPHYSICS

ACIDITY AND ALKALINITY

1 The reaction of the tissues and tissue fluids of the body is nearly natural excepting gastric juice The slight changes in reaction have a significant physiological effect. In case, the reaction of the solution is made slightly more acid or more alkaline, the heart stops

2 Acidity is considered by an excess of hydrogen ions (H^+) over hydroxyl ions (OH^-) and alkalinity by an excess of OH^- over H^+

3 The intensity of acidity depends upon the amount of hydrogen ions in excess, and the intensity of alkalinity depends upon the excess of hydroxyl ions. If the amounts of hydrogen and hydroxyl ions are the same, the solution is neutral Pure water is a neutral solution and it contains H^+ and OH^- in equal proportions It is never free from H^+ and OH The ions are formed by ionisation, thus

$$H_2O \rightleftharpoons H^+ + OH^-$$

4 The hydrogen ion (H^+ or proton) with its high ratio of charge cannot exist free in aqueous solution. It becomes associated with one or more water molecules to form a hydrated hydrogen ion called hydronium ion (H_3O^+)

5 The dissociation of an acid should be written as

$$H_2O + HA \rightleftharpoons H_3O^+ + A^-$$

But it is convenient to neglect the hydration and must be written as

$$HA \rightleftharpoons H^+ + A^-$$

6 The concentration of H^+ can be made increased without increasing OH^- by adding an acid which ionises to form H^+ Example .

$$HCl \rightleftharpoons H^+ + Cl^-$$

Similarly, the concentration of OH^- can be made increased without increasing H^+ by adding an alkali Example

$$NaOH \rightleftharpoons Na^+ - OH^-$$

7 It must be noted that even in the most acid solutions there are always some OH^- and in alkaline solutions there are always some H^+

8 Aqueous solution always contain both H^+ and OH^- because water always ionises to some extent.

9 The ionisation of water is a reversible reaction admitting the *law of Mass Action.* This clearly explains that the product of the concentrations of H^+ and OH^- maintains a constant ratio to the concentration of water in any system. This is expressed as

$$[H^+] \times [OH^-] = K[H_2O]$$

where K=constant, [ ]=concentration.

10 If H^+ is added to this above system, the left hand side of the equation will be increased To re-establish equilibrium, OH^- must decrease by combining with H^+ to form water until the equation is balanced In other words, if H^+ are added to a solution, the ionisation of water will be depressed and there will be fewer OH^- than in pure water Similarly, addition of OH^- will depress the ionisation of water and reduce the concentration of H^+

11. As a matter of fact, water is ionised to such a small extent that even if all the H^+ and OH^- are recombined to form water, the increase in the concentration of water molecules will be negligible Considering the effect of the addition of H^+ or OH^- to a system, it can be neglected that the addition will cause a minute increase in the concentration of H_2O

12 In pure water or a perfectly neutral solution, $[H^+]=[OH^-]$ Hence $[H^+]^2=Kw$.

13 If $[H^+]$ is greater than 10^{-7} (*e g* 10^{-4}), the solution is acid If $[H^+]$ is less than 10^{-7} (e g 10^{-11}), the solution is alkaline

14 HCl and NaOH are practically 100% ionised Solutions of these substances are almost completely ionised when the solutions are more dilute than N/10 The ionisation is not complete at concentration greater than this

15 With weak acids (e g acetic acid) 100% dissociation is attained when the solution is weaker than N/10 Therefore, N/10 acetic acid is much less acid than N/10 HCl

16. The reactions of all biological fluids occur between $[H^+]10^{-1}$ and 10^{-10}.

INDICATORS

Definition : Certain dyes which can determine the hydrogen ions in a solution are said to be Indicators

Characteristics :

1. The dyes are weak acids (or less commonly weak bases)

2 They have the property of dissociating in solution to yield ions of different colour from the undissociated molecules

3 The P^H at which dissociation will occur depends upon the strength of the acid , the stronger the acid the lower the P^H at which it dissociates

4 The free acid of methyl orange which is red can be represented as MH It is dissociated as below on dissolving in water

$$MH \rightleftharpoons H^+ + M^-.$$

M^- being a yellow ion The colour of the solution will depend upon the extent of ionisation, so that in very dilute solution it will be yellow.

5 It may he orange in stronger solutions with incomplete dissociation or red with no dissociation

6 If acid (i e H^+) is added, the ionisation will be depressed and MH will be reformed and the colour will be red But if alkali (i e OH^-) is added, the OH^- will combine with H^+ to form water and MH will ionise to the great extent. Hence, the colour will be yellow.

Uses ·

1. Indicators are used to determine the titratable acidity or alkalinity

2 When an indicator gives its intermediate colour (i e it is partially dissociated), it can be easily assumed that the pH of the solution is about the middle of the range

3 Solutions of mixed indicators which give a number of colour changes over a wide range of PH are called *"universal"* indicators They are used for indicating quickly in what part of the PH range the PH of a given solution lies. For accurate determination of PH, the "PH meter" is used

4 The following table gives a list of some common indicators which are used for the biological PH range

Indicator	PH *range*	*Colour change*
Methyl orange	3 1—4 4	Red—→Yellow
Bromphenol blue	3 0—4 6	Yellow—→Blue
Congo red	3 0—5 0	Blue—→Red
Topfer's reagent	2 9—4 0	Red—→Yellow
Methyl red	4 3—6 3	Red—→Yellow
Phenol red	6 8—8 4	Yellow—→Red
Thymol blue	8 0—9 6	Yellow—→Blue
Phenolphthalein	8 3—10 0	Colourless—→Red

PH

Definition

PH is defined as the negative of logarithm (base 10) of the hydrogen ion concentration

Or,

It is defined as the logarithm of the reciprocal of the hydrogen ion concentration

i e $P^H = -\log [H^+]$ or $P^H = \log \frac{1}{[H^+]}$

All vital activities are affected by H^+ concentration Hydrogen ion concentration must be ascertained before the PH is calculated For strong electrolytes, $[H^+]$ may be substantially the same as the total concentration, if complete ionisation is assumed But for weak electrolytes, $[H^+]$ must be obtained by the calculation from the ionisation constant

Variation in PH and Interpretation

A solution of PH 3 contains 10^{-3} gram H^+ Per litre

A solution of PH 5 contains 10^{-5} gram H^+ Per litre

A solution of P^H 8 contains 10^{-8} gram H^+ Per litre.

A solution of P^H 3 has 10 times the $[H^+]$ of one of P^H 4 and 100 times that of a solution of P^H 5. As $[H^+]$ increases, P^H decreases in such a way that for one unit increase in P^H the $[H^+]$ increases 10 times. It does mean that the higher the P^H the lower will he the acidity.

A neutral solution has P^H 7. Pure water (the neutral solution) is ionised as follows.

$$[H^+]\times[OH^-]=Kw.$$

or, $[H^+]\times[OH^-]=1\times10^{-14}$.

Putting logarithm on hoth sides,

$$\log[H^+]+\log[OH^-]=\log 1+\log 10^{-14}.$$

or, $-\log[H^+]-\log[OH^-]=0+14\log 10$. [multiplying hoth sides hy — sign]

or, $P^H+P^{OH}=14$ [Since, $P^H=7$].

$\therefore$ $P^H=P^{OH}$ i.e. $[H^+]=[OH^-]$.

Therefore, a solution with P^H less than 7 is acid and higher than 7 is alkaline. The P^H range is 0 to 14 only. It should be noted that the P^H scale is logarithmic, not numerical.

P^H 6.5 does not represent a $[H^+]$ half-way hetween 6 and 7.

Actually, P^H 6.5=$[H^+]$ 3.2×10^{-7}

P^H 6.0=$[H^+]$ 10×10^{-7}=$[H^+]$ 10^{-7}

Determinatlon of P^H :

For weak electrolytes with which the physiologic chemistry is concerned, this may he calculated hy the law of mass action.

$$HA=[H^+]\,[A^-]$$

$$\therefore\quad \frac{[H^+]\,[A^-]}{[HA]}=Ka$$

HA=Undissociated weak acid, Ka=Dissociation constant for the acid.

The table helow gives the P^H of some typical solutions.

Solution	P^H	*Solution*	P^H
N/10. HCl	1.04	Pure water	7.0
N/100. HCl ...	2.00	Milk	7.1
Gastric Juice (adult) ...	1.2—1.5	Tears	7.2
Gastric Juice (Infants)	5.0	Blood (average) ...	7.4
Urine (average) ...	6.0	Intestinal Juice ...	7.8
Saliva	6.8	Pancreatic Juice ...	8.0
		N/100. NaOH	12.0

Determination in a non homogeneous solution

Diffusion between two planes X and Y in a non homogeneous solution can be expressed quantitatively by Fick's law as follows

$$\frac{ds}{dt} = DA \frac{dc}{dx},$$

where, $\frac{ds}{dt}$ = The rate of movement of solute

D = Diffusion constant.

A = The area of the planes

$\frac{dc}{dx}$ = The concentration gradient i e the difference in concentration between X and Y/distance between X and Y

Physiological role

1 It helps in passive transport of substances across the cell membrane.

2 Substances move in the direction of the physical gradients which follows the rule of diffusion

OSMOSIS AND OSMOTIC PRESSURE

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

Osmosis occurs in the direction opposite to that in which diffusion occurs The animal membranes are not completely 'semi permeable' The artificial membrane $Cu_2Fe(CN)_6$ is the most selective This is prepared by keeping copper crystals in the pores and dissolved in $K_4Fe(CN)_6$ solution

Experiment

In the figure below, the semipermeable membrane is fitted at the mouth of the thistle funnel The thistle funnel is provided with a sucrose solution upto a mark and inverted over water in a trough After some times it has been found that the level of sucrose solution is increased in the thistle funnel owing to the entry of water through the semi permeable membrane The flow of water will be prevented when both the pressure (inside and outside) are equal The excess pressure can be measured by a mercury manometer

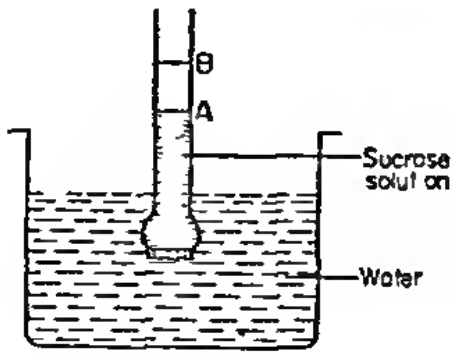


Fig 2 1 Osmosis and osmotic pressure

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

Point of some significance in the absorption of food

1 Osmotic pressure is only permanent if the membrane is truely semi-permeable, i e if it stops all solute molecules and only passes solvent molecules

2 In case of dialysis, if the collodion or cellophane bag is filled with a solution of a dye with small molecules and placed in contact with water, water will pass into the dye but at the same time water plus dye will pass out from the bag As water molecules are smaller than the dye molecules, water will pass into the bag more quickly than water plus dye will leave it Therefore, an osmotic pressure will be developed, but it will only be small and transient because the membrane is permeable to both water and dye But if the bag is filled with a dye of large molecules, the dye cannot pass out into the water but water will pass into the dye causing a permanent pressure difference If the bag is surrounded with a solution of NaCl of greater osmoticpressure than the dye, water will at first pass out of the bag faster but at the same time NaCl will pass into the bag The final equilibrium will be attained when the osmotic pressure of the salt solution outside the bag equals the osmotic pressure of salt inside, so that the ultimate permanent osmotic pressure difference will be that of the dye alone

Factors regulating osmotic pressure

1 Osmotic pressure depends on the number of solute molecules but not on the size of the molecules

Example A solution of urea (Mol wt 60) of 60 g per litre has the same osmotic pressure as a solution of cane sugar (mol wt 342) of 342 g per litre, because these two solutions contain the same number of molecules per litre

2. In case of salts which ionise, it is the number of ions plus molecules which count, so that fully ionised NaCl has twice the osmotic pressure it would have as judged by the number of molecules

3 Other substances, such as soaps, form molecular aggregates, so that their *solutions have lower osmotic pressure*

4 The osmotic pressure increases with the rise in temperature

Osmotic pressure in cells

1 If the cell is kept in a hypotonic solution, the cell wall and the vacuolar membrane both will allow water to pass into it and will set up an excess pressure in the interior of the cell causing the cytoplasm to be forced tightly against the cell wall In normal health, this condition is known as "Turgor and the cell is said to be turgid

2 If the cell is immersed in a concentrated solution (high osmotic pressure), water will pass out of the interior of the cell The cytoplasm will then shrink and detach itself from the cell wall This phenomenon is said to be ' plasmolysis".

Iso-osmotics—Solutions with the same pressure are termed iso-osmotics

Isotonic solution—A pair of solutions which produce no flow through a semi-permeable membrane are said to be isotonic solutions

Physiological importance

1 Absorption from gastro intestinal tract, fluid interchange in various compartments of the body follow the principles of osmosis.

2 The osmotic pressure of plasma proteins regulates water to flow from the protein—free intestinal fluid into the blood vessels

3 Living red cells if suspended in 0.92% NaCl solution they neither gain nor lose water Briefly speaking intracellular fluid of red cells is isotonic with the red cell membrane in 0.92% NaCl solution

Application of Osmosis

1 The purgative action of Epsom ($MeSo_4$) or Glauber's (Na_2So_4) salts is an osmotic phenomenon A strong solution of a salt in the intestine prevents absorption of water or withdraw of water from the body causing dilution of the intestinal contents

2 The pain caused by the contact of sugar with exposed nerves of teeth is due to the osmotic withdrawal of water from the exposed area by strong sugar solution

3 The pain experienced by the application of salt on the cut of the skin follows the same osmotic phenomenon as in No 2

4 Water or salts (chiefly NaCl) are excreted by the kidney to keep the blood isotonic with the cells Hence osmosis is of great importance in the process of urine secretion

5 The clinical application of osmotic force is the injection of hypertonic solution of magnesium sulphate to reduce the volume of the brain or lower the pressure of cerebrospinal fluid

6 Hemolysis is caused by the dilution of RBC by the osmotic phenomenon

SURFACE TENSION

Definition The force with which the surface molecules are held together is called the surface tension

Explanation The interior molecules of a homogeneous liquid are equally attracted in all directions by surrounding molecules They are free to move in all directions But the molecules in the surface of the liquid are attracted downward and sideways but not upward (except for the little attraction of air molecules) As a result the molecules of the surface are not so free to move They are held together and form a membrane over the surface of the liquid Therefore when finely powdered sulphur or other non wetting powders are sprinkled upon water they do not sink but are suspended on the surface

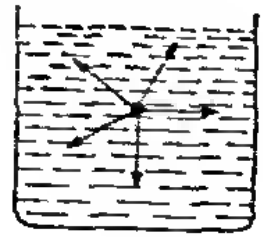


Fig. 2.2 Surface tension

A great part of the energy required to convert a liquid into a gas is essential to overcome surface tension and drag the molecules free from the surface of the liquid

There is also an interfacial tension which is biochemically very important especially in the process of adsorption This tension lies at the boundary between immiscible liquids e.g. oil drops emulsified in water This tension is due to unequal attraction of the film molecules as compared with the molecules in the interior of the liquid

Surface tension × Surface area=Surface energy A falling drop of liquid assumes a spherical form because the ratio of surface area and total free surface energy is the least

Method of determination of surface tension

$$\gamma = \frac{1}{2} hdgr$$

where, h=height of the liquid

d=density of the liquid

g=acceleration due to gravity

r=radius of the capillary tube

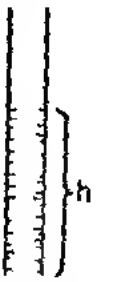


Fig 2 3 Capillary tube

Factors affecting surface tension

1 *Temperature*—Surface tension decreases with the increase in temperature

2 *Dissolved substances*—(a) Most inorganic salts slightly raise surface tension of water although potassium permanganate lowers

(b) Organic substances usually lower surface tension Soaps and bile salts are the most effective in this respect

(c) Alkalis increase surface tension but ammonia lowers it Strong mineral acids also decrease surface tension

(d) In liquid liquid and solid liquid systems dissolved substances generally lower interfacial tension

The Gibbs-Thomson principle in relation to surface tension

1 Substances that lower the surface tension become concentrated at the interface

2 Substances that increase surface tension tend to move away from the interface

3 Lipids and proteins which are both effective in lowering surface tension are found concentrated in the cell wall

Physiological importance

Surface tension is involved in the process of digestion because bile salts reduce the surface tension of lipids and thus assist emulsification

As a result, the surface area is increased which favours lipase activity on lipids

ADSORPTION

Definition The process of taking up substances from solution on surface is called adsorption

Characteristics

1 Adsorption is a surface phenomenon

2 The attractive forces on the surface are limited to distances of one molecule deep

3 The extent to which adsorption takes place is dependent upon the nature of both adsorbing agent and the substances adsorbed

4 The greater the surface of the adsorbing agent, the greater is the adsorption

5 Charcoal becomes activated when it is heated at 700°—800°C in a closed container and adsorption takes place on the activated charcoal due to the attraction of oppositely charged ions Salts, acids and alkalis restrict it

6 It has got much importance in industry

Principles governing adsorption

1 Adsorption is a reversible process

2 It decreases with the rise in temperature

3 This process takes place relatively quickly Equilibrium is reached within one hour

4 Adsorption is proportional to the surface area and it varies with the nature of the surface of the adsorbent and of the substances to be adsorbed

5 It proceeds best from dilute solutions

6 Narrow pores on the surface of the adsorbing agent are more effective than globular openings

7 Heat is given off in all adsorption

8 The molecules adsorbed on the surface are oriented and arranged in a definite manner

Importance of adsorption

1 Many chemical reactions are speeded up by the presence of adsorptive surface Oxygen and hydrogen are adsorbed together upon platinum black and combine rapidly at ordinary temperature to form water

2 Surface adsorption helps to combine enzymes with substrates to give reaction products

3 Adsorption processes taking place on the cell membranes promote many vital chemical reactions and also cause changes in surface tension and cell consistency

4 Drugs and poisons which are adsorbed on cell surfaces exert their effects from that location Selective adsorption may be related to specific action

5 The process of adsorption is applied in the purification of enzymes

Arrangement of molecules at an interface

Solute molecules accumulated at an interface tend to arrange themselves in a definite pattern if the molecules are unsymmetrical, (i e if they have —CooH, —OH, and —NH_2 groups), they have water attractions , whereas the arrangement is symmetrical within the solution

The unsymmetrical molecules align themselves, so that the polar groups are directed towards water and the non polar groups at the other end of the molecule remain away from it (orientation)

At an oil water interface the orientation would be especially favoured since the oil would attract the non polar groups in addition to the water attracting eht polar groups

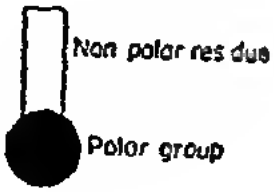


Fig 2 4

In the cell (which contains both water and lipids) it is likely that some cell constituents are oriented in this way The cell membrane absorbs oriented molecules Orientation is an important factor in adsorption and enzyme reactions In this way one part of a molecule can be presented to a reacting substance

HYDROTROPY

Introduction

Moore and Parker (1901) studied the solvent action of 5% aqueous solution of bile salts on fatty acids (and their salts) The solubility of acids in water was less than 0 1% in bile salt solution was 0 5% and the solubility was increased to 4 0% when 1% of lecithin was added The fatty acids could be readily recovered from solutions by extraction with solvents showing that the fatty acid molecule had not been altered

Neuberg (1916) suggested that substances having the power of making water insoluble substances water soluble should be called hydrotropic substances

Definition It is the process by which water insoluble substances are made water soluble by hydrotropic substances

Hydrotropic substances Cholic acids benzoic acids hippuric acids phenyl acetic acid and soaps of higher fatty acids

Water Insoluble substances Fats phospholipids sterols calcium carbonate calcium phosphate magnesium phosphate and uric acid

Biological importance

1 Substances so dissolved by hydrotropy are diffusible through membranes

2 In the body, hydrotropic substances are found not only in bile but also in intestinal juice, extracts of intestinal mucosa, blood plasma and places where such substances are particularly useful in helping absorption and transport of insoluble substances such as cholesterol and fatty acids formed by digestion

3 The hydrotropic substances have the power of decreasing surface tension, e g bile salts reduce the surface tension of fats and make emulsification for lipase action

VISCOSITY

Definition The resistance experienced by one layer of a liquid in moving over another layer is called "viscosity"

Viscosity varies greatly Ether and gasoline have little viscosity and are quite mobile But honey and coal tar have high viscosities The unit of viscosity is the 'Poise" named after Poiseuille It is expressed as

$$\eta(\text{eta}) = \frac{\pi P r^4}{8VL} t$$

Factors affecting viscosity

1 *Temperature* The viscosity of liquids decreases by about 2% for each degree rise in temperature

2 *Chemical composition* The viscosity of liquids generally depends upon the size shape and chemical nature of their molecules It is greater with larger than with smaller molecules, with elongated than with spherical molecules Large amounts of dissolved solids generally increases viscosity Small amounts of electrolytes lower the viscosity of water slightly

3 *Colloid systems* The viscosity of lyophilic colloid solution is generally relatively high

4 *Suspended material* Suspended particles cause an increase in the viscosity The viscosity of blood is important in relation to the resistance offered to the heart in circulating the blood The heart muscle functions best while working against a certain resistance The viscosity of blood is due largely to the emulsoid colloid system present in plasma and to the great proportion of suspended corpuscles

COLLOIDS

Introduction

Graham (1861) first distinguished two types of solutions—crystalloidal which diffused through a parchment or animal membrane, and colloidal which did not The difference is only one of the size of the solute particles and is unrelated to their chemical nature The particles are of the size of small molecules (like sugar or urea) and they form a crystalloidal or a true solution In such a solution, the difference in size of solute and solvent molecules is relatively small, so that the solution can he called homogeneous. But if the particles are large compared to the solvent molecules, the solution can he called heterogeneous and if the particles do not separate when the mixture is allowed to stand, this is called a colloidal solution

About 90% of the organic matter of living tissues is present in the colloidal state

The colloids much depend on surface area A physiological example of the importance of surface area may be pointed out in case of the R.B.C. which contains the oxygen carrying pigment hemoglobin As blood circulates through the capillaries of the lungs, oxygen diffuses the surface of the corpuscles and unite with the hemoglobin to form oxyhemoglobin This is performed by a quick process This is facilitated by the great surface area for taking up oxygen.

Definition Certain substances such as proteins, polysaccharides do not diffuse through percbment or animal membrane although form homogeneous or beterogeneous solution These substances are called colloids.

Size of the colloidal particle Generally the size of each particle is 10—100 nm (submicrons) Only visible under ultramicroscope

Determination of the size of colloidal particles

1 By ultrafiltration 2 By diffusion rate 3 By the ultracentrifuge 4 By light scattering 5 By X ray analysis 6 By osmotic pressure

The electric charge of colloidal particles

The colloidal particles are electrically charged This charge is of much importance in stabilizing colloidal solution They repel each other and remain in suspension The neutralization of the charges causes precipitation or floccula tion of colloids

Streaming potential or flow potential

When water is present in a glass capillary the walls of the capillary are negatively and the water positively charged If water is forced to flow through the capillary from left to right, a potential develops between the ends of the capillary This potential is referred to as the streaming or flow potential

It varies with the nature of the liquid and the capillary The glass capillary probably becomes negative by absorbing OH ions from water, leaving the H^+ ions to charge water molecules This positively charged water molecules are mobile As water flows through the capillaries, the positive charges accumulate in excess at one end leaving an excess of negative charges at the other This causes potential difference which opposes the flow

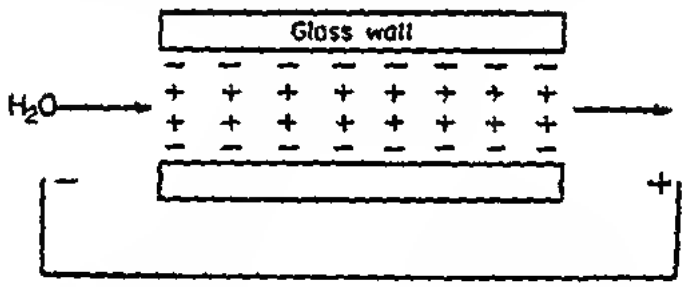


Fig 2 5 Streaming potential or flow potential

The flow of fluids through the membranes of tissues develops streaming potentials which contribute to the potential differences across such me nbranes

The precipitation or flocculation of colloidal particles

In order to precipitate emulsoids (hydrated colloids) by salts, sufficient salts must be added to dehydrate the emulsoid At the same time the positive or negative ion of the salt neutralizes the charge upon the particles leading to precipitation

The colloidal protein particles of blood serum may be precipitated by adding a large amount of $(NH_4)_2 SO_4$ The precipitations of emulsoids by adding large amount of soluble salts is referred to as 'salting out'

Protective colloids

1 When a gelatin solution is added to a gold sol the particles of the e nulsoids (gelatin) are adsorbed by the particles of the suspensoids (gold) and the gold particles become much more resistant to precipitation.

2. Protective colloids play an important role physiologically. Some of the calcium phosphate of blood is held in colloidal suspension by the protective action of the proteins present.

3. The bile salts also act as protective colloids to keep sparingly soluble cholesterol and the calcium salt of bilirubin in colloidal suspension. Gall stones may result from the precipitation of such substances in the absence of sufficient protective colloids.

4. Protective colloids in urine may prevent bladder stone formation.

General properties of colloidal solution :

A. *Brownian Movement* :

1. The continuous motion of the particles is known as Brownian Movement.

2. The particles are kept in movement by continuous buffeting by the solvent molecules which are themselves always in motion

3. The rate of brownian movement depends on the size of the particles, the smaller particles are more easily moved than the big ones.

4. If the particles are too large, they gradually sink under the influence of gravity.

5. Brownian movement is quite haphazard.

6 The particles move in a straight line with sudden irregular changes of direction.

B. *Osmotic pressure of colloidal solutions* :

1. Since the size of the colloidal particles are bigger than that of solvent molecules the number of colloidal particles are less relative to solvent molecules in a solution.

2. The osmotic pressure of a solution is directly proportional to the number but not the size of the dissolved particles. Therefore, the colloidal solutions have a low osmotic pressure.

3. The serum proteins which are present to the extent of 7% to 8% exert only an osmotic pressure of about 30 mm. Hg ; whereas the crystalloids of serum (0 9% NaCl) have an osmotic pressure of about 5200 mm. Hg. and 6% sucrose has an osmotic pressure of about 3,000 mm. Hg.

4. Soap solutions have small osmotic pressure because the soap molecules aggregate to form micelles of colloidal dimensions.

5. Although the osmotic pressure of colloidal solutions is very small, it has immense biological importance in providing the driving force for the passage of water and other substances through cell membranes.

C. *Dialysis* :

1. The process of separation of crystalloids from colloids by diffusion through a membrane by osmotic force is called dialysis.

2 The most usual membranes used to-day for dialysis are the various grades of collodion (made from solutions of cellulose—nitrates or acetates—in solvents such as alcohol, ether or acetic acid) or cellophane perchment.

3. Dialysis is particularly needed for removing salt from proteins after precipitation by "salting out".

4 The precipitate with a little water is placed in a collodion bag and immersed in water

5 The passage of salt into water is accelerated by keeping salt content of the water low, i e by running water

6 Arrangement must be made for a big volume increase inside the bag in the early stages of dialysis, since it is a strongly hypertonic solution and water molecules will pass in more quickly than salt molecules pass out

7 The pressure will only be transient if the water outside is frequently altered and the final pressure inside the bag may be very slightly more than the original

8 Dialysis is applied in medicine in the "artificial kidney"

9 This mechanism is inserted into the patient's circulation and urea passes out from the blood, substituting for the action of the faulty kidneys

10 *Dialysis of electrolytes can be done by passing an electric current* through the solution A cell consisting of three compartments separated by membranes is used, the colloidal solution is placed in the centre compartment and the electrodes in the outer ones Positive ions are attached to the cathode and negative ions to the anode This process is called electrodialysis

D *The Donnan membrane equilibrium*

1 If the membrane is not permeable to all the ions, i e if one of the ions is a colloid, the result becomes very interesting But if the membrane is freely permeable, equilibrium is reached easily

Let us take the case of solutions of NaCl and NaR (where R^- is an ion too large to penetrate the membrane) separated by a membrane The initial state can be represented

Na^+	Na^+
R^-	Cl^-
(1)	(2)

Since Na^+ and Cl^- can diffuse across the membrane and R^- cannot, equilibrium can be represented

Na^+	Na^+
R^-	Cl^-
Cl^-	
(1)	(2)

If we express the molar ion concentrations as C_1 and C_2 and assume that x ions of Na^+ and Cl^- diffuse from (2) to (1) This can be represented

Initial state				*Equilibrium*				
Na^+	R^-	Na^+	Cl^-	Na^+	R^-	Cl^-	Na^+	Cl^-
C_1	C_1	C_2	C_2	(C_1+x)	C_1	x	(C_2-x)	(C_2-x)

Therefore, $(C_1+x)x=(C_2-x)^2$

$$\therefore \quad x=\frac{(C_2)^2}{C_1+2C_2}$$

2 Let us consider the salt NaR and water through a membrane This can be represented

Initial state			*Equilibrium*	
Na^+	H^+		Na^+	Na^+
R^-	OH^-		R^-	OH^-
			H^+	

Here the P^H is changed The NaR side is acid and the alkali will be "excreted" across the membrane

3 Let us consider the salt, RCl and water through a membrane This can be represented

Here acid is ' excreted" across the membrane

4 It must be noted that in the body equilibrium may never be established owing to the removal of "exereted ' ions in other reactions This rather helps to accelerate "excretion"

Types of colloidal solution

There are two types of colloidal solution—Emulsoids and Suspensoids

A Suspensoids :

1 The surface tension and viscosity of suspensoids are nearly the same as those of the solvent.

2. The suspensoid particles carry a definite electric charge which determines the stability of the suspensoid.

3 They are very easily precipitated if the charge is neutralised

4 Once they are precipitated, they are not brought back into colloidal solution again

5 Suspensoids are not hydrated, hence they are said to be hydrophob e or lyopbobie colloids (water fearing colloids)

B Emulsoids :

1 They are very stable and not easily precipitated by salts.

2 If they are precipitated, they are easily redissolved to form a collo dal solution

3 They have a lower surface tension and much higher viscosity than the solvent.

4 The particles carry electric charges , some carry positive and negative charges simultaneously, e g proteins

5 The nature of the charge on a protein particle can be changed by altering the P^H of the solution

6 Practically, all the colloids of the living cell exist as emulsoids. They bave a great affinity for water, hence they are called hydropbilic.

7 The emulsoid particles are molecules surrounded by shells of adsorbed water.

8 An emulsoid may be changed into a suspensoid by debydration and tben is precipitated.

An emulsoid can exist in two forms—Sol and Gel

Sol I Sol can be converted into a gel by changes of temperature, bydrogen ion concentration or salt concentration

2 The continuous phase in sol is water (or a dilute solution), and the disperse phase is a concentrated solution

3. Addition of $CaCl_2$ to an alkaline solution of caseinogen gives a sol

Gel 1 Solutions stronger than about 1% form gels on cooling

2 Gels are quasi-solids

3 They do not assume the shape of the vessel in which they are placed

4 Great pressure is required to squeeze water out of a gel

5 In a gel, the concentrated solution forms the continuous phase and water the disperse phase.

6 Formaldehyde can remove water from the gel and hence it is used in the histological process of "fixing" tissues

7 Many tissue structures are essentially gels Cytoplasm may undergo sol⇌gel transformation

8 Tissues, especially the skin, are not dehydrated owing to the existence of gels

Imbibition. 1 Dried emulsoids take up (imbibe) water and swell considerably, this process is called imbibition

2 The process of germination of seeds is the taking up of water by imbibition

3 Heat is liberated during imbibition

4 Most dried animal and vegetable tissues show imbibition

5 In imbibition, water is not expelled by squeezing

TRANSPORT THROUGH BIOLOGICAL CELL MEMBRANE

The living organisms can be resolved into organs, glands, tissues, cells and organelles It is very interesting in biology to know how solutes and water get into and out of cells and organelles Most attention is to be paid to erythrocytes and mitochondrion The cell membrane is a complex lipoprotein structure

A. Passive diffusion :

1. Some solutes pass through cell membrane by simple diffusion with the concentration gradient This can be expressed by the modification of Fick's law

$$\frac{ds}{dt} = PA\ (C_0 - C_i)$$

where, P=the permeability coefficient

A=area of membranes.

C_0 and C_i=the concentration of solution outside and inside the membrane respectively.

$\frac{ds}{dt}$ =rate of movement of solute

2 Lipid-soluble solutes pass more readily through cell membranes than lipid-insoluble solutes Because the cell membrane consists of small water-filled pores of radius about 0 4 nm through which water-soluble solute of suitable

molecular size pass, surrounded by lipid areas through which lipid-soluble solutes penetrate

3 Water diffuses through the cell pores from a solution of low concentration to a solution of high concentration and this "bulk flow" of liquid across the membrane will speed up molecules diffusing in the direction of the flow and slow down those moving in the opposite direction. This "drag" effect is a second force acting in passive diffusion

4 The third force which may operate is an electric potential across the membrane Many cell membranes can maintain potential difference between inside and outside and the potential gradient acts as a driving force for passive transport across the cell The membrane acts as a passive barrier.

B Facilitated transfer :

1 Some compounds, e g sugar, aminoacids pass through membranes at a greater rate than expectations This is because of the effect of a carrier

2. The carrier in the membrane combines with the substance to be transported and in some way ferried through the membrane and released on the other side

3 In case of enzymic reactions, there is a "saturation effect" The rate of transport of the solute increases when the carrier, enzyme, is saturated This type is sometimes termed "catalysed diffusion".

4 Another mechanism is that the substance to be transferred is converted into another which will penetrate the membrane more easily, e.g the mitochondrial membrane is impermeable to acyl coenzyme A derivatives The acyl group is transferred to carnitine to form acyl carnitine derivative which can pass through the membrane The acyl coenzyme A derivative is then reformed on the other side of the membrane

Fatty acids can also be transferred into and out of mitochondria

Acetyl CoA within the mitochondria can be transferred to oxaloacetate to yield citrate to which the mitochondrial membrane is permeable The citrate passes out into the cytoplasm where it is split enzymically to give acetyl-CoA again

C Pinocytosis :

1 The cell membrane forms pockets or invaginations which can draw materials on the outside towards the cell interior

2 The vesicles extend into the cell where they are pinched off and finally release their contents into the cell by some unknown way

3 This process occurs in the foetal and newborn animals and helps the absorption of intact protein from the gut

D Transport of ions :

1 The membrane itself contains polar groups and is therefore electrically charged

2 The transport of most ions occur more slowly than the non-electrolytes But H^+, OH^- penetrate all cell membranes easily The red cell is easily penetrated by Cl^- and HCO_3^-

3 In the case of ions, especially, Na^+ and K^+, the permeability is very small The high concentration of K^+ and low concentration of Na^+ which are

often found in cells are maintained by special mechanism which involve the expenditure of energy

E **Active transport :**

1 The process by which solutes can often pass through membranes against their concentration gradient requires energy This process is termed active transport

2 Active transport is involved in the absorption from the small intestine of glucose and galactose, aminoacids and other substances important to the body

3 An active transport device which forces Na^+ out and K^+ in has been referred to as the "Sodium Pump'

4 The mechanism requires a carrier which can exist in two forms with different affinities for Na^+ and K^+ ATPase is involved in it (see active transport of glucose)

ISOTOPES

Definition Isotopes may be defined as atoms having the same atomic number but different atomic weights

They are the subspecies of the same chemical element and occupy the same position in the periodic table, but have different physical properties

The atomic constitution of three isotopes of hydrogen are illustrated below :

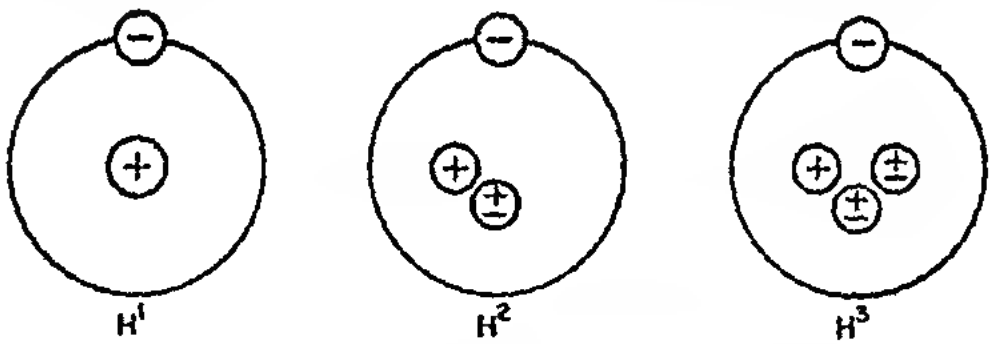


Fig 2 6

H^1 or ordinary hydrogen consists of a nucleus containing a proton (charge +1, mass I) around which revolves one electron (charge —1)

Heavy hydrogen contains an additional nuclear particle, a neutron (charge 0, mass 1), H^3 contains two neutrons

There are two classes of isotopes The "stable" isotopes which have no distinguishing characteristics other than their mass They are obtained from natural sources by fractional procedure

"Radioactive" isotopes not only differ in mass but also characterized by unstable nuclei This causes them to decompose spontaneously emitting radiation in the form of waves They occur in traces in nature They are prepared for experimental use by bombardment in the cyclotron or the atomic pile The concentration of radioactive isotopes is expressed in terms of intensities of radiation emitted The unstability of these isotopes is commonly expressed as their "half-life", the time required to decrease to half its initial value

Preparation of labelled material :

It can be prepared by Grignard reaction as follows :

$$R-CH_2-Mg-Br \xrightarrow[\text{then acidify}]{C^{14}O.} R-CH_2-C^{14}OOH$$

Methods of assay

1 Radioactive isotopes can be determined by means of Geiger-Muller counter

2. Particles or rays from the decay of the isotope enter an ionisation chamber and interact with the gas to form ions which causes a discharge of current by positive and negative plates

3 The current is amplified and registered automatically in a counting device which registers the total number of counts for any desired period of time.

4 Isotopes concentration can then be expressed as number of counts per unit time per unit weight of the sample.

Biochemical and diagnostic importance of isotopes

Biochemical importance :

1 I^{131} in the form NaI^{131} is administered in the body to study the thyroid physiology About $\frac{1}{3}$ of the iodine ingested in taken up by the thyroid and $\frac{2}{3}$ is excreted by the kidney in normal human adults.

2. Plasma volume is measured by using I^{131} labelled serum albumin and erythrocyte volume is measured by using Cr^{51} labelled erythrocytes Total body water is measured by using I^{131} labelled iodoantipyrin.

3 Absorption of fat is studied by using I^{131}-oleic acid, absorption of iron by using Fe^{59} ferrous salts and intestinal protein loss by intravenous injection of Cr^{51} labelled protein

4 The life span of RBC has been determined by tagging Cr^{51} to RBC.

5 Radioactive isotopes are widely applied to the study of the intermediary metabolism, almost every phase of metabolism, e g TCA cycle, amino acid metabolism, protein biosynthesis, nucleic acid synthesis fatty acid synthesis, biosynthesis of haem and cholesterol etc. have been studied by using compounds containing C^{14}, N^{15}, H^2, H^3, P^{32}, S^{35} etc. Mineral metabolism has also been studied by using Ca^{45}, Fe^{59}, I^{131}, Na^{24}, K^{42} and Cl^{35} etc.

Radioisotopes in Medical Science :

In recent years radioactive substances are used for the following purposes

1 Experimental
2 Diagnostic
3 Therapeutic.

1 Experimental purposes

(i) The research work needs radioisootpes to investigate absorption mobi lization, storage, and distribution of different substances in the body

(ii) The substance under investigation is 'tagged' with a radioactive isotope which acts as a tracer substance The tracer substance is detected in the body by the radiation it emits The best method used for the detection of the radioactive isotope is Geiger Muller counter and the less sensitive is auto radiograph method

(iii) The absorption, mobilization, and transport of iron have been studied by the use of radio active iron (Fe^{59})

(iv) Iodine clearance of blood is calculated by radio-active iodine (I^{131})

(v) The radio-active phosphorus (P^{32}) has been used for studying the for mation of antibody in the reticulo endothelial system

2. Diagnostic purposes

(i) The volume of fluid compartments of the body is measured by radio active isotopes

(ii) Thyroid function is determined by the use of radio iodine (I^{131})

(iii) The radio active cobalt (Co^{60}) is used for diagnosing pernicious anemia

(iv) The radio active sodium (Na^{24}) has been used for circulatory studies and diagnosis of arterial diseases

(v) The diagnosis for the localisation of brain tumours has been done by the use of radio-active di iodofluorescein

3 Therapeutic puruposes

(i) The isotopes which are selectively taken up by certain tissues and emit mainly β rays without causing any damage to the surrounding healthy tissues are only used for this purpose The most important radio isotopes for the purpose are radio-iodine (I^{131}), radiogold (Au^{198}), and radiophosphorus (P^{32})

(ii) The radio-active (I^{131}) as a solution of NaI^{131} is administered in the body after a light breakfast The effect of a dose is observed within 3 to 4 weeks and maximum effect within 3 to 4 months The second dose, if required, is given at the interval of 2 to 3 months It acts by emiting β and γ radiations This is used in the treatment of hyperthyroidism and thyroid cancers The usefulness of I^{131} depends on the capacity of the thyroid gland to capture and concentrate iodine from the blood stream NaI^{131}, in a very high dose, is used in the treat ment of congested heart failure or angina pectoris

I^{125} is more convenient than I^{131} because its half life is 7 times greater than that of I^{131}, the half life of I^{125} is 57 4 days, whereas the half life of I^{131} is 8 04 days I^{125} can be stored for more periods with loosing lesser activity and this causes lesser damage to the gland because it does not emit β radiation

(iii) Radio-active phosphorus (P^{32}) is administered in the form of sodium phosphate either orally or intravenously It emits β radiation and its half life is 14 3 days Its end product is S^{32} P^{32} is distributed to all tissues of the body as it has high metabolic role When administered, it is rapidly taken up by multiplying cells, bone marrow, spleen, and lymph nodes It is used in the treatment of various types of cancers Especially, it is used in polycythemia vera and chronic lymphatic leukemia

(iv) Radio-active gold (Au^{198}) emits β and γ rays and its half life is 2 69 days Its end product is Hg^{198} It is chemically and biologically inert It is deposited in tissue area when injected in the body It produces local irradiation since very little of it enters the blood The β rays emitted by it are completely trapped by tissues It is used in the treatment of malignant pleural and peri toneal effusions

(v) Radioactive cobalt (Co^{60}) is the most important isotope in clinical medicine It emits both β- and γ rays and its half life is 5 3 years It can be used as a substitute for sodium in interstitial surface It has been found to be cheaper and does not require any closed container

Exercise

1 What are colloids ? Discuss some specific properties of colloids How can these properties be related to proteins and other macro-molecules in the biological system ? (P U 69S)

2 What do you mean by permeability of membranes ? Illustrate your answer with examples from biological materials Have P^H change composition of the membrane of electrolytes any role on the permeability of the membrane ? (P U 69A)

3 What are buffers ? How do you derive Henderson Hasselbalch Equation (Mith 75A)

4 Define P^H What is the P^H of blood ? How is it regulated ? (Luck 65A , 70A P U 60S)

5 What is osmotic pressure ? Discuss its role in maintenance of fluid volume State the application of osmosis (P U 63S)

6 Describe the properties of colloids What is protective colloid ? (P U 64A)

7 What do you mean by co-efficient of viscosity ? How is viscosity of blood determined ? Describe its physiological importance (Luck 67S)

8 Define buffer Name the important buffer systems of the body (P U 73A)

9 Discuss the following

(a) P^H of blood is 7 4 (P U 69A)

(b) Osmotic pressure of blood is seven times the atmospheric pressure

(c) Membrane of RBC is semi permeable

10 Write short notes on

(i) Surface tension (C U 1981) (M U 73A R U 79S)

(ii) P^H (C.U 1981)(R U 66S 69A Mith 74A)

(iii) Radioactive isotope (C.U 1981) R U 65A)

(iv) Diffusion (M U 73S R U 64A M th 60S)

(v) Buffers (P U 75A Bh U 75A R U 64S)

(vi) Colloidal osmotic pressure (P U 75A)

(vi Osmos s and osmotic pressure (C U 1981 1983)(R U 70A , Mith 62A)

(viii) Protective colloids (M th 73A 75A)

(ix) Colloids (Mith 61A)

(x) Hydrotrophy (Bh U 75S M U 73A P U 68A)

(xi) Adsorption (Bh U 75A)

(xii) Brownian movement (Mith 74A)

EXERCISE

(xiii) Donnan membrane equilibrium (Bh U 74S)

(xiv) Viscosity (P U 71S)

(xv) Gibb s adsorption equation (C U 1983)

(xvi) Coefficient of viscosity and application of viscosity (C U 1983)

11 What is active transport? Discuss the mechanism by which this process operate in the body (C.U 1981)

12 Describe the principles of buffer action Write the major constituents of a buffer solution (C U 1983)

13 State the laws of osmotic pressure Write any two factors controlling O P of blood (C U 1981)

14 What is meant by protective action of colloids? How 'protective action is expressed? (C U 1981)

15 What is meant by a colloidal solution? Discuss the properties of a colloidal solution having special emphasis on electrical properties of colloids (C U 1981) (C U 1983)

16 Give briefly the properties of colloidal solution (C U 1981)

17 Indicate "True ' or ' False'

(a) Radioactive iodine has a halflife of 15 days

CHAPTER 3

CARBOHYDRATES

The carbohydrates are widely distributed both in animal and in plant tissues. In plants, they are produced by photosynthesis In animal cells, carbohydrate in the form of glucose and glycogen serves as an important source of energy for vital activities Some carbohydrates have highly specific functions e g. ribose in the nucleoproteins of the cells galactose in certain lipids and the lactose of milk

Definition

Carbohydrates may be defined as polyhydroxy aldehydes or ketones and their derivatives

Classification

Carbohydrates are divided into 4 major groups as follows

1 Monosaccharides
2 Disaccharides
3 Oligosaccharides
4 Polysaccharides

1 *Monosaccharides* These are often called "simple sugars" which cannot be hydrolyzed into a simpler form. The general formula is $C_n(H_2O)_n$ These can be further subdivided as follows

Carbon atoms	*Aldoses*	*Ketoses*
Trioses	Glyceraldehyde or Glycerose	Dihydroxyacetone
Tetroses	Erythrose	Erythrulose
Pentoses	Ribose Xylose Arabinose	Ribulose Xylulose
Hexoses	Glucose Galactose. Mannose.	Fructose
Heptoses	Glucoheptose Galactoheptose	Sedoheptulose

2. *Disaccharides* These carbohydrates produce two molecules of the same or of different monosaccharides on hydrolysis. The general formula is $C_n(H_2O)_{n-1}$ Examples—Lactose, Maltose, Sucrose

3 *Oligosaccharides* : These carbohydrates yield 2-6 monosaccharide units on hydrolysis.

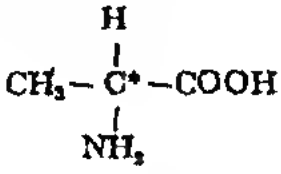
4 *Polysaccharides* These carbohydrates yield more than 6 molecules of monosaccharides on hydrolysis The general formula is $(C_6H_{10}O_5)x$ These may be classified as homopolysaccharides and heteropolysaccharides depending on the presence of either the same monosaccharides or more than one simple sugar in alternating repeating sequence Examples are

Homopolysaccharides Starch, Glycogen, Cellulose, Dextrin

Heteropolysaccharides Mucopolysaccharides

Asymmetric carbon atom ·

A carbon atom to which four different atoms or groups of atoms are attached is said to be asymmetric carbon atom



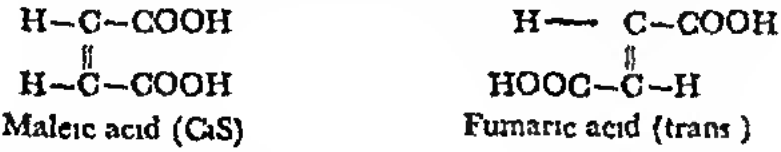
Many biochemicals contain 2 or more asymmetric C atoms

Isomerism

The presence of asymmetric carbon atoms allow the formation of isomers The compounds which have the same structural formula but differ only in spatial configuration are called Stereoisomers or Geometric isomers Glucose with 4 asymmetric carbon atoms has 2^n (16) isomers, n is the number of asymmetric carbon atoms

CiS-Trans Isomerism :

Cis-trans isomerism (Latin CiS "this side", trans "across") occurs in compounds with double bonds As the double bond is rigid, the atoms attached to it are not free to rotate like those attached to a single bond The structures are as



Maleic acid (CiS) Fumaric acid (trans)

These structures have different chemical and physiologic properties Fumaric acid is physiologically active

1. D and L ·

The designation of an isomer as D- or L- form is determined by its spatial configuration to the parent compound When the OH group around the carbon atom adjacent to the terminal primary alcohol carbon (carbon atom 5 in glucose) is on the right, the sugar is a member of the D series When it is on the left, it is a member of the L series The majority of the monosaccharides occurring in mammalian metabolism are of the D configuration

Optical isomer—When a beam of polarized light is passed through a solution exhibiting optical activity, it will be rotated to the right or to the left in accordance with the type of compound i e the optical isomer, which is present. A compound

which causes rotation of polarized light to the right is said to be dextrorotatory and a plus (+) sign is used to designate the fact. Rotation of the beam to the left (levorotatory action) is designated by a minus (−) sign. When equal amounts of dextrorotatory and levorotatory isomers are present, the resulting mixture has no optical activity since the activities of each isomer cancel one another. Such a mixture is said to be racemic or a DL mixture. Stereoisomerism and optical isomerism are independent properties. Thus a compound might be designated as D (−) or L (+). Synthetically produced compounds are necessarily racemic. The separation of optically active isomers from a racemic mixture is called resolution i.e. the racemic mixture is said to be "resolved" into the optically active components.

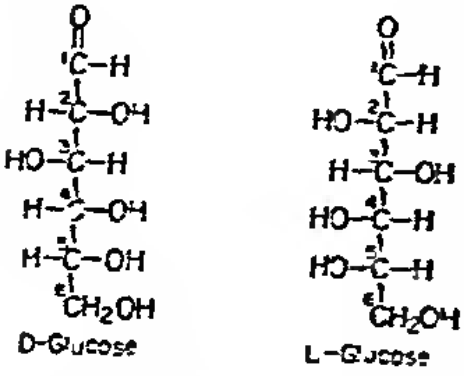


Fig. 3.1

2 α- and β-anomers :

The cyclic structure of glucose is retained in solution, but isomerism takes place about position 1. This is accomplished by optical rotation (mutarotation) by which the positions of −H and −OH groups are changed around carbon 1.

Let us take D-glucose as an example. As soon as D-glucose is dissolved in water its specific rotation is +111°. Gradually, and quicker if ammonia is present, this value falls, finally remains constant at +52.5°. This phenomenon is said to be mutarotation. If D-glucose be first recrystallised from boiling pyridine, on first dissolving in water is not +111°, but +19°. This solution also shows mutarotation, the specific rotation finally becomes constant at the same value, +52.5°. The two forms are referred to as *α- and β- glucose* respectively and the solution giving +52.5° is an equilibrium mixture of the two forms.

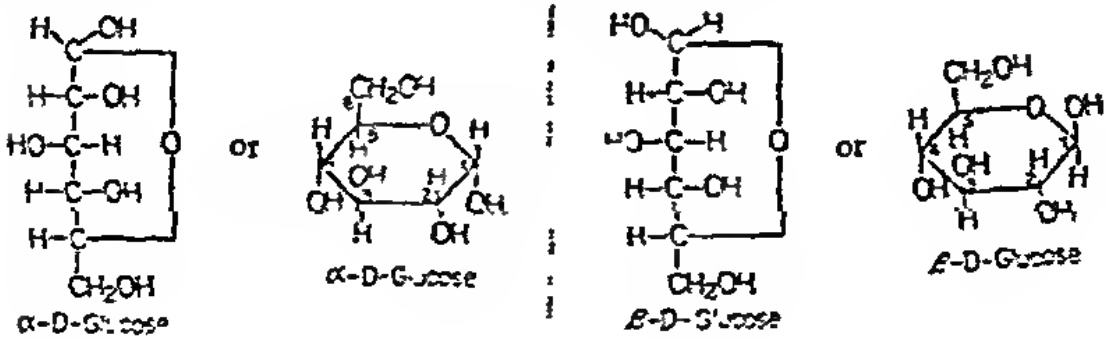


Fig. 3.2

3. Epimers :

Isomers formed as a result of interchange of the −OH and −H on carbon atoms 2, 3 and 4 of glucose are known as epimers. Biologically, the most important epimers of glucose are mannose and galactose formed by epimerization at carbons 2 and 4 respectively. In the body epimerization takes place by the enzyme *epimerase*.

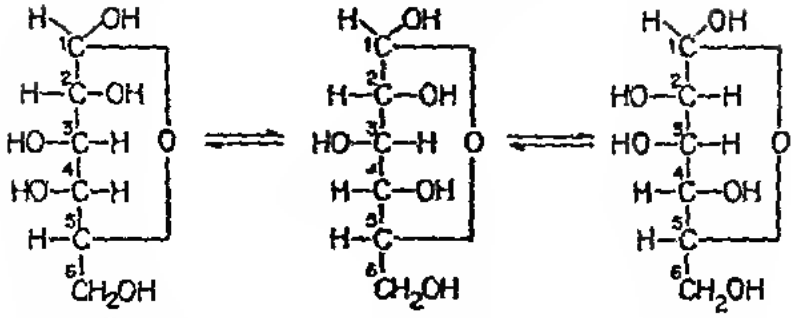


Fig 3.3

4 **Pyranose and furanose ring structures :**

Haworth in 1929 proposed a scheme in which all sugars forming six-membered rings are called pyranoses from their relation to pyrane and those forming five-membered rings furanoses after furane. The amylene oxide form of glucose would be called glucopyranose and the butylene oxide form glucofuranose. If a sugar is referred to by its ordinary name only, the pyranose form is to be inferred. The pyranose and furanose forms of some sugars are set out below in straight chain formulae as well as in ring formulae.

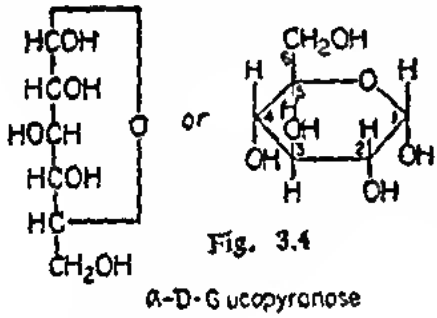
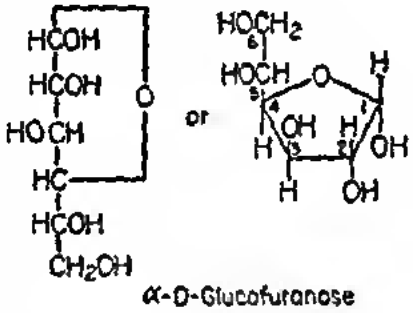
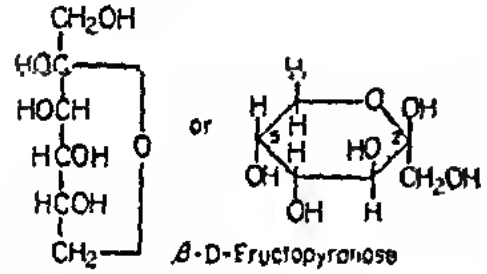


Fig. 3.4





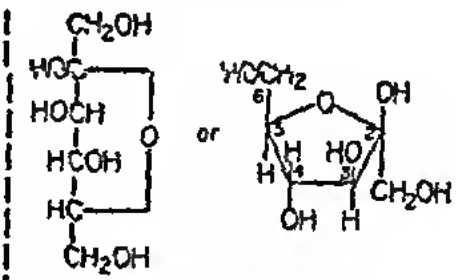


Fig 3.5

5. **Aldose-ketose isomerism :**

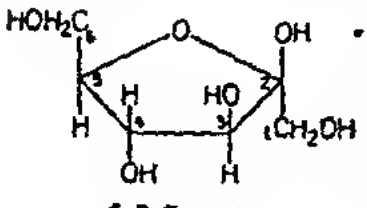


Fig 3.6

Fructose has the same molecular formula as glucose but differs in its structural formula since carbon 2 is a part of a >Co group, which makes fructose a ketose rather than an aldose. Generally, there is a free —H on carbon 1, the sugar is an aldose, but if a $-CH_2OH$ group is substituted, the sugar is a ketose (figure below).

THE KILIANI SYNTHESIS

A method for the synthesis of monosaccharides was first proposed by Kiliani. It is based upon the addition of HCN to the carbonyl group of aldehyde or ketones The application of the Kiliani synthesis to the production of the two tetroses, erythrose and threose from glycerose is shown below.

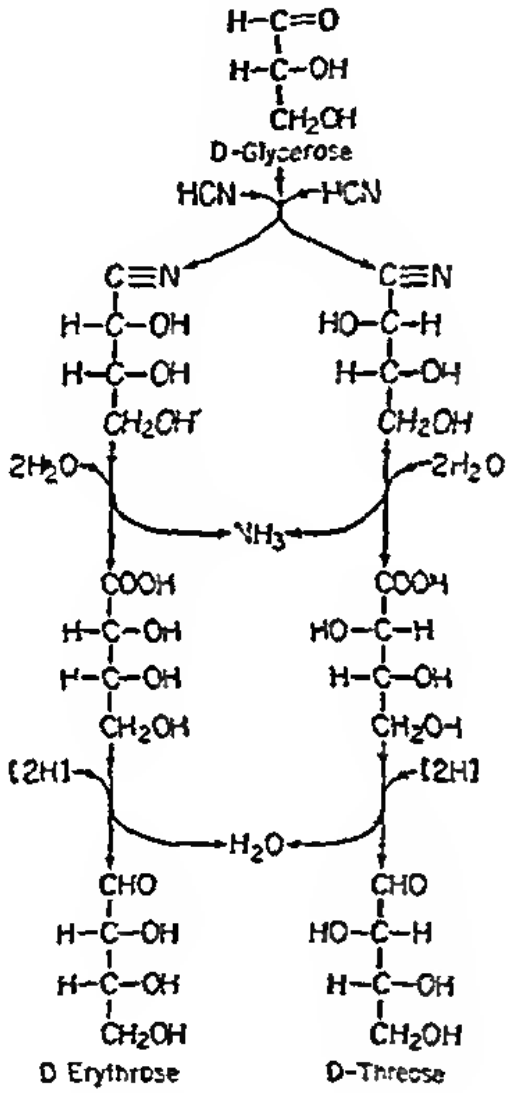


Fig 37

MONOSACCHARIDES

Trioses and tetroses :

Phosphates of the two trioses, glyceraldehyde and dihydroxyacetone, are formed from fructose-1,6-diphosphate by glycolysis 3-phosphoglyceraldehyde and erythrose-4 phosphate are formed by the hexose monophosphate shunt.

Pentoses :

Ribose and Deoxyribose are important constituents of nucleic acids and many coenzymes. The structures are given below. Deoxyribose is lacking in one atom of oxygen from carbon No. 2.

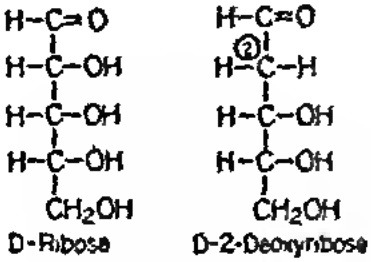


Fig. 3.8

TABLE 1 : Pentoses of physiologic importance

Sugar	*Source*	*Importance*	*Reactions*
D-Ribose.	Nucleic acids.	Structural elements of nucleic acids and coenzymes e.g. ATP, NAD, NADP, flavoproteins.	Reduce Benedict's, Fehling's, Barfoed's solution. Form osazones with phenylhydrazine.
D-Ribulose.	Formed in metabolic processes.	Intermediates in hexose monophosphate shunt.	Those of keto sugars.
D-Arabinose.	Plum and cherry gums.	Used in bacterial metabolism. No known physiological functions in man.	With orcinol –HCl reagent gives colours : violet, blue, red, green.
D-Xylose.	Wood gums.	Same as above.	Gives red colour with phloroglucinol –HCl.
D-Lyxose.	Heart muscle.	A constituent of a lyxoflavin isolated from human heart muscle.	

Hexoses

TABLE 2 · Hexoses of physiologic importance

Sugar	*Source*	*Importance*	*Reactions*
D-Glucose	Fruit juices, Hydrolysis of starch, cane-sugar, maltose and lactose	It is carried by the blood and used by the tissues Its presence in the urine causes glycosuria	Reduces benedict's, Barfoed's reagent. Gives osazones with phenyl hydrazine. Forms saccharic acid with HNO_3
D-Fructose	Fruit Juices, Honey, Hydrolysis of cane-sugar and of Inulin	It can be changed to glucose in the liver and intestine and thus used in the body	Reduces Benedict's, Barfoed's reagents Forms osazone with phenylhydrazine Gives red colour with Selwanoff's reagent.
D-Galactose	Hydrolysis of lactose	It can be changed to glucose in the liver and metabolized. Synthesized in the mammary gland to form lactose of milk It is the constituent of glycolipids and glycoproteins	Reduces Benedict's, Barfoed's reagents Forms osazone with Phenylhydrazine. Forms mucic acid with HNO_3 Not fermented by yeast
D-Mannose	Hydrolysis of plant mannosans and gums	It is the constituent of prosthetic polysaccharides of albumins, globulins, mucoproteins It frequently occurs in glycoproteins	Reduces Benedict's, Barfoed's reagents Forms same osazone as glucose.

Structures

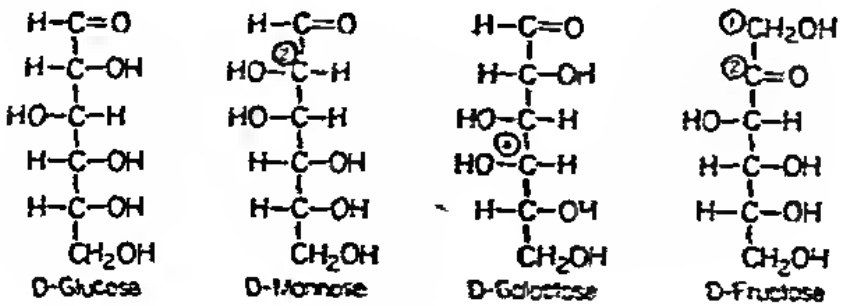


Fig. 3.9

Heptoses :

Sedoheptulose is a ketoheptose found in plants. Its phosphate is important as an intermediate in the hexose monophosphate shunt and has been identified as a product of photosynthesis.

GLYCOSIDES

Glycosides are compounds formed by the condensation reaction between a sugar and the hydroxyl group of a second compound or aglycone which may or may not be another sugar. If the carbohydrate portion is glucose, the resulting compound is a glucoside : if galactose, a galactoside etc.

All reducing sugars will condense with dry methyl alcohol under catalytic action of dry HCl to form glycosides. Thus, glucose forms α- and β- methyl glucosides.

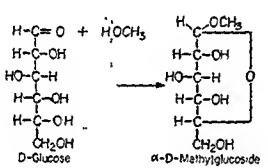


Fig 3.10

The aglycone may be —OH containing substances such as methyl alcohol, glycerol, a sterol, a phenol or another sugar (as in the disaccharides).

Importance :

(i) Glycosides are found in many drugs, spices and in the constituents of animal tissues.

(ii) The glycosides which are important in medicine because of their action on the heart (cardiac glycosides). All the glycosides contain steroids as the aglycone component.

(iii) The glycosides include derivatives of digitalis and strophanthus such as *Ouabain*, which is an inhibitor of the Na^+— K^+— ATPase of cell membranes.

(iv) Other glycosides include antibiotics such as *streptomycin.*

AMINO SUGARS (HEXOSAMINES)

Sugars containing an amino group are called amino sugars. Examples are D-Glucosamine, D-Galactosamine, and D-Mannosamine, all have been identified in nature. These amino sugars are formed by the replacement of

hydroxyl group attached to carbon atom 2 of the sugar by an amino group. The structure of an amino sugar is given below.

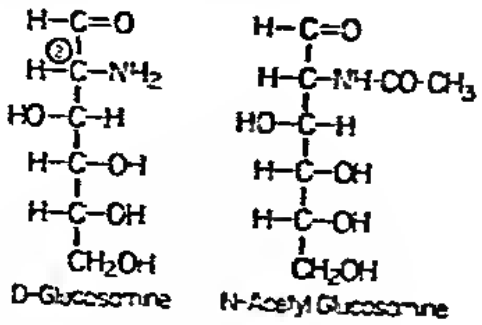


Fig. 3.11

Importance :

(i) Glucosamine is a constituent of hyaloronic acid. It is the organic constituent of lobster shell and fairly distributed in nature.

(ii) Galactosamine is a constituent of glycoproteins and of chondroitin as the N-acetyl derivative

(iii) Mannosamine is an important constituent of mucoprotein.

(iv) Several antibiotics (erythromycin, carbomycin) contain amino sugars. Erythromycin contains a dimethylamino sugar. Carbomycin contains the first known 3-amino sugar, 3-amino-D-ribose. The amino sugars are believed to be related to the antibiotic activity of these drugs.

IMPORTANT CHEMICAL REACTIONS OF MONOSACCHARIDES

(i) Iodo compounds :

An aldose sugar when heated with concentrated hydriodic acid (HI) loses all of its oxygen and is converted into an iodo compound (glucose to iodohexane, $C_6H_{13}I$).

(ii) Ester formation :

Sugars, by virtue of the alcohol groups, readily form esters with acids. All the free —OH groups are replaceable The greater biochemical significance is the ester with phosphoric acid and to a lesser extent with sulphuric acid. Pentose phosphates are involved in the formation of nucleic acids.

(iii) Acetylation :

The acetylation with acetylchloride indicates the presence of —OH group present in the sugar. The presence of 5 OH groups of glucose results in a penta acetate.

(iv) Oxidation

Oxidation of the aldehyde group forms "aldonic acids' If the aldehyde group remains intact and the primary alcohol group is oxidized 'uronic acids are formed Oxidation of galactose with concentrated HNO_3 yields the dicarboxylic *mucic acid* This compound crystallizes readily

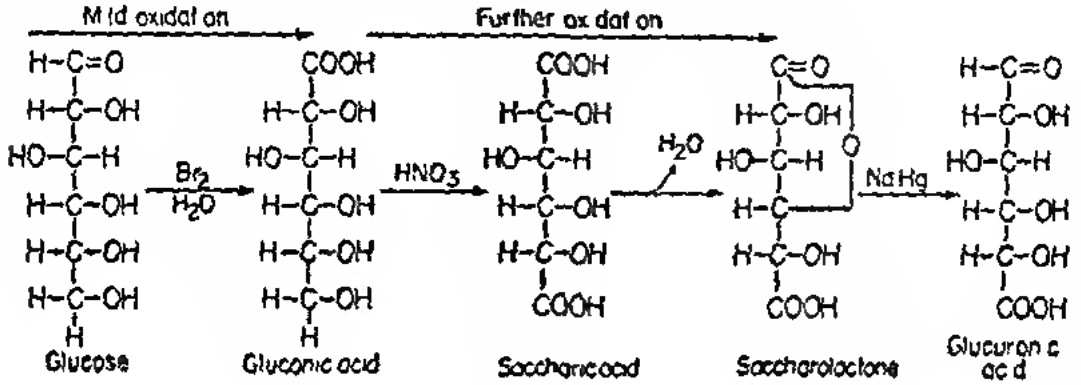


Fig 3 12

(v) Reduction

The monosaccharides are reduced to their corresponding alcohols by reducing agents such as sodium amalgam

Thus Glucose yields sorbitol
Galactose yields dulcitol
Mannose yields mannitol
Fructose yields mannitol and sorbitol

(vi) With strong mineral acids

There is a change of hydroxyl groups towards and of hydrogen away from the aldehyde end of the chain Reaction products with acids will condense with certain organic phenols to form compounds having characteristic colours

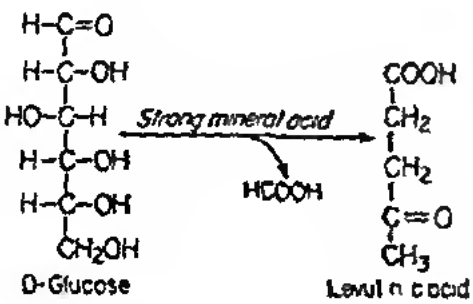


Fig 3 13

(vii) Heat

Gluconic acid on heating produces lactones These are cyclic structures which resemble pyranoses and furanoses

(viii) With alkali :

Monosaccharides react in various ways.

(a) In dilute alkali the sugar will change to the cyclic alpha and beta structures, with an equilibrium between the 2 isomeric forms.

On standing, a rearrangement will occur which produces an equilibrated mixture of glucose, fructose and mannose through the enediol form.

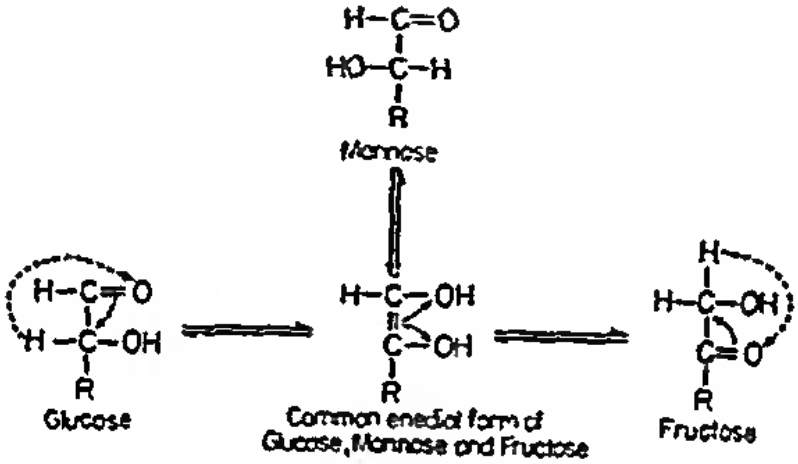


Fig 3.14

(b) In concentrated alkali, sugar produces a series of decomposition products. Yellow and brown pigments develop, salts may form, many double bonds between carbon atoms are formed, and carbon-to-carbon bonds may rupture.

(ix) Osazone formation .

It is nothing but the formation of crystalline derivatives of the sugars which are valuable in the identification of sugars.

These crystals are obtained by adding a mixture of phenylhydrazine hydrochloride and sodium acetate to the sugar solution and heating in a boiling water bath. The carbonyl group (i e. aldehyde or ketone group) and the next adjacent carbon are involved in this reaction. With an aldose the reaction is shown below. The hydrazone then reacts with two additional molecules of phenylhydrazine to form the osazone. The ketoses also show the similar reaction.

From the comparison of their structures it may be noted that glucose, fructose and mannose form the same osazone ; whereas *galactose* forms a different

osazone because *the part (carbon 4)* in the structure of galactose which differs from that of glucose fructose and mannose remains unaffected in osazone formation

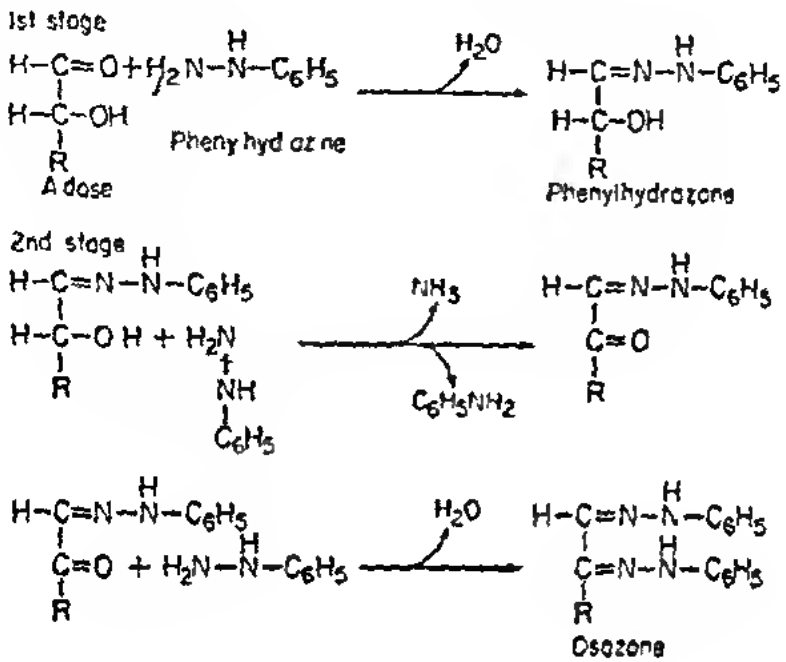


Fig 3 15

(x) Other reactions

Various other reactions take place due to the presence of aldehyde or ketone groups of the sugars which are important for analytical purposes

The best known tests are *reduction* of metallic hydroxides together with oxidation of the sugar The alkaline metal is kept in solution with sodium potassium tartrate (*Fehling's solution*) or sodium citrate (*Benedict s solution*) Other metallic hydroxides may be used (Bismuth Ammoniacal silver, Tollen's test, *Nylander s test)*

Barfoed s test distinguishes between monosaccharides and disaccharides The copper acetate in dilute acid is reduced in 30 seconds by monosaccharides , whereas reduction of the same takes place several minutes by disaccharides

DISACCHARIDES

The disaccharides are composed of two monosaccharide units united by a glycosidic linkage The physiologically important disaccharides are maltose, lactose and sucrose

Maltose=1 mol glucose+1 mol glucose

Lactose=1 mol glucose+1 mol galactose

Sucrose =1 mol glucose+1 mol fructose

1 Maltose (malt sugar)

(i) It does not occur in the body

(ii) The sources of it are germinating cereals and malt and it is the intermediate product in the breakdown of starch by amylase in the alimentary canal

(iii) It is hydrolysed to glucose by the enzyme maltase and the products are absorbed

(iv) It has one free aldehyde group and hence shows mutarotation and the final rotation of the solution is +130° It can exist in α or β forms

(v) It can reduce Fehling's and Benedict s solutions since it is a reducing sugar but cannot reduce Barfoed's solution

(vi) It forms an osazone with phenylhydrazine

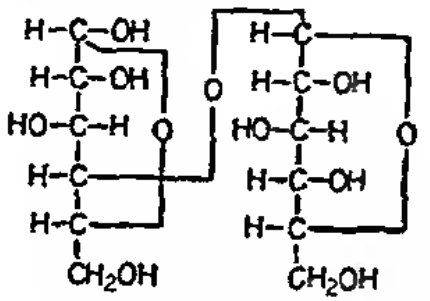


Fig 3 16

2 Lactose (milk sugar)

(i) It is present in milk and formed in the lactatating mammary gland It may occur in urine during pregnancy

(ii) It is hydrolysed to glucose and galactose by the enzyme lactase in the alimentary canal and the products are absorbed

(iii) It has free aldehyde group and hence shows mutarotation and the final constant specific rotation of the solution is +55 2° It can also exist in α or β forms

(iv) Since it is a reducing sugar it can reduce Fehling s and Benedict s solutions but cannot reduce Barfoed s solution

(v) It forms an osazone with phenylhydrazine

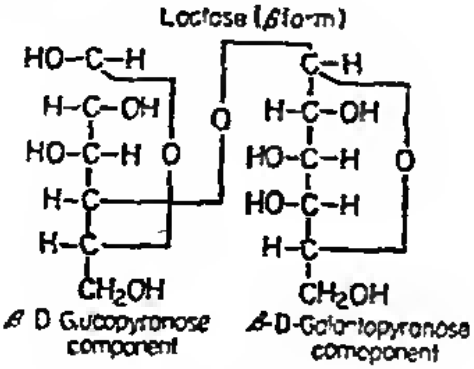


Fig. 3 17

3 Sucrose (cane sugar)

(i) It does not exist in the body but occurs in cane sugar, pineapple, carrot roots, sweet potato and honey

(ii) It is hydrolysed to glucose and fructose by the enzyme invertase (sucrase) in the alimentary canal. The products of hydrolysis are absorbed.

(iii) It has no free aldehyde or keto group because *the linkage is between the aldehyde group of glucose and keto group of fructose. Hence a non-reducing sugar.* It does not exhibit mutarotation and cannot exist in α or β forms.

(iv) Since it is a *non-reducing* sugar it does not reduce Fehling's or Benedict's solutions. It cannot reduce Barfoed's solution too.

(v) It cannot form osazone with phenylhydrazine.

(vi) The specific rotation of sucrose solution is +66.5°. During hydrolysis this rotation changes to −19.5° ; since the laevorotation of fructose is greater than the dextrorotation of glucose. The product of the hydrolysis is used to be referred to as "*invert sugar*".

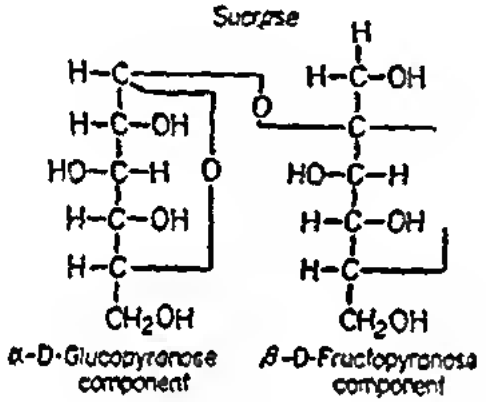


Fig 3.18

POLYSACCHARIDES

Polysaccharides are classified as homopolysaccharides and heteroolysaccharides.

The physiological important homopolysaccharides are Cellulose, Glycogen, Starch, Dextrins, and Inulin.

1. Cellulose :

(i) It is the main constituent of the supporting tissues of plants and forms a considerable part of our vegetable food. It does not occur in the animal body.

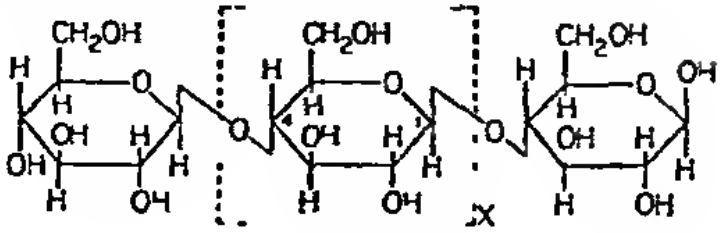
(ii) It is made up of β-glucose molecules which are linked by 1 : 4 linkages.

(iii) Owing to the difference in chemical structure, cellulose is not acted upon by amylases present in the digestive juices.

(iv) It is of considerable human dietetic value only because it adds "*bulk*" to the intestinal contents, thereby stimulating peristalsis and elimination of food residues.

(v) It is insoluble in ordinary solvents and gives no colour with iodine.

(vi) Cotton and filter paper are nearly pure cellulose.



Cellulose

Fig 3 19

2 Glycogen (animal starch)

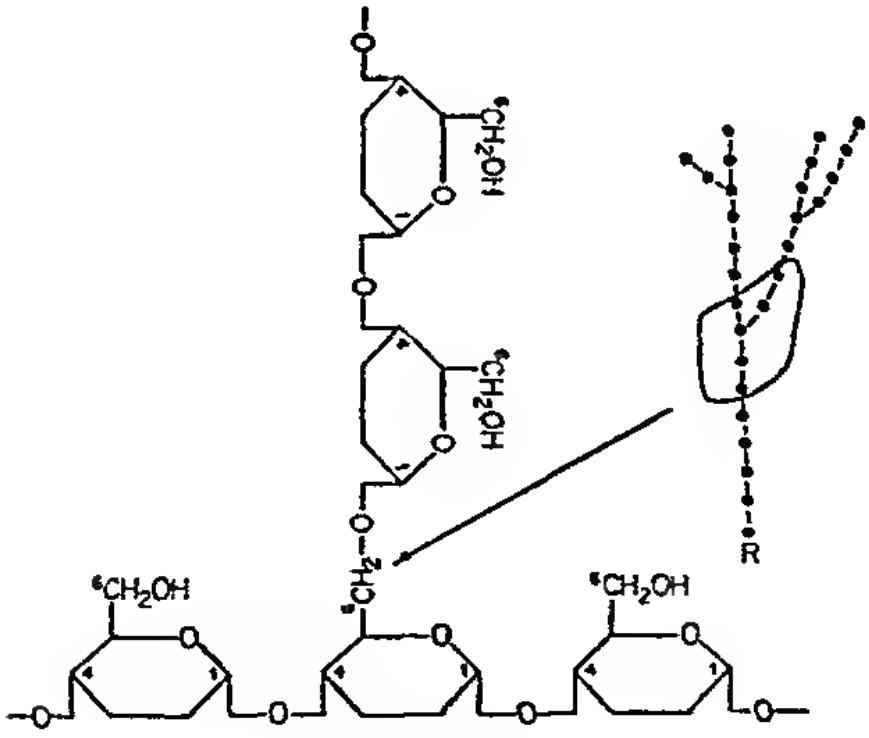
(i) It is the reserve carbohydrate found in liver and muscles of animals and human beings The glycogen content of liver is more than that of muscle

(ii) It is also fonnd in plants which have no chlorophyll system (e g fungi and yeasts), but not in green plants

(iii) The molecular weight of glycogen obtained from different sources may range from 10^5 to 10^8 and each molecule contains from 5,000 to 10,000 glucose molecules

(iv) It has a branched structure with straight chain units of 12-18-α-D-glucopyranose [in a 1-4 glucosidic linkage] with branching by means of a [1-6]-glucosidic bonds

(v) It is nonreducing, readily soluble in water and gives a red colour with iodine



(Structure of Glycogen)

Fig. 3.20

3 Starch

(i) It is the store carbohydrate of chlorophyll-containing plants In plants, the starch is laid down in the cells in granules The microscopic form of the granules is characteristic or the source of the starch

(ii) It is formed by an α-glucosidic chain Such a compound which produces only glucose on hydrolysis is called a *glucosan*

(iii) It is the most important source of carbohydrate in our food and is found in cereals, potatoes, legumes and other vegetables in high concentrations

(iv) It is a mixture of two substances—*amylose and amylopectin*—both are composed of glucopyranose units In the amyloses, the glucose units are joined by 1, 4-α links to form unbranched chains which are in the form of a helix with six glucose units per turn Their molecular weights are about 60,000 which are equivalent to about 300-400 glucose units and are responsible for the development of blue colour with iodine

Amylopectins have much larger molecular weights of about 5,00,000 and the chains have at least 80 branches, each branch is at an interval of 24-30 glucose units The point of branching is the sixth carbon atom of glucose

(v) Raw starch is insoluble in cold water owing to the resistance of the outer cellulose layer of the granule When this is ruptured by heating in water, starch is soluble Concentrated solutions gelatinise on cooling and are used as an adhesive—starch paste

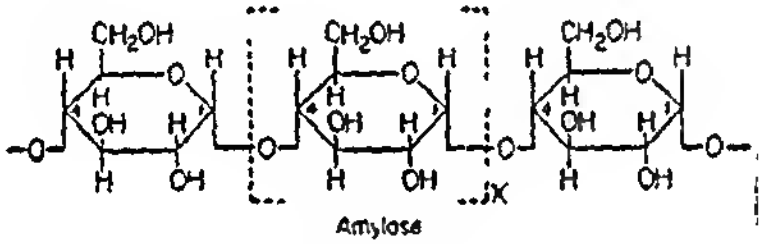


Fig 3.21

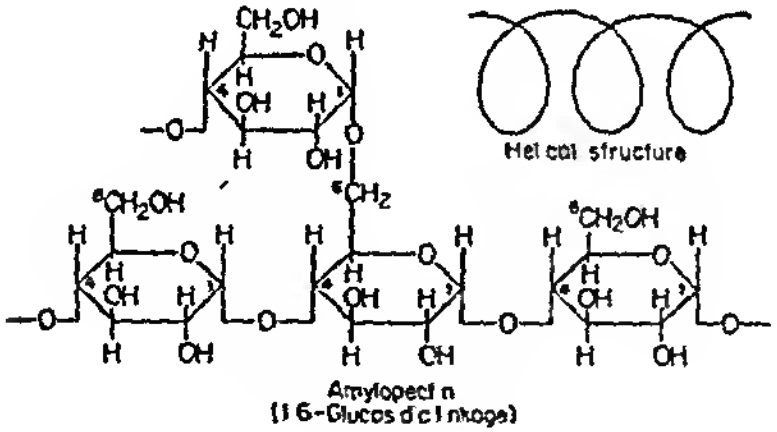


Fig. 3 22

4. Dextrins :

(i) Dextrins are formed by the partial hydrolysis of starch by an enzyme (salivary amylase), dilute mineral acids or heat

(ii) Amylodextrin, erythrodextrin and achrodextrin give blue, red brown and no colour respectively with iodine. Achrodextrin being the simplest.

(iii) If they have reducing properties at all, they are very feeble.

(iv) They have faint sweet taste.

(v) They form sticky solutions in water and are frequently used as adhesive e.g. on postage stamps.

(vi) The final product of hydrolysis of starch by an amylase is maltose which is hydrolysed to glucose by maltase.

5 Inulin

(i) It is found in tubers and roots of dahlias, and dandelions.

(ii) It is hydrolysed to fructose, hence it is a fructosan.

(iii) It does not produce any colour with iodine.

(iv) It is easily soluble in warm water.

(v) It is used in physiological investigation for the determination of the rate of glomerular filtration.

MUCOSUBSTANCES

Mucosubstances are having the property of forming slimy, viscous solutions which occur in the body. They have structural and protective functions. They are divided into Glycoproteins and Mucopolysaccharides.

1 Glycoproteins (mucoproteins)

(i) They are protein-polysaccharide compounds occurring in the tissues, particularly in mucous secretions.

(ii) They do not contain uronic acids although they contain acetyl hexosamines such as N acetylglucosamine and N acetylgalactosamine. Hexoses such as mannose or galactose are also found. In addition, a methyl pentose (L-fucose) and the sialic acids commonly occur in these conjugated proteins.

The sialic acids are actually a family of compounds derived from neuraminic acid. They are widely distributed in vertebrate tissues and have also been isolated from certain strains of bacteria. N acetylneuraminic acid is an example of sialic acid. Enzymes which are identified in the liver of the rat can accomplish the biosynthesis of N acetylneuraminic acid.

(iii) Examples of glycoproteins are also found among the alpha$_1$ and alpha$_2$ globulins of the plasma.

(iv) They form viscous solutions which function as lubricants and protective "screens" in the body. The continuously secreted mucus of the respiratory tract

is a protection against invasion by bacteria and the uterus is protected from the vaginal microbial flora by the cervical mucus Intestinal mucus constitutes a protection for the intestinal cells against mechanical damage

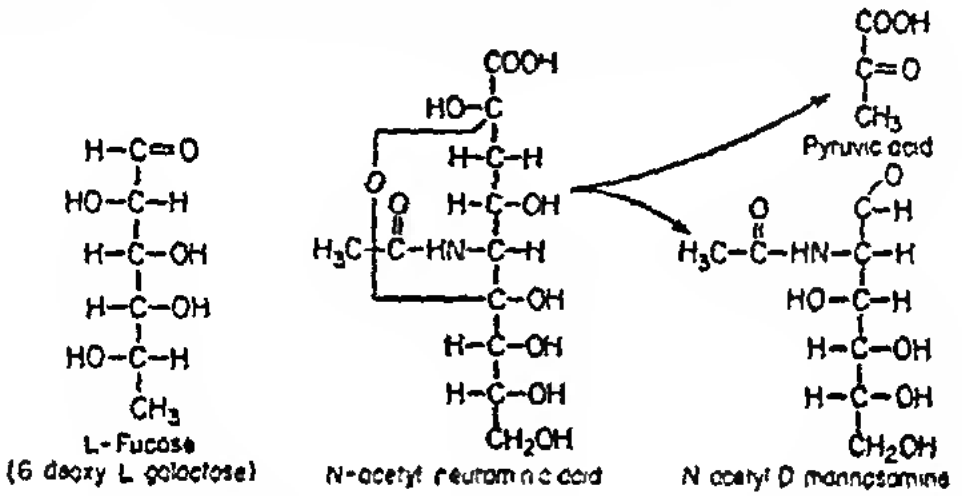


Fig 3 23

2 Mucopolysaccharides (Heteropolysaccharides)

A *Hyaluronic acid*

(i) It has a molecular weight of 1 to 4 million

(ii) It consists of alternate units of glucuronic acid and N acetylglucosamine linked to give a thread like structure

(iii) It forms viscous solutions in water and is important in the body as a lubricant

(iv) It has an enormous capacity to hold water

(v) It occurs in synovial fluid in the skin and vitreous humour

(vi) Its presence affects the rate of diffusion of materials in the skin

(vii) The presence of the enzyme hyaluronidase increases the rate of diffusion *of substances through tissues containing hyaluronic acid The richest source of* the enzyme in mammals is the testis and its presence in the semen facilitates fertilization It is secreted by some pathogenic organisms which can therefore invade the tissues of the host animal more easily

B *Chondroitin sulphates*

(i) They occur in the ground substance of connective tissue and they are the components of cartilage, tendon and skin

(ii) Chondroitin sulphates A and C consist of alternate units of glucuronic acid and 2 N acetylamino galactose

(iii) Chondroitin sulphate B contains iduronic acid in place of glucuronic acid

(iv) In all three forms one hydroxyl group of each amino sugar is esterified with sulphuric acid and the molecules are long and linear

(v) They have a marked capacity to bind water and contribute to the resistance to compression of connective tissue

(vi) Since they are polyvalent anions they may also regulate the flow and concentration of cations round the cells

C. *Heparin*

(i) It occurs in most cells and is present in liver, lung and the arterial wall

(ii) It consists of an unbranched chain of alternate units of glucosamine and glucuronic acid joined mainly by 1, 4-α links. Sulphate groups are linked to the hydroxyl group of carbon atom 6 and the amino group of the glucosamine and to some of the glucuronic acid units.

(iii) Its molecular weight is about 17,000 and the molecule is highly sulphated.

(iv) It is used in medicine as an anticoagulant

D. *Blood group substances*

(i) They consist of N-acetylglucosamine, galactosamine, galactose, fucose and sialic acid

(ii) They are used to determine blood group

E. *Keratosulphate*

(i) It consists of N acetylglucosamine, galactose sulphuric acid

(ii) It is the component of cartilage and cornea.

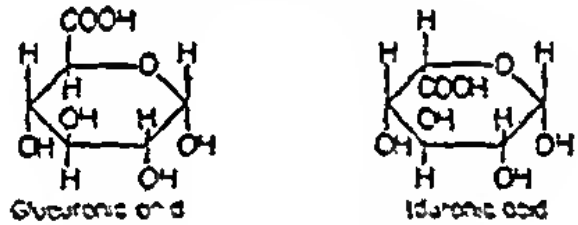
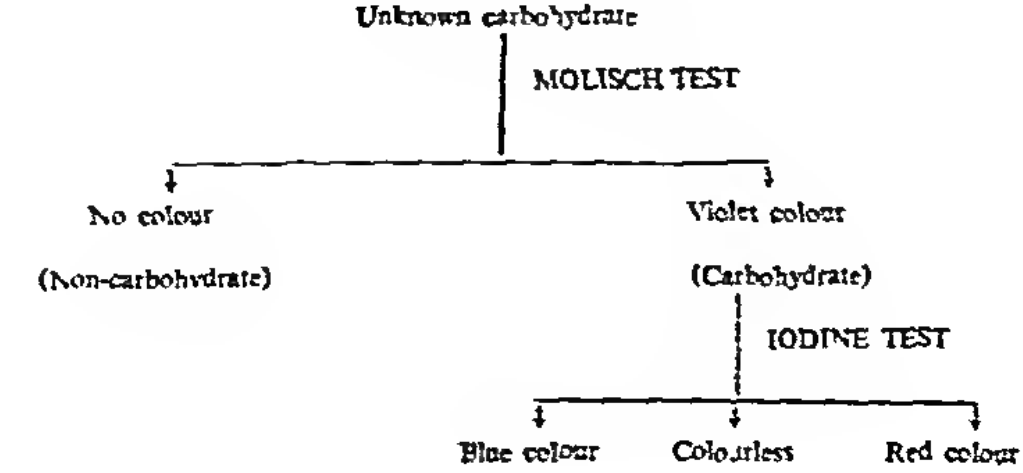
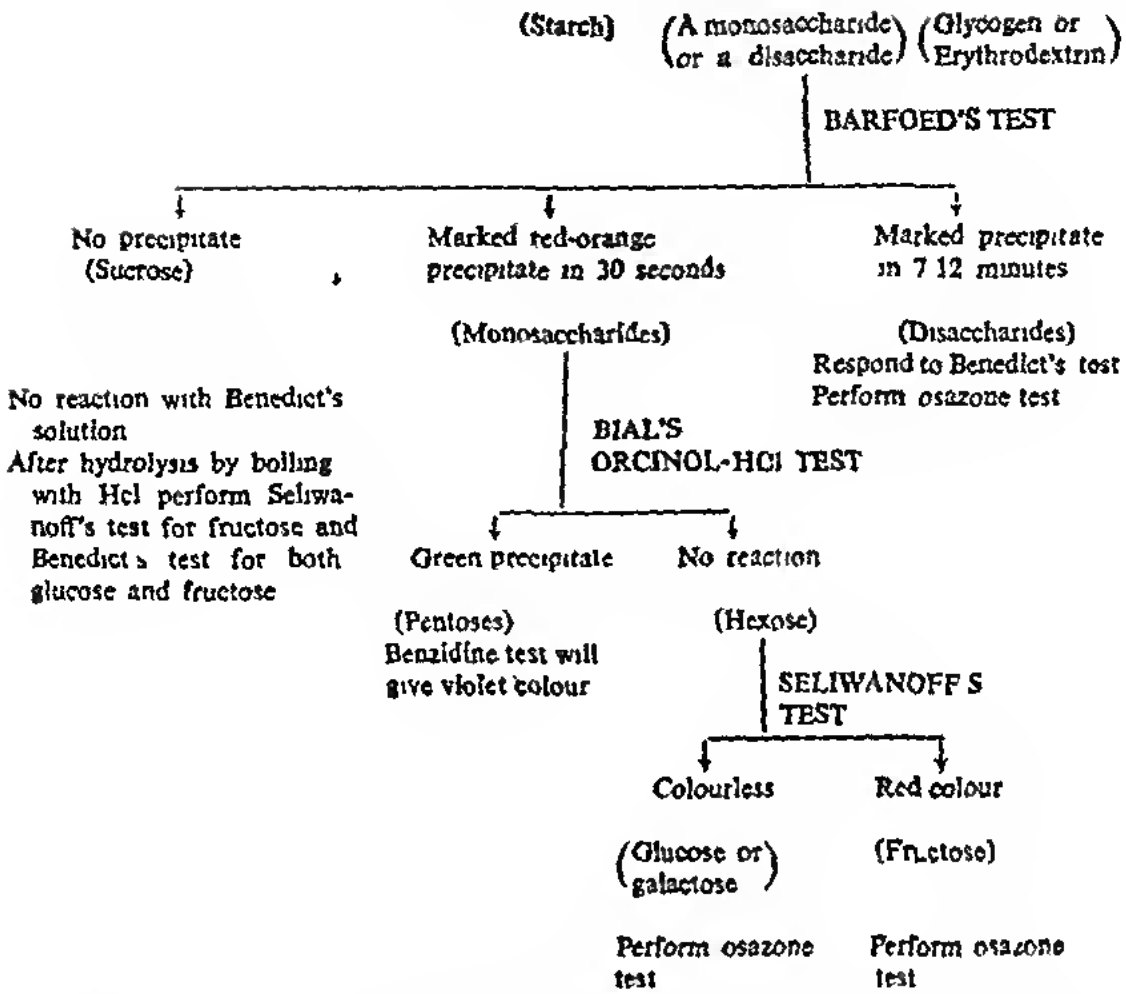


Fig 3.24

QUALITATIVE TESTS FOR IDENTIFICATION OF CARBOHYDRATES





Benedict's test is preferred than the Fehling's test for the detection of glucose in urine due to the following causes

Benedict's Qualitative solution is composed of copper sulphate, sodium citrate and sodium carbonate

Fehling's solution is composed of copper sulphate, sodium potassium tartrate, and potassium hydroxide

(i) Benedict's test is less likely to give weakly positive results with concentrated urine due to the action of creatinine and uric acid

(ii) It is more sensitive to small quantities of glucose because of the weaker alkali it contains

(iii) The strong alkali (KOH) of Fehling's solution can polymerize small quantities of glucose when vigorously boiled

Exercise

1 Define carbohydrates classify them and describe the properties of Monosaccharides (P U 68A ; R U 67A , Mith 64S)

2 Write short notes on

(a) Disaccharides (Bh U 76A)
(b) Starch (Mith 73A)
(c) Mucopolysaccharides (Bh U 75A , R U 68A ; P. U 72S)
(d) Lactose (Muz 74A)
(e) Glycogen (M U 73A , Mith 62A)
(f) Benedict's test (P U 68S)

3. Mention five characteristic reactions of an aldose hexose which occurs in our body. (C.U. 1981)

4. Give the structural formula of the following: (C.U. 1981)
   (a) Lactic acid
   (b) Fructose

5. What products will be produced by acid hydrolysis of lactose? (C.U. 1981)

6. With examples write explanatory notes on optical isomerism. (C.U. 1981)

7. Explain the mutarotation of carbohydrates. Describe in brief the structure and important peopertues of the following (C.U. 1982)
   (a) Glycogen
   (b) Starch.
   (c) Cellulose

8 Indicate 'True' or 'False'

In lactose molecule there is one β-1, 2 glucosidic linkage ..... . (C U. 1981)

Suggest the nature and molecular formula of A from the following data: (C.U 1981)

(i) A $\xrightarrow[\text{solution}+\text{heat}]{\text{Fehling's}}$. No reduction

(ii) A $\xrightarrow[\text{heat}]{\text{dil HCl}}$ B+C

(iii) B $\xrightarrow[\text{solution}+\text{heat}]{\text{Fehling's}}$ reduction (Red ppt.)

C $\xrightarrow[\text{solution}+\text{heat}]{\text{Fehling's}}$ reduction (Red ppt)

(iv) Both B and C separately on reduction produces hexahydric alcohol.

(v) B $\xrightarrow{\text{Strong oxidation}}$ on dicarboxylic acid with six C-atom.

(vi) C $\xrightarrow{\text{Strong oxidation}}$ two dicarboxylic acids—one with two C-atoms and the other with four C-atoms.

9 Fill up the blanks with appropriate words (C.U. 1981)

(a) Sucrose solution is — rotatory but after hydrolysis with dil. HCl it shows — rotation

(b) Lactose solution exhibits — rotation and after treatment with dil. HCl it shows — rotation.

CHAPTER 4

LIPIDS

Definition

The lipids are a heterogeneous group of compounds related to the fatty acids and are insoluble in water but soluble in solvents such as ether, chloroform and benzene

Sources The lipids occur widely in plants and animal kingdom

Examples The lipids include fats, oils, waxes and related compounds

Oils are liquids at 20°C but fats are solids at 20°C

Biological importance

1 In the body, fat serves as an efficient source of energy when stored in adipose tissue

2 The fat soluble vitamins and the essential fatty acids are found with the fat of natural foods

3 It serves as an insulating material in the subcutaneous tissues and around certain organs

4 The phosphatides of blood platelets are involved in the production of thromboplastin activity in the early stages of blood clotting

5 Lipoproteins (combinations of fat and protein) and glycolipids (combinations of fat and carbohydrate) are essential for maintaining cellular integrity

6 It provides building blocks for different high molecular weight substances e g acetic acid can be used for the synthesis of cholesterol and certain hormones

7 They produce metabolites through oxidation in the tissues which are used in the interconversion of substances

Classification

Bloor has proposed the following classification of lipids

1 *Simple lipids* *Ester of fatty acids with various alcohols*

(a) Fats—Esters of fatty acids with glycerol

(b) Waxes—Esters of fatty acid with higher alcohols other than glycerol

2 *Compound lipids* Esters of fatty acids containing groups in addition to an alcohol and a fatty acid

(a) Phospholipids—An alcohol + fatty acids + a phosphoric acid + a nitrogen-containing base and other substituents

(b) Glycolipids (cerebrosides)—Fatty acids + carbohydrate + nitrogen-containing base

(c) Other compound lipids—Sulpholipids, aminolipids and lipoproteins

3 *Derived lipids* Substances derived from the above groups by hydrolysis

These include fatty acids, glycerol, sterols, glycerol steroids, fatty aldehydes, ketone bodies

Glycerides (acylglycerols) cholesterol and cholesteryl esters are neutral lipids because they are uncharged

SIMPLE LIPIDS

A. Fats :

(a) They are esters of fatty acids with glycerol.

(b) They are enormously found in nature.

(c) They are the best reserve food material in the human body.

(d) They act as insulator for the loss of body heat.

(e) They act as a padding material for protecting internal organs.

The chemical structure of fat (triglyceride) consists of three different molecules of fatty acids with one molecule of glycerol. The three differeot fatty acids (R_1, R_2, R_3) are esterified with the three hydroxyl groups of glycerol.

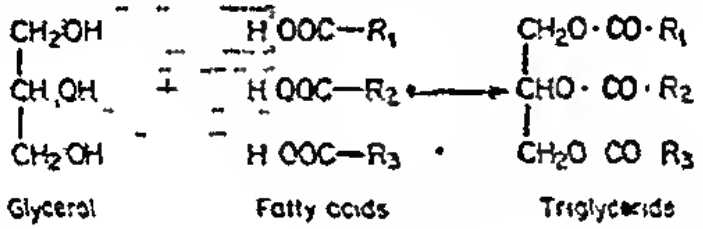


Fig 4 1

Physical Properties of Fats :

(a) The fats are insoluble in water, but readily soluble in ether, chloroform, benzene, carbon tetrachloride.

(b) They are readily soluble in hot alcohol but slightly soluble in cold.

(c) They are themselves good solvents for other fats, fatty acids etc.

(d) They are tasteless, odourless, colourless and neutral in reaction.

(e) Several neutral fats are readily crystallised, e g. beef, mutton.

(f) Their melting points are low.

(g) The specific gravity of solid fats is about 0.86. So the fat people float in water more readily than thin ones.

(h) They spread uniformly over the surface of water ; so the spreading effect is to lower surface tension.

Chemical Properties :

1. *Hydrolysis :* Hydrolysis of triacylglycerol takes place by lipases producing fatty acids and glycerol. Phospholipases attack the ester linkages of phospholipids.

2. *Soponification* : Hydrolysis of a fat by alkali is called saponification. The products are glycerol and the alkali salts of the fatty acids which are called *soaps*.

3. *Saponification number* . The number of milligrams of KOH required to saponify 1 gram of fat or oil.

4 *Acid number* : The number of milligrams of KOH required to neutralize the free fatty acids of 1 gram of fat.

5. *Iodine number* : This is the amount (in grams) of iodine absorbed by 100 grams of fat. This is the measure of the degree of unsaturation of a fat.

6 *Acetyl number* The number of milligrams of KOH required to neutralize the acetic acid obtained by saponification of 1 gram of fat after it has been acetylated This is a measure of the number of hydroxy acid groups in the fat

7 *Polenske number* The number of milliliters of 0 1 normal KOH required to neutralize the insoluble fatty acids from 5 grams of fat

8 *Reichert-Meissl number* This is the same as the Polenske number except that the soluble fatty acids are measured by titration of the distillate obtained by steam distillation of the saponification mixture

9 *Halogenation* Chlorine, bromine, iodine atoms may be added to the double bonds of unsaturated fatty acids containing fats

10 *Rancidity* Nearly all natural fats are oxidized when exposed to air, light, moisture, particularly if warm developing an unpleasant odour and taste This happens so due to the formation of peroxides at the double bonds of unsaturated fatty acids Vitamin E is an important natural antioxidant

B Waxes

(1) They are esters of fatty acids with higher alcohols and formed as secretions, which are mostly protective in function, by many animals

(2) They resemble the fats and are usually solid

(3) In the human body, the commonest waxes are esters of cholesterol

COMPOUND LIPIDS

A Phospholipids (phosphatides)

(i) They are esters of fatty acids with glycerol containing an esterified phosphoric acid and a nitrogen base

(ii) They are present in large amounts in nerve tissue, brain, liver, kidney, pancreas and heart

Biological functions of phospholipids

(i) They increase the rate of fatty acid oxidation

(ii) They act as carriers of inorganic ions across the membranes

(iii) They help blood clotting

(iv) They act as prosthetic group to certain enzymes

(v) They form the structures of membranes, matrix of cell wall, myelin sheath, microsomes and mitochondria

The phospholipids include the following groups

1 *Phosphatidic acid and phosphatidyl glycerols*

Phosphatidic acid is important as an intermediate in the synthesis of triacylglycerols and phospholipids

SIMPLE LIPIDS

A Fats

(a) They are esters of fatty acids with glycerol

(b) They ore eoormously found in nature

(c) They are the best reserve food material io the humao body

(d) They act as insulator for the loss of body heat

(e) They act as a padding material for protectiog internal orgaos

The chemical structure of fat (triglyceride) consists of three differeot molecules of fatty acids with ooe molecule of glycerol The three differeot fatty acids (R_1 R_2 R_3) are esterified with the three hydroxyl groups of glycerol

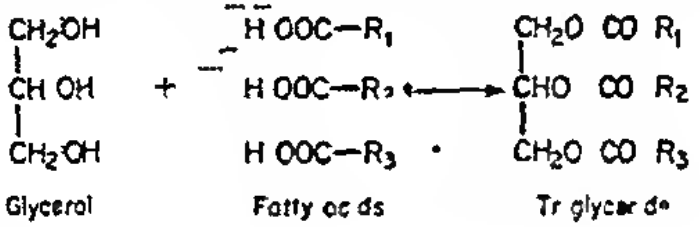


Fig 4 1

Physical Properties of Fats

(a) The fats are insoluble io water, but readily soluble in ether chloroform, beozeoe, carbon tetrachloride

(b) They are readily soluble in bot alcobol but slightly soluble io cold

(c) They are tbemselves good solvents for other fats fatty acids ete

(d) They are tasteless, odourless, colourless and neutral in reactioo

(e) Several neutral fats are readily crystallised e g beef mutton

(f) Their melting poiots are low

(g) The specific gravity of solid fats is about 0 86 So the fat people float in water more readily than thin ones

(h) They spread uniformly over the surface of water , so the spreading effect is to lower surface tension

Chemical Properties

1 *Hydrolysis* Hydrolysis of triacylglycerol takes place by lipases producing fatty acids and glycerol Phospholipases attack the ester linkages of phospholipids

2 *Saponification* Hydrolysis of a fat by alkali is called saponificatioo The products are glycerol aod the alkali salts of the fatty acids which are called *soaps*

3 *Saponification number* The number of milligrams of KOH required to saponify 1 gram of fat or oil

4 *Acid number* The number of milligrams of KOH required to oeutralize the free fatty acids of 1 gram of fat

5 *Iodine number* This is the amount (in grams) of iodine absorbed by 100 grams of fat This is the measure of the degree of unsaturation of a fat

6 *Acetyl number* The number of milligrams of KOH required to neutralize the acetic acid obtained by saponification of 1 gram of fat after it has been acetylated This is a measure of the number of hydroxy acid groups in the fat

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Phosphatidic acid is important as an intermediate in the synthesis of triacylglycerols and phospholipids

Cardiolipin which is found in mitochondria is formed from phosphatidylglycerol

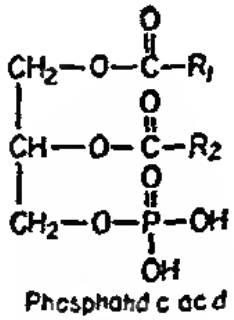


Fig 4 2

2 *Lecithins (phosphatidylcholine)*

The lecithins contain glycerol and fatty acids, phosphoric acid and choline (nitrogenous base) Lecithins generally contain a saturated fatty acid at α position and an unsaturated fatty acid at β position They can exist in α or β forms

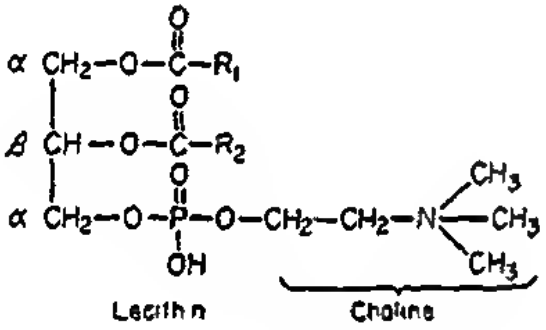


Fig 4 3

Physical properties

(i) Lecithins are waxy, white substances but become brown soon when exposed to air

(ii) They are soluble in ordinary fat solvents except acetone

(iii) They decompose when heated

(iv) They constitute valuable agents for the emulsification of fats and oils

Chemical properties •

(i) Lecithins on boiling with alkalis or mineral acids not only choline is split off but the phosphatidic acid is also hydrolyzed to give glycerophosphoric acid and fatty acids

(ii) They have the property of forming complexes with many different substances especially with other lipids, proteins, carbohydrates, and various heavy metals This property is concerned in the ionisation and function of many macromolecular cellular components

(iii) When the alcoholic hydroxyl group of choline is acetylated, acetylcholine is formed

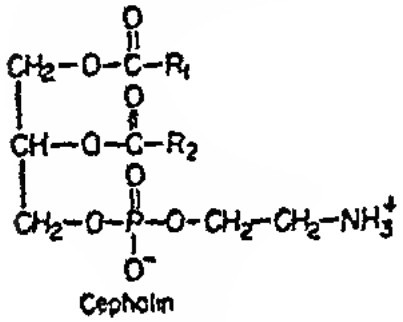
Physiological functions

(i) They facilitate combinations with proteins to form lipoproteins of plasma and cells

(ii) Acetylcholine formed from choline has an important role in the transmission of nerve impulses across synapses and form nerve endings to the muscle inervated

(iii) Choline is the most important lipotropic agent

3 *Cephalins (phosphatidyl ethanolamine)*



They always occur in the tissues in association with lecithins and are very similar in properties The only difference in the nitrogenous base

Fig 4 4

4 *Phosphatidyl serine*

A cephaline like phospholipid found in tissues

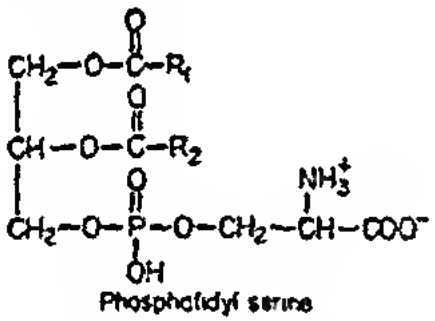


Fig 4.5

5 *Phosphatidyl inositol (Lipositol or Phosphoinositides)*

(i) They are found in brain, liver, heart and soyabean

(ii) They contain no base but have inositol in its place

(iii) They are more acidic than the other phospholipids

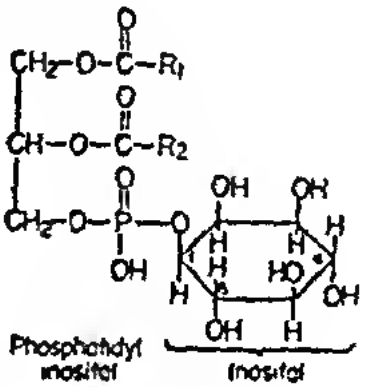


Fig 4 6

6 *Lysophospholipids*

These are phosphoacylglycerols containing only one acyl radical, e g Lysolecithin

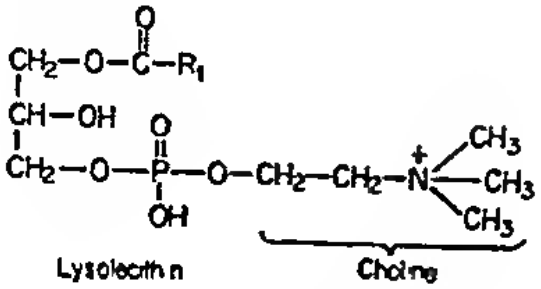


Fig 4 7

7 *Plasmalogens*

(i) These are the contents of brain and muscle.

(ii) Structurally, these resemble lecithins and cephalins but give a positive reaction when tested for aldehydes with Schiff's reagent (fuchsin-sulphurous acid) after pretreatment of the phospholipid with mercuric chloride

(iii) They possess an ether link in α position instead of ester link. The alkyl radical is an unsaturated alcohol

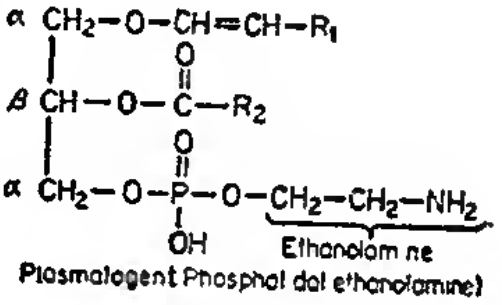


Fig 4 8

8 *Sphingomyelins*

(i) These are found in large quantities in brain and nerve tissue

(ii) The concentrations of these phospholipids are increased in Niemann–Pick disease in the liver and spleen

(iii) These contain a complex amino alcohol (sphingosine) only found in animal tissues, a fatty acid, a phosphoric acid and choline No glycerol is present

(iv) At least three types of sphingomyelins are known

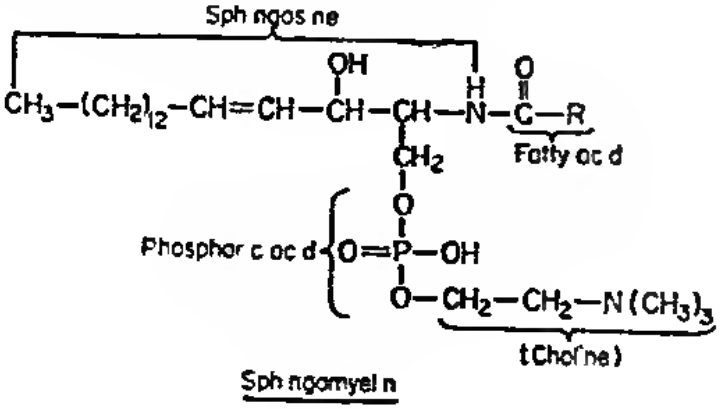


Fig. 4 9

B. Glycolipids

These contain an amino alcohol (sphingosine or iso sphingosine) attached with an amide linkage to a fatty acid and glycosidically to a carbohydrate moiety (sugars, amino sugar, sialic acid)

These are further classified into—(i) Cerebrosides, (ii) Gangliosides

(i) *Cerebrosides*

(a) Cerebrosides contain galactose a high molecular weight fatty acid and sphingosine Therefore, they may also be classified as sphingolipids

(b) They are the chief constituent of myelin sheath

(c) They may be differentiated by the type of fatty acid in the molecule These are

Kerasin—Containing lignoceric acid [$CH_3-(CH_2)_{22}-COOH$]

Cerebron—Containing a hydroxylignoceric acid (cerebronic acid)
[$CH_3-(CH_2)_{21}-CH(OH)-COOH$]

Nervon—Containing an unsaturated homologue of lignoceric acid called nervonic acid [$CH_3-(CH_2)_7-CH=CH-(CH_2)_{13}-COOH$]

Oxynervon—Containing hydroxynervonic acid
[$CH_3-(CH_2)_7-CH=CH-(CH_2)_{12}-CH(OH)-COOH$]

(d) Stearic acid is a major component of the fatty acids of rat brain cerebrosides

(e) Cerebrosides, specially cerebronic acid increases in Gaucher's disease and the Kerasin is characterized by glucose replacing galactose

(f) The cerebrosides are in much higher concentration in medullated than in nonmedullated nerve fibers

(ii) *Gangliosides*

(a) These are glycolipids occurring in the brain

(b) Gangliosides contain ceramide (sphingosine + fatty acids), glucose galactose N acetylgalactosamine and sialic acid

(c) Some gangliosides also contain dihydrosphingosine or Ganglionine in place of sphingosine

(d) Most of the gangliosides contain a glucose, two molecules of galactose, one N acetylgalactosamine and upto three molecules of sialic acid

3 A Lipoproteins

(i) Triacylglycerol (45%) phospholipids (35%) cholesterol and cholesteryl esters (15%) free fatty acids (less than 5%) and also protein combine to form a hydrophillic lipoprotein complex

(ii) Since pure fat is less dense than water the proportion of lipid to protein in lipoproteins if increases the density decreases This property is used to separate various lipoproteins in plasma by ultracentrifugation

(iii) The density of lipoproteins increases as the protein content rises and the lipid content falls and the size of the particle becomes smaller

(iv) Lipoproteins may be separated on the basis of their electrophoretic properties and may be identified more accurately by means of immuno-electrophoresis.

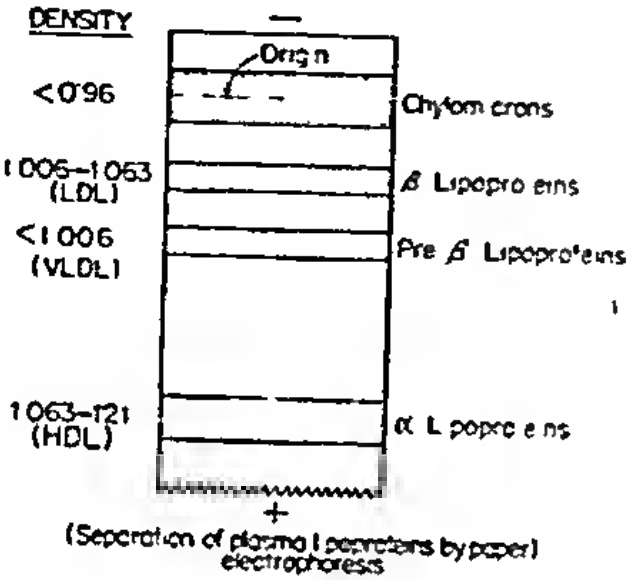


Fig. 4.10

(v) Four major groups of lipoproteins have been identified which are important physiologically and in clinical diagnosis in some metabolic disorders of fat metabolism

These are (a) Chylomicrons
(b) Very low density lipoproteins (VLDL or pre β-lipoproteins)
(c) Low density lipoproteins (LDL or β-lipoproteins)
(d) High density lipoproteins (HDL or α lipoproteins)

(vi) Chylomicrons and VLDL —Predominant lipid is triacylglycerol (50%) and cholesterol (23%). The concentrations of these are increased in atherosclerosis and coronary thrombosis etc

LDL —Predominant lipid is cholesterol (46%) and phospholipids (23%). Increased in atherosclerosis and coronary thrombosis etc.

HDL —Predominant lipid is phospholipid (27%) and proteins (45%)

(vii) The protein moiety of lipoprotein is known as an apoprotein which constitute nearly 60% of some HDL and 1% of chylomicrons. Many lipoproteins contain more than one type of apoprotein polypeptide

(viii) The larger lipoproteins (such as chylomicrons and VLDL) consist of a lipid core of nonpolar triacylglycerol and cholesteryl ester surrounded by more polar phospholipid, cholesterol and apoproteins

Importance :

(i) To transport and deliver the lipids to tissues

(ii) To maintain structural integrity of cell surface and subcellular particles like mitochondria and microsomes

(iii) The β lipoprotein fraction increases in severe diabetes mellitus,, atherosclerosis etc Hence determination of the relative concentrations of α and β lipoproteins and pre β lipoproteins are of diagnostic importance

B Aminolipids

Phosphatidyl ethanolamine and serines are aminolipids and sphingomyelins and gangliosides contain substituted amino groups

C. Sulpholipids (Sulphatides)

(i) These have been isolated from brain and other animal tissues

(ii) These are sulphate derivatives of the galactosyl residue in cerebrosides

DERIVED LIPIDS

1 Fatty acids

(i) These are obtained by the hydrolysis of fats

(ii) Fatty acids occurring in natural fats usually contain an even number of carbon atoms because they are synthesized from 2 carbon units and are straight chain derivatives

(iii) The straight chain may be saturated (containing no double bonds) or unsaturated (containing one or more double bonds)

(iv) Carbon atoms of fatty acids are numbered from the carboxyl carbon (Carbon No 1) The carbon atom adjacent to the carboxyl carbon (Carbon No 2) is also known as the α carbon Carbon atom No 3 is the β carbon and the end methyl carbon is known as the ω carbon

(v) Various convensions are used for indicating the number and position of the double bonds e g Δ^9 indicates a double bond between carbon atoms 9 and 10 of the fatty acid

A. Saturated fatty acids

General formula for saturated fatty acids is $C_nH_{2n+1}COOH$ Other higher fatty acids occur in waxes A few branched—chain fatty acids have also been isolated from both plant and animal sources

Saturated fatty acids

Acid	*Formula*	*Carbon atoms*	*Sources*
Acetic	CH_3COOH	2	Product of carbohydrate fermentation by rumen organisms
Propionic	C_2H_5COOH	3	-do-
Butyric	C_3H_7COOH	4	Butter
Caproic	$C_5H_{11}COOH$	6	Product of carbohydrate fermentation by rumen organisms
Caprylic	$C_7H_{15}COOH$	8	Butter
Decanoic (Capric)	$C_9H_{19}COOH$	10	Butter

Acid	*Formula*	*Carbon atoms*	*Sources*
Lauric	$C_{11}H_{23}COOH$	12	Coconut oils.
Myristic	$C_{13}H_{27}COOH$	14	Coconut oils
Palmitic	$C_{15}H_{31}COOH$	16	Animal and Plant fats
Stearic	$C_{17}H_{35}COOH$	18	-do-
Arachidic	$C_{19}H_{39}COOH$	20	Peanut oil
Behenic	$C_{21}H_{43}COOH$	22	Seeds
Lignoceric	$C_{23}H_{47}COOH$	24	Peanut oil, Cerebrosides.

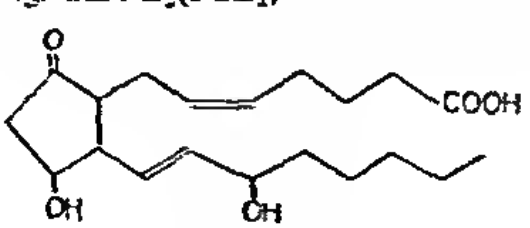
B. Unsaturated fatty acids

General formula $C_nH_{2n-1}COOH$

Type of acid	*Acid*	*Formula*	*Unsaturation at carbon atoms*	*Number of double bonds*	*Sources*
Mono-unsaturated	Palmitoleic	$C_{15}H_{29}COOH$	Δ^9	1	Nearly all fats.
,,	Oleic	$C_{17}H_{33}COOH$	Δ^9	1	-do-
Poly-unsaturated	Linoleic	$C_{17}H_{31}COOH$	Δ^9 & Δ^{12}	2	Animal and plant fat.
,	Linolenic	$C_{17}H_{29}COOH$	Δ^9, Δ^{12}, Δ^{15}	3	-do-
,,	Arachidonic	$C_{19}H_{31}COOH$	Δ^5, Δ^8, Δ^{11}, Δ^{14}	4	Peanut oil

A group of compounds known as prostaglandins are synthesized from arachidonic acid in the body They have pharmacologic and biochemical activity.

Example Prostaglandin E_2(PGE_2)



Prostaglandin E_2 (PGE_2)

Fig 4.11

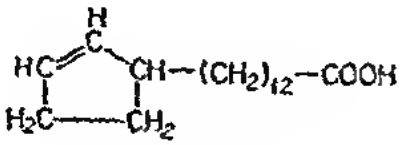
C Many other fatty acids -

(i) These have been detected in biologic material

Example : Fish oil contains 5 and 6 unsaturated fatty acids having carbon atoms 22

(ii) Various other structures with hydroxy groups (ricinoleic acid) or cyclic groups have been found in nature

Example of cyclic groups is chaulmoogric acid which was used many years ago in the treatment of leprosy



Chaulmoogric acid

Fig 4.12

ESSENTIAL FATTY ACIDS

Burr and Burr (1930) introduced the term "Essential Fatty Acids" (EFA) on the basis that they are essential for the growth and health of young albino rats These polyunsaturated fatty acids which are not synthesized in the body but are taken from natural sources are called essential fatty acids They are :

Essential fatty acids	*Chemical name*	*General formula*	*No of double bonds*	*Structure*	*Sources*
Linoleic	9, 12 Octadecadienoic acid	$C_nH_{2n-3}COOH$	2 (Δ^9, Δ^{12})	$CH_3-(CH_2)_4(CH=CH-CH_2)_2(CH_2)_6COOH$	Corn Peanut, Cottonseed, Soyabean oil
Linolenic	6 9, 12-Octadecatrienoic acid	$C_nH_{2n-9}COOH$	3 (Δ^6, Δ^{12}, Δ^{15})	$CH_3-(CH_2)_1(CH=CH-CH_2)_3(CH_2)_3COOH$	Found frequently with linoleic acid but particularly in linseed oil
Arachidonic	5, 8, 11, 14 eicosatetraenoic acid	$C_nH_{2n-7}COOH$	4 (Δ^5, Δ^8, Δ^{11}, Δ^{14})	$CH_3-(CH_2)_4(CH=CH-CH_2)_4(CH_2)_2COOH$	Found in small quantities with linoleic and linolenic acids but particularly in peanut oil

Linolenic and arachidonic acids are formed from linoleic acids provided linoleic acids are available in the body in sufficient quantities

Properties :

(i) The essential fatty acids of vegetable oils have low melting points and iodine number

(ii) They become saturated fatty acids on hydrogenation and the oils become solid fats

Functions :

(1) The essential fatty acids in high concentration along with the lipids constitute the structural elements of the tissues

(2) The lipids of gonads also contain a high concentration of polyunsaturated fatty acids which suggest the importance of reproductive function

(3) They effect the prolongation of clotting time and increase the fibrinolytic activity

(4) They retard atherosclerosis being esterified and emulsified with cholesterol and are incorporated into lipo-proteins for transport to the liver for further oxidation

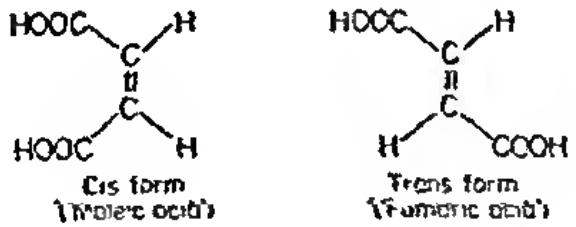
(5) They cure skin lesions

(6) The deficiency of these acids in the diet of babies causes eczema

ISOMERISM IN UNSATURATED FATTY ACIDS

Variations in the location of the double bonds in unsaturated fatty acid chains produce isomers Oleic acid has 15 different positional isomers

Geometric isomerism depends on the orientation of radicals around the axis of double bonds If the radicals which are being considered are on the same side of the bond, the compound is called "cis", if on opposite side, "trans". This can be illustrated with maleic acid and fumaric acid



Cis form ('Maleic acid') Trans form ('Fumaric acid')

Fig 4 13

There are more geometric isomers in case of acids with greater degree of unsaturation The unsaturated long chain fatty acids, occurring in nature are nearly all of the 'cis' form and the molecules are "bent" at the position of the double bond Thus, arachidonic acid is U-shaped.

REFINED AND HYDROGENATED OILS

Refined oil : It is prepared in the following manner :

(i) Free fatty acids are removed by alkali treatment

(ii) Colouring matter is removed by activated carbon

(iii) Odour is removed by superheated steam.

Hydrogenated oils The refined oils are hydrogenated under optimum temperature and pressure with hydrogen in the presence of nickel catalyst Unsaturated fatty acids are converted into saturated fatty acids

$$\text{Oleic acid} \xrightarrow{\text{Hydrogenation}} \textit{Stearic acid}$$

The liquid oil becomes solid fat and the unsaturated fatty acid content decreases Vanaspati is hydrogenated refined groundnut oil

2 ALCOHOLS

Alcohols found in lipid molecules include glycerol, cholesterol and higher alcohols (cetyl alcohol), usually found in the waxes

The unsaturated alcohols are important pigments Phytyl alcohol is a constituent of chlorophyll and lycophyll ($C_{40}H_{56}O_2$), a polyunsaturated dihydroxy alcohol occurs in tomatoes as a purple pigment

3 STEROIDS

The steroids are often found in association with fat They have a similar cyclic nucleus resembling phenanthrene (rings A, B °C) to which a cyclopentane ring (D) is attached The parent substance is better designated as cyclopentanoperhydrophenanthrene The positions on the steroid nucleus are numbered as shown in the figure below

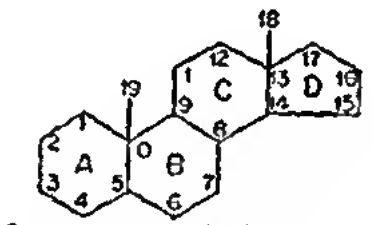


Fig 4 14

Methyl side chains occur typically at positions 10 and 13 (constituting C atoms 19 and 18) A side chain at position 17 is usual (as in cholesterol) If the compound has one or more hydroxyl groups and no carbonyl or carboxyl groups, it is a *sterol,* and the name terminates in —OL

Steroids may be divided in the following manner

Sterols—cholesterol, ergosterol, coprosterol

Bile acids—Glycocholic acid and taurocholic acid

Sex hormones—Testosterone, Estradiol

Vitamin D—Vit D_2 and D_3

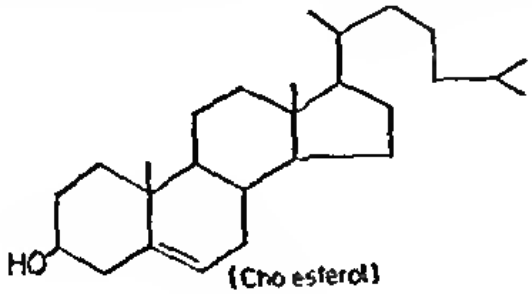
Adrenocortical hormones—Corticoster

Cardiac glycosides—Stropanthin

Saponins—Digitonin

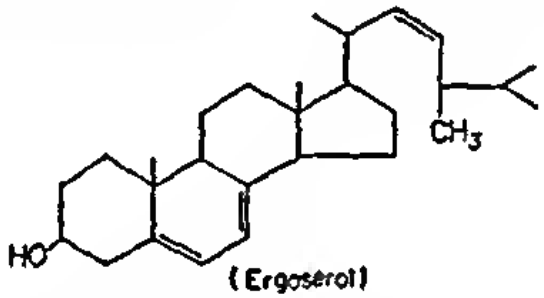
Cholesterol :

It is widely distributed in all cells of the body It occurs in animal fats but not in plant fats Its structure is given below The metabolism of cholesterol is discussed in lipid metabolism chapter



Ergosterol Fig 4 15

(i) It occurs in ergot and yeast
(ii) It is the precursor of vitamin D
(iii) It acquires antirachitic properties with the opening of ring B when irradiated with ultraviolet light



Coprosterol Fig 4 16

It occurs in feces as a result of the reduction by bacteria in the intestine of the double bond between C_5 and C_6 of cholesterol

Important tests

(1) Greese spot test A drop of oil is placed over a piece of ordinary paper A translusent spot is visible *This indicates the presence of fat*

(2) Emulsification test 2 ml water is taken in one test tube and 2 ml of diluted bile salt solution in another test tube Add 3 drops of the given oil to each test tube and shake vigorously Note the stability of the emulsification formed

(3) Saponification test Take 10 drops of coconut oil in a test tube Add 20 drops of 40% NaoH and 2 ml of glycerol to it Gently boil for about 3 minutes until complete saponification occurs If oil globules are visible, boiling must be continued Divide the solution into 3 parts to carry the following experiments in test tube 1, 2, 3

To test tube No 1 add saturated solution of Nacl Note that the soap separates out and floats to the surface (salting out process)

To test tube No 2 add a few drops of Conc Hcl An oily layer of the fatty acids rises to the surface

To test tube No 3 add a few drops of $CaCl_2$ solution The insoluble calcium soap is precipitated

Unsaturatioo test Add 10 drops of Huble s iodine reageot to 10 ml of chloroform The chloroform assumes a pink colour due to the free iodine The solution is divided equally into three test tubes as (a) (h) and (c) and three types of oil are added

Add the oil No 1 to the test tube (a) drop by drop shaking the tube vigorously after each addition till the pink colour of the solution just disappears The number of oil drops required are noted The experiment is repeated hy oil 2 and 3 adding to test tubes (h) aod (c) respectively The more the number of drops required to discharge the piok colour, the less is the unsaturation

Colour reactions to detect sterols

Liebermano Burchard reaction A chloroform solution of a sterol wheo treated with acetic anhydride aod sulphuric acid gives a greeo colour This reaction is the hasis of a colorimetric estimatioo of hlood cholesterol

Salkowski test A red to purple colour appears wheo a chloroform solutioo of the sterol is treated with an equal volume of concentrated sulphuric acid

Exercise

1 What are lipids ? How are they classified ? Discuss the characteristics and functions of a class of lipids having nitrogen hase and phosphoric acid as constitueots (P U 69S)

2 What are lipids and classify them Describe some important tests for lipids (Mith 65S)

3 Give the classificatioo and characteristics of lipids Discuss briefly the chemistry and importance of phospholipids (Bh U 75A)

4 Write short ootes on

(i) Essential fatty acids (Mith 75A , Bh U 76S , P U 74A)
(ii) Sterol (Bh U 76A)
(iii) Phospholipids (M U 73A , P U 68A , 76A)
(iv) Saponification number (Bh U 75S)
(v) Hydrogenated fats (Muz. 74A)
(vi) Lecithin (P U 72A , Mith 62S)
(vii) Iodine number (R U 71S)
(viii) Lipoproteins (R U 72S)
(ix) Unsaturated fats (Muz 75A)
(x) Saponification (C U 1981)
(xi) Rancidity (C U 1981)
(xii) Cholesterol (C.U 1981)

5 Give the structural formula of α glycerophosphate (C.U 1981)

6 How the l pids can be classified? Enumerate some neutral fats present in our body (C U 1981)

7 Name and with tests and reactions the presence of characteristics functional group occurring in glycerol (C.U 1981)

8 With structures give examples of two unsaturated fatty acids essential for our body (C.U 1981)

9 Fill up the gaps with correct words (C.U 1981)

(i) Oils and fats are — of fatty acids Oils are generally more — than fats (C.U 1981)

(ii) On boiling with NaOH solution a fat will produce — and —

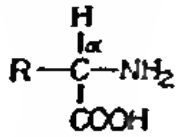
CHAPTER 5

AMINO ACIDS AND PEPTIDES

Definition

Amino acids are the monomers of proteins. Alpha amino acids have an amino and a carboxyl group attached to the same (α) carbon atom.

Although, there are over 200 different amino acids found in nature, only about one tenth (20) of these occur in proteins. Proteins of plant, animal and microbial life contain the same 20 amino acids



(α-amino acid)

Fig. 5.1

Complete acid, base-, or enzyme-catalyzed hydrolysis of proteins produces the naturally occurring 20 L α-amino acids. D amino acids are also found in some natural peptides.

Common properties of amino acids

1. Protonic equilibria of amino acids

(a) Amino acids have at least two ionizable groups i.e. —COOH and $-NH_3^+$. The former dissociates more easily than the latter. In solution, two forms of these groups one charged and one neutral, exist in protonic equilibrium with each other

$$R—COOH \rightleftharpoons R—COO^- + H^+$$
$$R—NH_3^+ \rightleftharpoons H^+ + R—NH_2$$

Fig. 5.2

R—COOH and $R—NH_3^+$ represent the protonated or acid partners in these equilibria. R—COO and $R—NH_2$ are the conjugated bases (i.e. proton acceptors) of the corresponding acids. Although both R—COOH and $R—NH_3^+$ are weak acids R—COOH is a several thousand times stronger acid than is $R—NH_3^+$.

(b) At P^H 7.4, carboxyl groups exist almost entirely as the conjugated base i.e. $R—COO^-$. Most amino groups exist in the form, $R—NH_3^+$. In blood and most tissues amino acid structures are drawn as follows

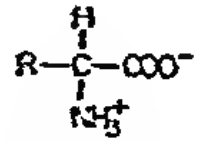
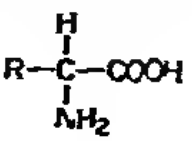


Fig. 5.3

The following structure cannot exist at any P^H but is frequently used as a convenience when the chemistry of amino acids is discussed



(c) P^K of an acid is simply the negative log of the dissociation constant

$$P^K = -\log K$$

P^K values for α amino groups of free amino acids is about 9 8

(d) The *isoelectric* P^H (P^I) of an amino acid is that P^H at which it has no net charge and hence does not move in an electric field

Addition of acid or alkali depresses one type of ionisation so that the amino acid behaves as a base or an acid The ion at the isoelectric point which carries + and — charges internally neutralised is called a 'Zwitterion' The three types of ions are represented below

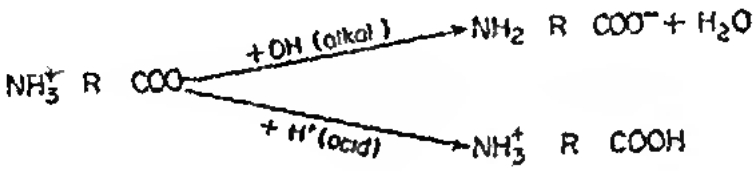


Fig 5 5

In an acid solution, the amino acid acts as a base yielding cations When current is allowed to pass through the solution, the amino acid migrates to the cathode or positive pool In an alkaline solution, it behaves as an acid forming anions. In the electric field, the amino acid migrates to the anode or negative pool On account of these opposite reactions depending on the acidity or alkalinity of the solution the amino acids are called ampholytes

Since Pk_1 (RCOOH)=2 35 and Pk_2 (RNH_3^+)=9 69, the isoelectric P^H (P^I) of alanine is

$$P^I = \frac{Pk_1 + Pk_2}{2} = \frac{2\,35 + 9\,69}{2} = 6{\cdot}02$$

P^I of Aspartic acid $= \frac{Pk_1 + Pk_2}{2} = \frac{2\,09 + 3\,86}{2} = 2\,98$

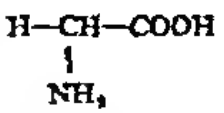
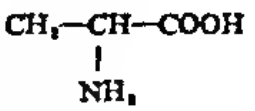
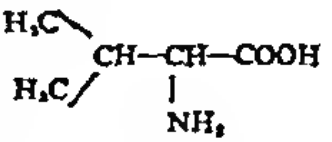
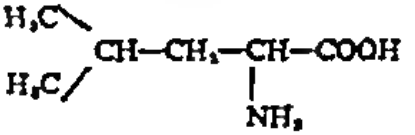
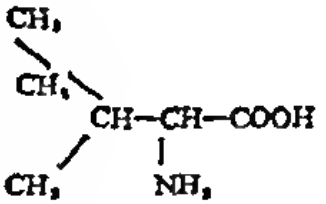
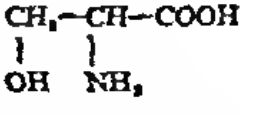
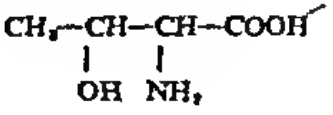
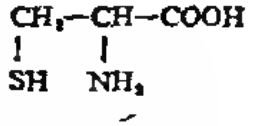
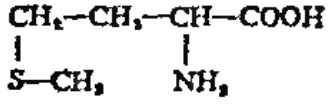
Thus P^I of lysine and arginine is 9 7 and 10 8 respectively

The ability to perform calculations of this type is of significant value in the clinical laboratory to assess the mobility of known compounds in electric fields and to select appropriate buffers for separation of one from another

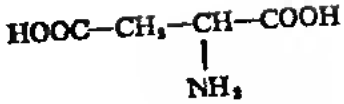
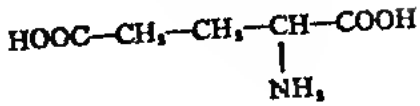
2. Structures of amino acids

For many purposes it is convenient to subdivide the amino acids in proteins into 7 classes as in the following table In addition to their common names, systematic chemical names are also included in this table

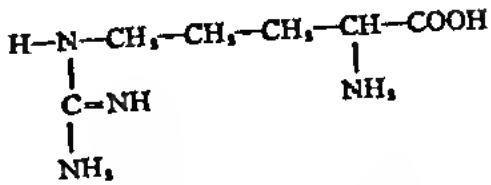
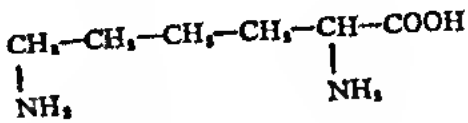
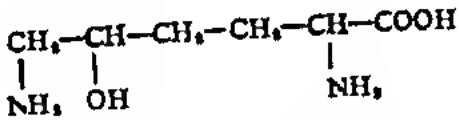
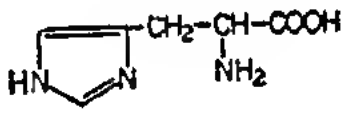
TABLE - L-α-amino acids found in proteins

Group	*Trivial name*	*Symbol*	*Systematic name*	*Structural formula*
I. With aliphatic side chains.				
	Glycine	Gly (G)	Amino acetic acid	
	Alanine	Ala (A)	2-Amino propanoic acid	
	Valine	Val (V)	2-Amino-3-methyl butanoic acid	
	Leucine	Leu (L)	2-Amino-4-methyl pentanoic acid	
	Isoleucine	Ile (I)	2-Amino-3-methyl pentanoic acid	
II	With side chains containing hydroxyl (OH) groups.			
	Serine	Ser (S)	2-Amino-3-hydroxy propanoic acid	
	Threonine	Thr (T)	2-Amino-3-hydroxy butanoic acid	
III	With side chains containing sulfur atoms.			
	Cysteine	Cys (C)	2-Amino-3-mercapto propanoic acid	
	Methionine	Met (M)	2-Amino-4-(methylthio) butanoic acid	

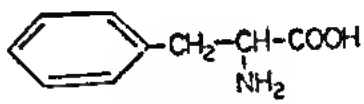
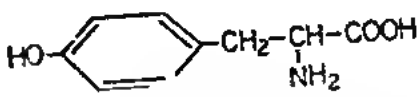
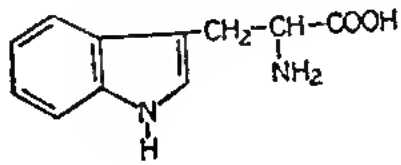
IV With side chains containing acidic groups.

Aspartic acid	Asp (D)	Amino succinic acid	
Glutamic acid	Glu (E)	2-Amino glutaric acid	

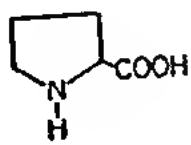
V With side chains containing basic groups.

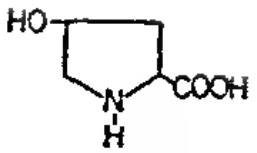
Arginine	Arg (R)	2-Amino-5-guanido valeric acid	
Lysine	Lys (K)	2, 6-Diamino hexanoic acid	
Hydroxy lysine	Hyl	2, 6-Diamino-5-hydroxy-hexanoic acid	
Histidine	His (H)	2 Amino-1H-imidazole-4-propanoic acid	

VI Containing aromatic rings

Phenyl alanine	Phe (F)	2-Amino-3-phenyl-propanoic acid	
Tyrosine	Tyr (Y)	2-Amino-3-(4-hydroxy phenyl) Propanoic acid	
Tryptophan	Trp (W)	2-Amino-3-(3-indolyl) propanoic acid	

VII Imino acids

Proline	Pro (P)	2-Pyrrolidine carboxylic-acid	

4 hydroxy Prol ne	Hyp	4 hydroxy-2 pyrolidine-carboxyl c acid	

3 Optical isomers of amino acids

Except glycine, each amino acid has at least one asymetric carbon atom and hence is optically active Although D amino acids occur in cells and even in polypeptides they are not present in proteins

Various other amino acids e g Homocysteine, Homoserine, Ornithine, Citrulline, Arginosuccinic acid, Dopa, 3 monoiodotyrosine, 3, 5 Diiodotyrosine 3, 5, 3 triiodotyrosine, Thyroxine, β Alanine, Taurine etc , in free or combined states fulfill important functions in metabolic processes other than as constituents of proteins Many additional amino acids occur in plants or in antibiotics Over 20 D amino acids occur naturally

PHYSICAL PROPERTIES OF AMINO ACIDS

(i) Amino acids are soluble in polar solvents such as water and ethanol but they are insoluble in nonpolar solvents such as benzene or ether

(ii) Their melting point is above 200°C.

(iii) The aromatic amino acids tryptophan tyrosine, histidine and phenyl alanine absorb ultraviolet light

CHEMICAL REACTIONS OF AMINO ACIDS

(i) Ninhydrin test Ninhydrin is a powerful oxidizing agent which causes oxidative decarboxylation of α amino acids yielding CO_2 NH_3 and an aldehyde The reduced ninhydrin then reacts with the liberated ammonia forming a blue complex Proline and hydroxyproline produce a yellow rather than a purple colour with ninhydrin

(ii) A variety of colour reactions specific for particular functional groups in amino acids are known which are useful in both qualitative and quantitative identification of particular amino acids These are given below

Name of the test	*Regents*	*Colour*	*Amino acid detected*
Nitroprusside	Sodium nitroprusside in dil NH_4OH	Red	Cysteine
Hopkins Cole	Glyoxylic acid in 36N H_2SO_4	Purple	Tryptophan
Millon	$HgNO_3$ in HNO_3 heat	Red	Tyrosine.
Xanthoproteic	Boiling conc. HNO_3	Yellow	Tyrosine Tryptophan, Phenylalanine
Sakaguchi	α Naphthol and Sodium hypochlorite	Red	Arginine
Pauly	Diazotized sulfanilic acid in alkaline solution	Red	Histidine

(iii) Formation of peptide bonds: Peptide bond formation involves removal of one mole of water between the α-amioó group of one amino acid and the α-carboxyl group of a second amino acid.

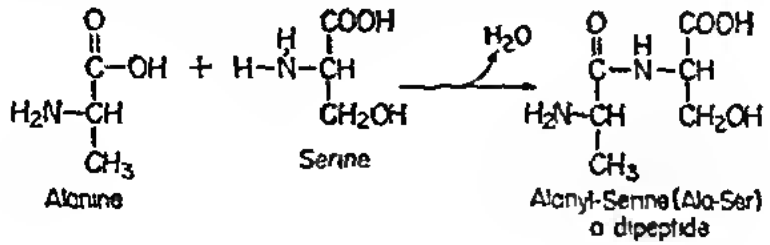


Fig. 56

PEPTIDES

Definition : A peptide consists of two or more amino acids linked by a peptide bond formed between the carboxyl group of one amino acid and the amino group of another with the removal of one mole of water during a peptide bond.

Polypeptides are formed by long peptide chains containing large numbers of peptide bonds. Polymers of 100 amino acids are termed polypeptides and those with more than 100 are generally termed proteins.

Primary structure of peptides :

The sequence of amino acids in a polypeptide is referred to as its primary structure. Chemical methods are required to determine the primary structure. Physical technics such as X-ray crystallography are employed for higher order of protein structure.

The N-terminal (amino terminal) amino acid is always shown at the left and the C-terminal (carboxyl terminal) amino acid at the right of the polypeptide chain.

Fig. 57

Even small changes in the primary structure of proteins may produce remarkable physiologic effects. Substitution of a single amino acid for another in a sequence of 100 or more amino acids may abolish biologic activity with serious consequences (e.g. Sickle cell disease) The biochemical basis for many inherited metabolic diseases are known owing to the introduction of new chemical and physical methods to determine protein structure.

DETERMINATION OF THE PRIMARY STRUCTURE OF PEPTIDES

The primary structure of peptides is determined by

(A) Qualitative identification and estimation of the amino acids

(B) Determination of sequence

A Determination of the number and kinds of amino acids present

(i) The peptide bonds are first broken by hydrolysis by acid, alkali or enzymes

(ii) The free amino acids are then separated from one another and identified by chromatography and electrophoresis

(A) *Hydrolysis by acid* Most proteins are completely hydrolyzed by 6N Hcl at 110°C for 20-70 hours in a sealed evacuated tube to exclude oxygen by preventing oxidative side reactions

(B) *Hydrolysis by alkali* All amino acids are racemized by alkaline hydrolysis with 5N NaoH.

(C) *Hydrolysis by enzymes* All peptide bonds are catalytically hydrolyzed by a variety of bacterial peptidases , but the reaction is slow

Separation and identification of the amino acids present

The free amino acids after hydrolysis of peptides are separated and identified by chromatography or by electrophoresis

1 Paper chromatography

(i) A small volume (about 0 005 ml containing about 0 01 mg) of amino acids is applied at a marked point 5 cm from the end of filter paper strip

(ii) The filter paper strip is suspended in a sealed cylindrical jar or cabinet.

(iii) One end of the strip is dipped into a solvent consisting of water, an acid or base, an organic substance such as n butyl alcohol

(iv) The solvent is placed in a trough from which the paper strip hangs (descending paper chromatography) or the strip may be suspended from the top of the jar and dipped into a trough at the bottom of the jar (ascending paper chromatography)

(v) Strips are removed when the solvent migrates the marked distance and dried

(vi) Then the strips are sprayed with 0 5% ninhydrin in acetone and heated for a few minutes at 80-100°C.

(vii) Purple spots will indicate the presence of amino acids

(viii) The amino acids are identified by the R_f value which is determined as follows

$$R_f = \frac{\text{Distance travelled by an amino acid from the marked point of application}}{\text{Distance travelled by the solvent from the marked point of application}}$$

Thin layer chromatography :

Thin layer chromatography may be employed in place of paper chromatography.

(i) The absorbents (e.g. cellulose powder, alumina, a cellulose ion exchange resin, sephadex) are spread as a slurry on 8×18 inch glass plates.

(ii) The plates are dried and used like paper strips of paper chromatography.

(iii) The advantage in this is in the choice of adsorbents and in the rapidity of separation. Amino acid mixtures require 3 hours for separation instead of 18 hours.

(iv) Lipids including sterols are rapidly and clearly separated.

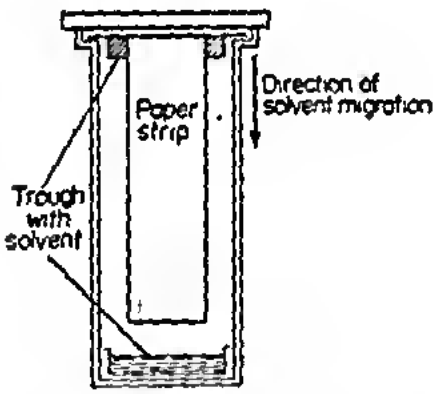


Fig. 5.8 Descending paper chromatography

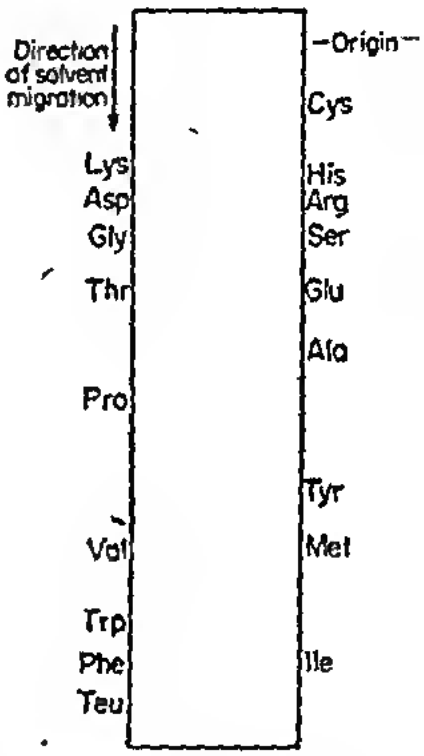


Fig. 5.9 Identification of amind acids present in protein after desending paper chromatography

2. Electrophoresis :

(i) Amino acid mixtures are applied at the centre of the paper or thin layers of powdered cellulose.

(ii) This is then moistened with buffer of an appropriate P^H connected to buffer reservoirs by paper wicks and covered with a glass plate to reduce evaporative loss of buffer during passage of the current.

(iii) The current is applied with 2000—5000 volts for 0.5—2 hours. The molecules at the net positive charge at the selected P^H more toward the cathode and those with n net negative charge toward the anode.

(iv) The electrogram (paper or thin layers of powdered cellulose) is dried and treated with ninhydrin to see the separated nmino acids.

(v) The amino acids are separated readily on the basis of their difference in molecular weight.

(vi) P^{H} 3 5 is better for the separation of the peptides formed from the enzymic digestion of a protein

B Determination of seqnence

The sequence of amino acids of peptides is determined by many chemical technics One tecbnic is mentioned below

Treatment with 1-fluoro-2, 4-dinitrobenzene (Sanger's Reagent)

(i) All free amino groups produce yellow 2, 4-dinitrophenyl amino acids which are readily quantitated by spectrophotometry

(ii) Only tbe N terminal residue can form a 1-fluoro-2, 4-dinitrobenzene derivative wbicb may be separated and identified by bydrolysis

SYNTHESIS OF PEPTIDES

Peptides may be syntbesized by a reaction between an activated carboxyl group such as an acid cbloride of one amino acid and tbe amino group of another, e g cysteine acid chloride and lysine

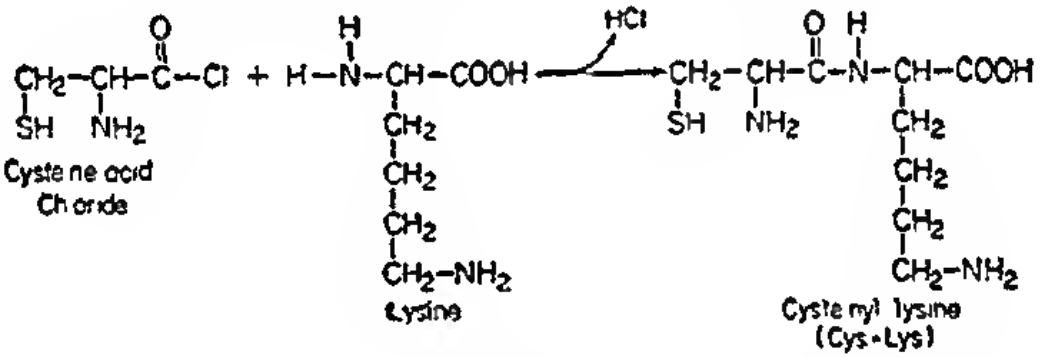


Fig 10

After the peptide bond is formed, the blocking group is removed, leaving the desired peptide

PHYSIOLOGICALLY ACTIVE PEPTIDES

(i) In some cases, they are the basis of products of protein

(ii) In others, they may be hormones, antibiotics, precursors of bacterial cell walls, or even potent poisons

(iii) The widely distributed tripeptide glutathione is required for the action of several enzymes including insulin Glutathione reductase functions either in insulin degradation or in the formation of disulfide bonds.

(iv) Other naturally occurring important peptides are brady—Kinin and Kallidin which are smooth muscle hypotensive agents are liberated from specific plasma proteins by treatment with snake venom

CHAPTER 6

PROTEINS

Definition :

Proteins may be defined as the high molecular weight mixed polymers of α-amino acids joined together with peptide linkage (—CO—NH—).

Proteins are the chief constituents of all living matter. They contain carbon, hydrogen, nitrogen and sulphur and some contain phosphorus also.

Biological importance or biochemical functions :

1. Proteins are the essence of life processes.
2. They are the fuodamental constituents of all protoplasm and are involved in the structure of the living cell aod in its function.
3. Enzymes are made up of proteins.
4. Many of the hormones are proteins.
5. The cement substances and the reticulum which bind or hold the cells as tissues or organs are made up partly of proteins.
6. They execute their activities in the transport of oxygen and carbon dioxide by hemoglobin and special enzymes in the red cells.
7. They function in the homostatic control of the volume of the circulating blood and that of the interstitial fluids through the plasma proteins.
8. They are involved in blood clotting through thrombin, fibrinogen and other protein factors.
9. They act as the defence against infections by means of protein antibodies.
10. They perform hereditary transmission by nucleoproteins of the cell nucleus.

CLASSIFICATION :

1. Simple Proteins :

Class of Proteins	*Characteristics*	*Examples*
(i) Albumins ..	Soluble in water, coagulable by heat and precipitated at high salt concentrations.	Serum albumin, egg albumin, lactalbumin (Milk), leucosin (wheat), legumelin (soyabeans)
(ii) Globulins ..	Insoluble in water, soluble in dilute salt solutions and precipitated by half saturated salt solutions	Serum globulin, vitellin (egg yolk), tuberin (potato), myosinogen (muscle), legumin (peas).
(iii) Glutelins ..	Insoluble in water but soluble in dilute acids and alkalis Mostly found in plants.	Glutenin (wheat), oryzenin (rice).

Class of Proteins	*Characteristics*	*Examples*
(iv) Prolamines	Insoluble in water and absolute alcohol but soluble in 70 to 80 per cent alcohol	Gliadin (wheat), zein (maize).
(v) Protamines	Basic proteins of low molecular weight Soluble in water, dilute acids and alkalis Not coagulable by heat	Salmine (salmon sperm).
(vi) Histones	Soluble in water and insoluble in very dilute ammonium hydroxide	Globin of hemoglobin and thymus histones
(vii) Scleroproteins	Insoluble in water, dilute acids and alkalis	Keratin (hair, horn, nail, hoof and feathers) collagen (bone, skin) elastin (ligament)

2. Conjugated Proteins

Class of Proteins	*Characteristics*	*Examples*
(i) Nucleoproteins	Composed of simple basic proteins (protamines or histones) with nucleic acids, found in nuclei Soluble in water	Nucleoprotamines and nucleohistones
(ii) Lipoproteins	Combination of proteins with lipids such as fatty acids, cholesterol and phospholipids etc	Lipoproteins of egg-yolk, milk and cell membranes lipoproteins of blood
(iii) Glycoproteins	Combination of proteins with carbohydrate (mucopolysaccharides)	Mucin (saliva), ovomucoid (egg white) osseomucoid (bone) tendomucoid (tendon)
(iv) Phosphoproteins	Contain phosphorus radical as a prosthetic group	Casemogen (milk), ovovitellin (egg yolk)
(v) Metalloproteins	Contain metal ions as their prosthetic groups The metal ions generally are Fe, Co, Mg, Mn, Zn, Cu etc.	Siderophilin (Fe), ceruloplasmin (Cu)
(vi) Chromoproteins	Contain porphyrin (with a metal ion) as their prosthetic groups	Haemoglobin, myoglobin, catalase, peroxidase, cytochromes
(vii) Flavoproteins	Contain riboflavin as their prosthetic groups.	Flavoproteins of liver and kidney

3 Derived Proteins

Class of Proteins	Characteristics	Examples
A Primary derivatives		
(i) Proteans	Derived in the early stage of protein hydrolysis by dilute acids enzymes or alkalis	Fibrin from fibrinogen
(ii) Metaproteins	Derived in the later stage of protein hydrolysis by slightly stronger acids and alkalis	Acid and alkali metaproteins
(iii) Coagulated proteins	They are denatured proteins formed by the action of heat X rays ultraviolet rays etc	Cooked proteins, coagulated albumins
B Secondary derivatives (Due to cleavage of peptide bonds)		
(i) Proteoses	Formed by the action of pepsin or trypsin Precipitated by saturated solution of ammonium sulphate incoagulable by heat	Albumose from albumin globulose from globulin.
(ii) Peptones	Further stage of cleavage than the proteoses Soluble in water incoagulable by heat and not precipitated by saturated ammonium sulphate solutions	
(iii) Peptides	Compounds containing two or more amino acids They may be di tri and polypeptides	Glycyl-alanine leucyl glutamic acid

Protein hydrolysing enzymes

(i) Pepsin—In Gastric Juice
(ii) Trypsin, Chymotrypsin and Carboxypeptidases—In Pancreatic Juice
(iii) Amino-peptidases, Dipeptidases and Polypeptidases—In Intestinal Juice

BONDS RELATING TO PROTEIN STRUCTURE

Protein structures are confirmed by

1 Strong bonds—Peptide and disulphide
2 Weak bonds—Hydrogen and hydrophobic

1 Strong bonds

A Peptide bonds

The primary structure of proteins is derived from peptide bonds which can be established by the following evidences.

(i) Proteases produce polypeptides by hydrolyzing proteins. They can also hydrolyze peptide bonds.

(ii) The protein, insulin, is synthesized by linking amino-acids by peptide bonds.

(iii) Polypeptides like proteins react with biuret reagent which is suggestive of 2 or more poptide bonds.

B. Disulphide bond :

(i) The disulphide bond may interconoect two parallel chains throngh cysteine within each polypeptide sbown in the figure below :

(ii) The bond is not broken uoder the usual conditions of denaturation.

(iii) Performic acid is used to oxidize insulin to separate tbe protein molecule into its constituent polypeptide chaios without affecting the other part of the molecule.

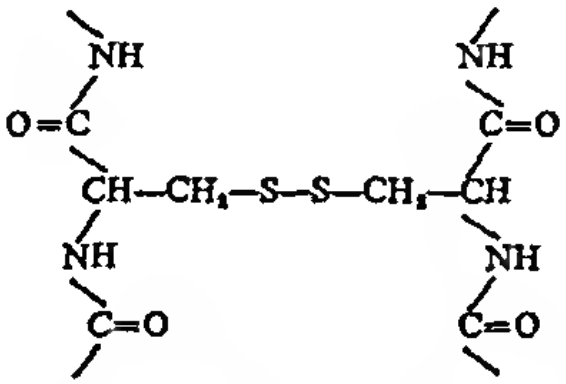


Fig. 6.1 (Two peptide chains united by a disulphide linkage)

2. Weak bonds :

A. Hydrogen bonds :

(i) The hydrogen bond appears from the shariog of hydrogen atoms between the nitrogen and the carbonyl oxygen of different peptide bonds as shown below by star mark.

(ii) Each hydrogen bood is quite weak.

(iii) During denaturation of a protein, the hydrogen and hydrophobic bonds are broken.

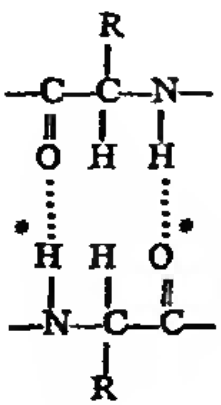


Fig. 6.2

B. Hydrophobic bonds :

(i) The nonpolar side chains of neutral amino acids are closely associated with one another in proteins.

(ii) No true bond exists.

(iii) Nonetheless, they play an important role in maintaining protein structure.

STRUCTURE OF PROTEINS

The structure of protein is considered by several level of organization on the basis of modern views. The levels of organization are

1. Primary level of organization.
2. Secondary level of organization.
3. Tertiary level of organization.
4. Quarternary level of organization.

1. Primary structure :

Peptide bond is formed by the amino acids linked by carbox amino acid with the α-amino group of another amino acid as sho

$$H_2N-CH_2-CO-NH-CH_2-COOH.$$

Glycyl—Glycine.

Thus, they form peptide bonds by several amino acids as

—Ala—Gly—gly—His—leu—
—Ala—Gly—His—Gly—leu—

The primary structure ultimately becomes as

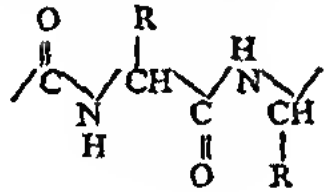


Fig. 6.3

2. Secondary structure :

Globular proteins indicate a coiled structure in which peptide bonds are folded in a regular manner. The foldings are the results of linking of the carboxyl and amino groups of the peptide chains by means of hydrogen bonds and disulphide bonds. Such foldings are referred to as the secondary structure of the protein. Present evidence suggests that in many proteins, the bydrogen bonding produces a regular coiled arrangement, called α-helix.

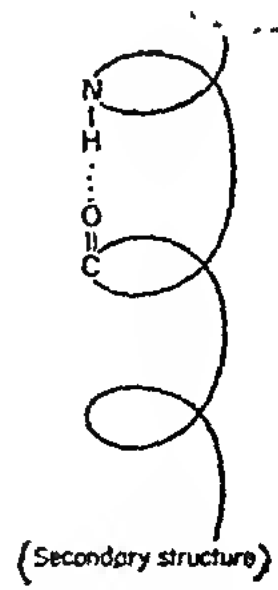


Fig 6.4

3 Tertiary structure

The globular protein if completely is composed of a series of single helix, these molecules will have elongated structures with a larger axial ratio (length breadth) Many proteins are spherical The special structure in three dimensions is maintained by covalent or other bonds Many globular proteins lack in disulphide bonds yet they are stable in solution The other bonds like hydrogen bonds, salt bonds and hydrophobic or non polar bonds are also involved in the stability of the molecule This has been described as the tertiary structure of protein

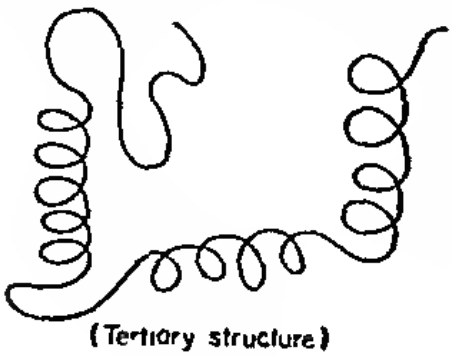


Fig 6 5

α helix

(i) Pauling and Corey proposed that the polypeptide chain of α keratin is arranged as an α helix

(ii) In the structure of α helix it has been found that R groups on the α-carbon atoms exist outward from the centre of the helix

(iii) There are 3 6 amino acid residues per turn of the helix.

(iv) The distance traveled per turn is 0 54 nm

(v) The spacing per amino acid residue is 0 15 nm

(vi) The α helix is stabilized by hydrogen bonds

(vii) Since α helix is the lowest energy it forms spontaneously

(viii) The right handed helix which occurs in proteins is significantly more stable than the left handed helix when the residues are L amino acids

(ix) Certain amino acids like proline tend to disrupt the α helix

The β Pleated Sheet

(i) Pauling and Corey also proposed a second ordered structure, the β pleated sheet (β because it was their second structure, the α helix being the first) In the α helix the polypeptide chain is condensed, in the β pleated sheet it is almost fully extended (Fig 6 6)

(ii) In a β-pleated sheet when the adjacent polypeptide chains run in opposite directions (N to C terminus), the structure is termed an antiparallel β-pleated sheet (Fig 6 6) But when the chains run in the same direction, it is termed parallel

(iii) Regions of β pleated structure are present in many proteins, and both parallel and antiparallel forms occur

(iv) The α-helix is stabilized by hydrogen bonding between peptide bonds 4 residues apart in a primary structural sense, stabilization of the β-pleated sheet results from formation of hydrogen bonds between peptides far removed from one another in a primary structural sense

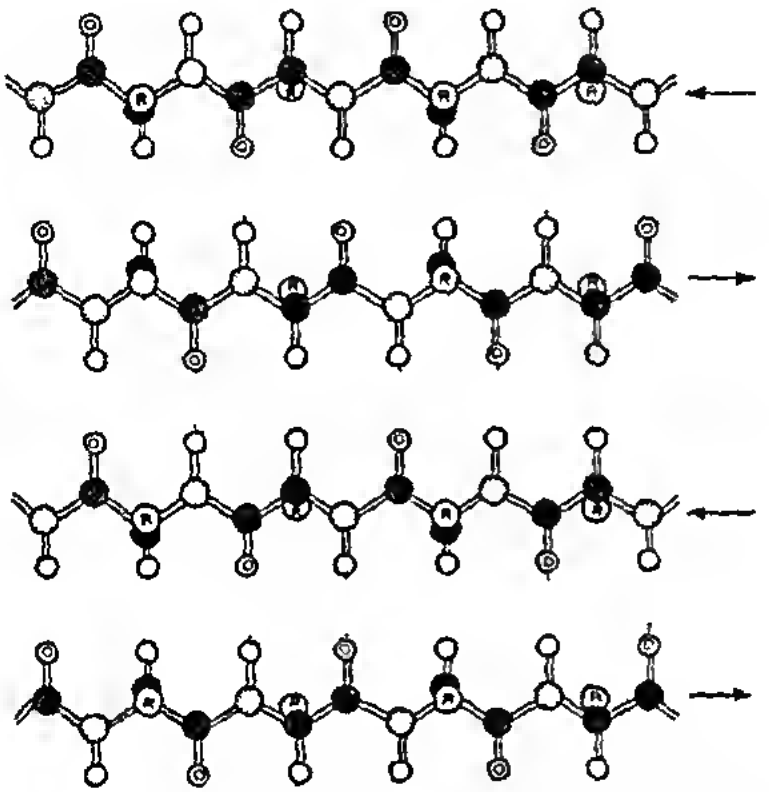


Fig 6 6 Antiparallel β pleated sheet Adjacent strands run in opposite directions. Hydrogen bonds between NH and CO group of adjacent strands stabilize the structure The side chains (R) are above and below the plane of the sheet ● =Carbon atoms ○ =Nitrogen atoms O=Hydrogen atoms

4 Quarternary structure .

In addition to primary, secondary and tertiary structures, many proteins exhibit a fourth level of organization wherein several monomeric units, each with appropriate primary, secondary and tertiary structures, may combine The polymerization of these monomers or protomers or subunits is described as the quarternary structure of the protein

Glutamic dehydrogenase molecule is a tetramer and aldolase molecule is a trimer Haemoglobin contains four subunits identical in pairs In the adult, the *molecule consists of two α and two β chains* , Foetal haemoglobin contains two α and two γ chains When haemoglobin takes up oxygen there is a change in

the structure due to the β chains moving closer together without structural alteration in the chains themselves

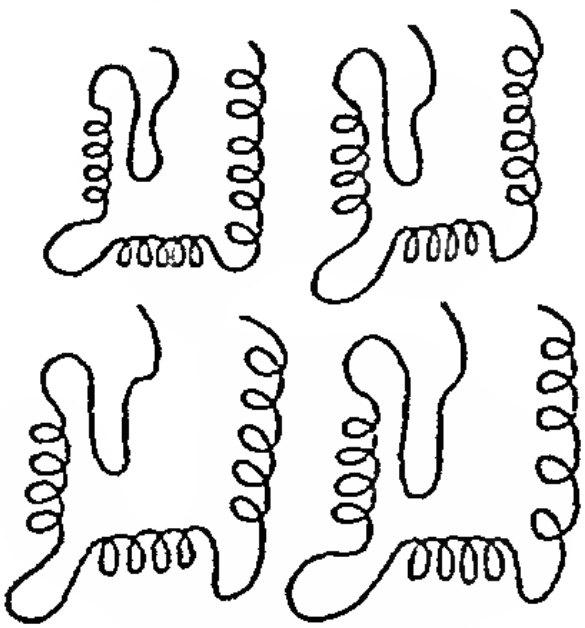


Fig. 6.7

DENATURATION OF PROTEINS

Denaturation may be defined as the disruption of the secondary, tertiary and quarternary structure of the native protein resulting in the alterations of the physical, chemical and biological characteristics of the protein by a variety of agents.

The native proteins are said to be the proteins occurring in animal and plant tissues They possess many characteristic properties such as solubility, viscosity, optical rotation, sedimentation rate, electrophoretic mobility etc.

For an oligomeric protein, denaturation may involve dissociation of the protomers with or without subsequent unfolding or with or without undergoing changes in protomer conformation.

Denaturing agents .

(i) *Physical agents—Heat, surface action, ultraviolet light, ultrasound, high* pressure etc.

(ii) Chemical agents—Acids, alkalis, heavy metal salts, urea, ethanol, guanidine, detergents etc.

Urea and guanidine probably interfere with the hydrogen bonds between peptide linkages Acids and alkalis probably attack directly the hydrogen bonds in the secondary and tertiary structure of proteins.

Physical alterations .

Many proteins, especially of the globular type, can be crystallized in the native state. But denatured proteins cannot be crystallized.

Chemical alterations -

The denatured protein is greatly decreased in solubility at its isoelectric point. The chemical groups are exposed to chemical reactions and more readily detected as a result of the unfolding process in denaturation. Among these are sulphydryl group of cysteine, the disulphide group of cystine and the phenolic group of tyrosine.

Biological alterations

The digestability of certain denatured proteins by proteolytic enzymes is increased Enzymatic or hormonal activity is usually destroyed by denaturation The antigenic or antibody functions of proteins are frequently altered

If the denaturation is *severe*, the protein molecules becomes insoluble and precipitation results as well as the changes in the properties of the proteins are permanent and ' irreversible' In case of *mild* denaturation, there is "reversible denaturation" leading to the slight changes in the properties of the protein which can be restored to the native state after suitable treatment

Significance

(i) The precipitation of the native protein as a result of denaturation is used to advantage in the clinical laboratory

(ii) Blood or serum samples to be analyzed for small molecules (e g glucose, uric acid, drugs) generally are first treated with acids such as trichloroacetic acid, phosphotungstic acid or phosphomolybdic acid to precipitate most of the proteins present in the sample This is removed by centrifugation and the protein free supernatant liquid is then analyzed

(iii) Denaturation is used to know the enzyme catalyzed reaction of an extract at the loss of the enzyme activity when boiled or acidified

Denaturation and Renaturation of Proteins

Bovine ribonuclease of single polypeptide chain of 124 amino acid residues with small molecular weight contains four disulphide bonds When it is treated with β mercaptoethanol in 8 M urea, the disulphide bonds are reduced to —SH groups as a result of the denaturation of the enzyme and the enzyme activity is also lost

Denatured ribonuclease when freed from urea and β mercaptoethanol by dialysis slowly regains enzymic acitivity as the SH groups are oxidized by oxygen of air to form S S bonds But if the reduced ribonuclease in 8 M urea solution is reoxidized it loses its enzymic activity almost completely as wrong disulphide bonds are formed resulting in 'scrambled' ribonuclease

Similarly, when egg albumin is heated till it is coagulated, the denaturation is irreversible and the secondary and tertiary structure of the proteins are completely lost resulting in a mixture of randomly arranged polypeptide chains

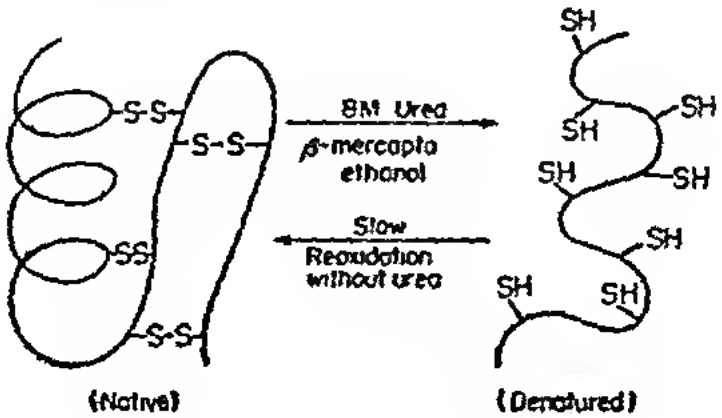


Fig 6.8

IMPORTANT TESTS OF PROTEINS :

A. Colour reactions :

(i) *Biuret test* : To 2 ml. of test solntioo add ao equal volume of 10 % NaOH and one drop of 10% $CuSO_4$ solutioo. A violet colour formation iodicates the presence of peptide liokage.

(ii) *Ninhydrin test* : To 1 ml. Ninhydrio solution add 1 ml. protein solution and heat. Formation of violet colour indicates the presence of α-amino acids.

B. Coagulation reactions :

Heat coagulation : Take the test solution upto $\frac{2}{3}$ of the test tube and heat the upper portion of the solutioo holding the lower part of the test tube. An opalesceot appears which hecomes deep on the additioo of a few drops of 2% acetic acid. This indicates the presence of proteio (alhumio).

C. Precipltation reactioo :

(i) Full saturatioo : Saturate 5 ml. of test solution in n test tube with solid ammonium sulphate. A gelatinous precipitate appears indicating the presence of albumin.

(ii) Half saturation : Add 3 ml. saturated solution of ammonium sulphate to 3 ml. of test solution in n test tube and shake vigorously. A' gelatinous precipitate forms indicating the presence of globulin.

D. Tests for colour reactions of amino acids : See chapter of amino aeids and peptides (Page 62).

ESTIMATION OF PROTEINS

Quantitative estimations of proteins of foods and other biological materials are performed by the following methods.

1. Kjeldahl method.
2. Colorimetric method.
3. Electrophoretic method.

1. Kjeldahl method :

(i) This method consists in digesting a known weight of the material with concentrated sulphuric acid and potassium sulphate with copper sulphate, mercuric oxide or seleoium dioxide as a catalyst :

(ii) The amino groups of the amino acids aod N present io the heterocylic rings of histidine, tryptophan, proline aod hydroxyproline are converted into ammonia as well as carhon is oxidized to carbon dioxide.

(iii) The ammonia preseot as sulphate is estimated after distillation hy titration with standard acid or colorimetrically using Nessler's reagent.

(iv) The oitrogeo cootent of the sample is calculated and converted into 'Crude Protein' content by multiplying by the factor 6.25.

Defects :

(i) The factor 6.25 is only approximate for the same proteins.

(ii) The nitrogen of urea, creatinine and other N compounds (which have no nutritive value) present in animal foods, fish and milk in small amounts also forms part of the total nitrogen i.e. crude protein.

2. Colorimetric method :

(i) This method is entirely based on the Biuret reaction or Folin's phenol reagent.

(ii) This method is generally used for the estimation of concentration of protein of serum.

3. Electrophoretic method :

(i) This method is used for the separation and estimation of protein in serum, tissues and foodstuffs.

(ii) In case of tissues and foods, the proteins are extracted with suitable solvents before separating them electrophoretically.

(iii) The electrophoretic separation can be performed using filter paper, agar gel and starch gel.

(iv) The proteins after separation are treated with suitable dye and the intensity of the colour of the individual dye-protein complex bands are measured using a densitometer.

ALBUMIN

Chemistry :

1. Albumins are simple proteins, soluble in water, coagulated by heat and precipitated by saturated salt solutions.

2. The glycine content of many albumins is low.

Sources :

They are frequently found in serum, muscle, milk and egg white. They are also widely distributed in plants especially in the seeds and fruits.

Characterstics :

1. Albumin is more readily affected by nutritional factors e.g. restricted protein intake.

2. Because of its small molecular size, it is lost from the circulating plasma more easily than the other proteins by passage through capillary walls of increased permeability as in inflammation and nephrosis (albuminuria).

3. In sudden haemodilution or severe malnutrition, in the majority of the disease states, the plasma proteins are altered and the albumin is decreased.

4. It has the property of osmotic pressure and fatty acid transport.

Clinical Importance :

1. The plasma albumin concentration falls somewhat in the later stages of normal pregnancy owing to haemodilution and decreased synthesis.

2. The major causes for abnormal decrease in albumin (hypoalbuminemia) are outlined below :

(i) Excessive loss in urine.

(ii) Inadequate protein supply (dietary protein restriction, vomiting, diarrhea etc.).

(iii) Impaired synthesis (liver dysfunction, chronic infection and severe anemia etc.).

(iv) Sudden plasma dilution (following sudden recovery from dehydration, infantile diarrhea etc)

3 In the prominent clinical manifestation of hypoalbuminemia edema occurs as a result of the decrease in plasma colloid osmotic pressure which favours retention of water in tissue spaces

4 The investigation of albumin is of much importance not only in the case of edema but also of the state of liver function and of protein nutrition

5 Pathological proteinuria may occur as a result of

(i) Increased glomerular permeability

(ii) Renal tubular damage with defective reabsorption of serum albumin (nephrosis)

(iii) Disease of the lower urinary tract.

(iv) Abnormal protein in the plasma.

GLYCOPROTEINS

1 The polypeptide chain is attached to one or more heterosaccharide units by covalent links

2. Some glycoproteins have many identical disaccharide units attached to the polypeptide chain

3 In addition to the more common sugars such as glucose, mannose, N-acetyl glucosamine, and N acetylgalactosamine, other units also occur e.g L-fucose (a methyl pentose) and sialic acids (acyl derivatives of neuraminic acid which is formed by the condensation of pyruvic acid and mannosamine)

4 The carbohydrate part is bound to the protein by a glycosidic link between N acetylglucosamine and the amide group of asparagine, this link is more stable than a peptide bond

5 The carbohydrate is in the form of disaccharide units 600-700 such units are attached to the peptide chain, one per 6 4 amino acid residues

6 They are secreted by the submaxillary glands of various animals

Functions

1 They form viscous solutions which function as lubricants and protective "screens" in the body

2 The continuously secreted mucus of the respiratory tract is a protection against invasion by bacteria and the uterus is protected from the vaginal microbial flora by the cervical mucus

3 Intestinal mucus makes a protection for the intestinal cells against mechanical damage

CHROMOPROTEINS

These are conjugated proteins composed of simple proteins united with a coloured prosthetic group Many biologically important proteins belong to this group They are

(i) Hemoglobins—These are the respiratory proteins in which the prosthetic group is the iron containing porphyrin complex heme

(ii) Cytochromes—These are cellular oxidation—reduction proteins in which the prosthetic group is heme

(iii) Flavoproteins—These are cellular oxidation—reduction proteins in which the prosthetic group is riboflavin

Chromoproteins of certain animal fibres such as black wool and hair in which the prosthetic group is melanin

Visual purple of the retina, a chromoprotein in which the prosthetic group is a carotenoid pigment

Catalase, a chromoprotein in which the prosthetic group is heme Peroxidase has a similar composition

SCLEROPROTEINS (ALBUMINOIDS)

(a) These proteins are similar to albumins and globulins

(b) They are characterised by great stability and insolubility in water and salt solutions

(c) They form the supporting structures of animals e g collagen in cartilage and white fibres of connective tissue and in bones and teeth, elastin in the yellow or elastic fibres, keratins in horn, hair, wool, real silk and feathers

(d) The keratins differ from the collagen like members in having high sulphur content, chiefly in the form of cystine

(e) They are readily attacked and dissolved by alkali sulphides

(f) The collagen of animal hides can be preserved in flexible condition giving the substance known as leather when treated with tannic acid, alum or various metal salts

(g) By long boiling with water collagen is partly hydrolysed giving a soluble product known as gelatin

Exercise

1 What are proteins ? Classify them and give important tests for it Describe briefly the biological importance of proteins (Bihar 60S)

2 What is the primary structure of proteins ? Discuss the biochemical functions of proteins (Patna 70A , Ranchi 66S, 70A)

3 Discuss in brief the classification and biological importance of proteins (Patna 68S)

4 What are proteins and their broad classification ? What are they composed of ? What are the special features of their structures ? Name some enzymes which can hydrolyse proteins specifically (Patna 69S , Lucknow 58A)

5 Describe the structure of proteins Discuss how proteins are precipitated (M U 76A)

6. Write short notes on —
   (a) Denaturation (Muz 75S)
   (b) Basic proteins (R U 70A)
   (c) Isoelectric point (P U 71A , 73A)
   (d) Glycoproteins (R. U 68A)
   (e) Chromoproteins (M U 75A)
   (f) Conjugated proteins (Mith 74A)
   (g) Zwitterion (G.U 1981) (P U 75A , M U 75A)
   (h) Biuret reaction and its use (G U 1981)

7 Write names and structures of two 'essential' amino acids (C.U 1981)

8 What is meant by primary structure of protein? Write an account on methods of isolation of protein (C.U 1982)

9 Discuss how structure of protein determines biological function (C.U 1983)

10 Write True or False (C.U 1983)

(a) Methionine is an aromatic amino acid——

(b) The nitrogen content of protein is usually about 6.25%——

(c) Biuret test is a test for urea —— (C.U 1981)

11 Fill up the gaps with correct words

(i) In special reactions of amino acids, Sakaguchi test is positive with amino acid —— and in reactions of protein, Newmann's test is positive with —— (C.U 1982)

(ii) On heating near 100°C a protein solution gets ——, the enzyme —— hydrolyses —— protein (C.U 1981)

CHAPTER 7

NUCLEOTIDES

Definition

These are the compounds constituted by purine or pyrimidine bases, rhose or deoxyribose sugars and phosphoric acid

Biological importance

1 The nucleotides are important intracellular molecules of low molecular weight

2 They play an important role in carbohydrate, fat and protein metaholism

3 The best role of purine and pyrimidine nucleotides is to serve as the monomeric precursors of RNA and DNA

4 The purine nucleotides also act as the high energy source ATP, cyclic AMP[cAMP] in a wide variety of tissues and organisms and as components of coenzymes FAD, NAD, NADP and of an important methyl donor, S adenosylmethionine.

5 The pyrimidine nucleotides also act as high energy intermediates such as UDP glucose and UDP galactose in carbohydrate metabolism and CDP-acylglycerol in lipid synthesis

Structure

The structures of purine and pyrimidine bases are given below The direction of the numbering of the purine ring is differ ent from that of the pyrimidine ring But the number 5 carbon is the same in both

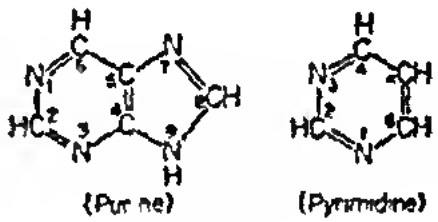


Fig 7 1

Pyrimidine bases

1 Cytosine (2 oxy-4 aminopyrimidine)
2 Thymine (2, 4-dioxy 5-methylpyrimidine)
3 Uracil (2 4-dioxypyrimidine)

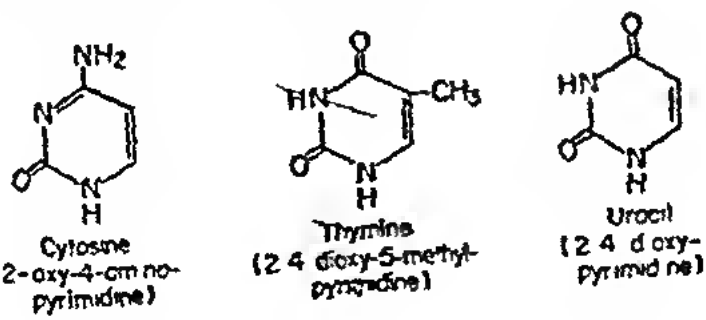


Fig. 7.2

Purine bases :

1. Adenine (6-aminnpurine)
2. Gnanine (2-amino-6-oxypurine)

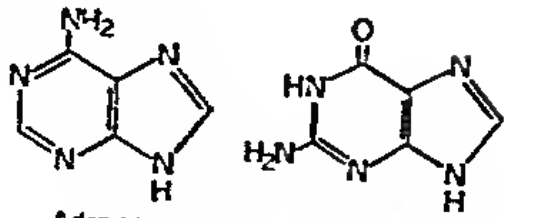
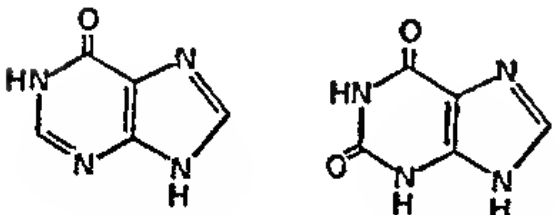


Fig. 7.3



These are the twn major purines found in living nrganisms Two other purine bases (*Hypoxanthine and Xanthine*) also occur as intermediates in the metabolism of adenine and guanine

Fig 7 4

Because of their resonant structures, they can exist in a lactim or lactam form The lactam form is the predominant tautomer of uracil nr thymine under physiologie conditinns

In plants, a series nf purine bases containing methyl substituents occurs. Many have pharmacolngie properties

Examples are

Cnffee which contains caffeine (1, 3, 7-trimethylxanthine)

Tea which contains theophylline (1, 3-dimethylxanthine)

Cocoa which contains theohromine (3, 7-dimethylxanthine)

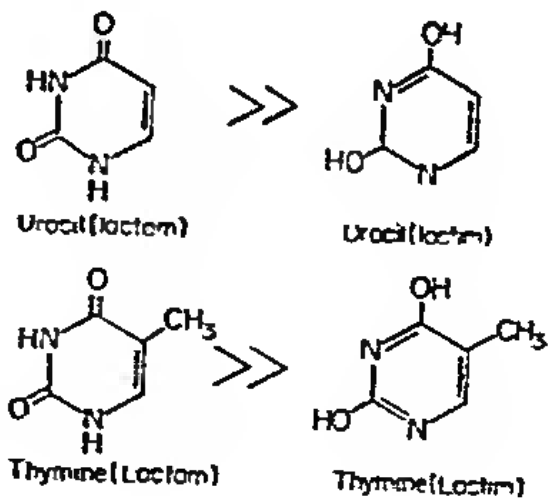


Fig. 7 5

In natural materials, many minnr bases occur Snme of these unusual substituted hases are found nnly in the nucleic acids of hacteria and viruses *5-methyl-cytosine and 5-hydroxymethylcytosine* are significant components nf hacteria and bacteriophage respectively

More recently, N^6 methyladenine, N^6-dimethyladenine and N^7-methylguanine have been found in the nucleic acids of mammalian cells.

NUCLEOSIDES AND NUCLEOTIDES

The free bases occur in nature are less in abundance thao are their oucleosides and nucleotides

A nucleoside is composed of a purine or a pyrimidine base aod a ribose or a deoxyribose sugar The composition of some nucleosides are given below :

Name of the Nucleosides	*Bases*	*Sugars*	*Attachment at the position*
1 Nocleosides containing purine bases			
Adenosine	Adenine	D-ribose	9
Guanosine	Guanine	D-ribose	9
Deoxyadenosine	Adenine	2-deoxyribose	9
Deoxyguanosine	Guanine	2-deoxyribose	9
Inosine	Hypoxanthine	D-ribose	9
2. Nocleosides containiog pyrimidine bases			
Cytidine	Cytosine	D-ribose	1
Deoxycytidine	Cytosine	2-deoxyribose	1
Uridine	Uracil	D-ribose	1
Deoxythymidine	Thymine	2-deoxyribose	1

Structures :

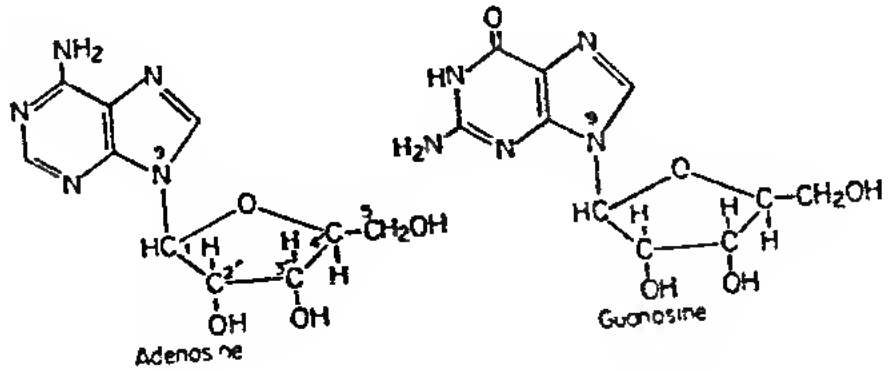


Fig. 7 6

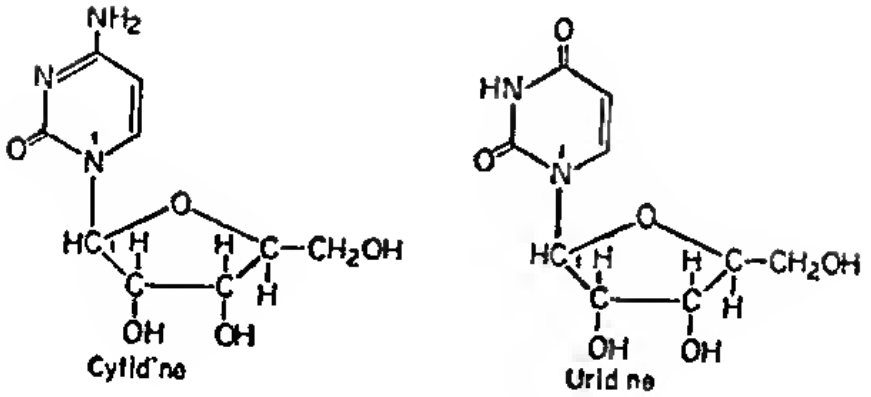


Fig 77

The *anti* form is necessary for the proper positioning of the complementary purine and pyrimidine bases in the double stranded form of deoxyribonucleic acid The structures of *syn* and *anti* configurations of adenosine are given below

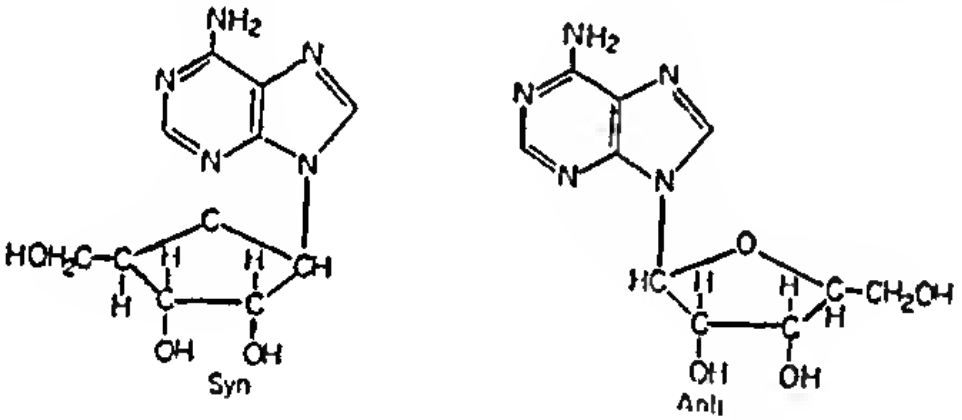


Fig 78

Nucleotides are the nucleosides phosphorylated on one or more of the hydroxyl groups of the sugar Thus,

Adenosine monophosphate (AMP or Adenylate)=Adenine+ribose+phosphate

2-deoxyadenosine monophosphate (dAMP or deoxyadenylate)=Adenine+2 deoxyribose+phosphate

Thymidylic acid (TMP)=Thymine+2-deoxyribose+phosphate

Uridylic acid (UMP)=Uracil+ribose+phosphate

Cytidylic acid (CMP)=Cytosine+ribose+phosphate

or

Cytidine monophosphate

Guanylic acid (GMP)=Guanine+ribose+phosphate

or

Guanosine monophosphate

Inosinic acid (IMP)=Inosine+ribose+phosphate

or

Inosine monophosphate

Deoxyribonucleic acid (DNA) = Adenine, Guanine, Cytosine, Thymine } + 2 deoxyribose + phosphate

Ribonucleic acid (RNA) = Adenine, Guanine, Cytosine, Uracil } + ribose + phosphate

Structure

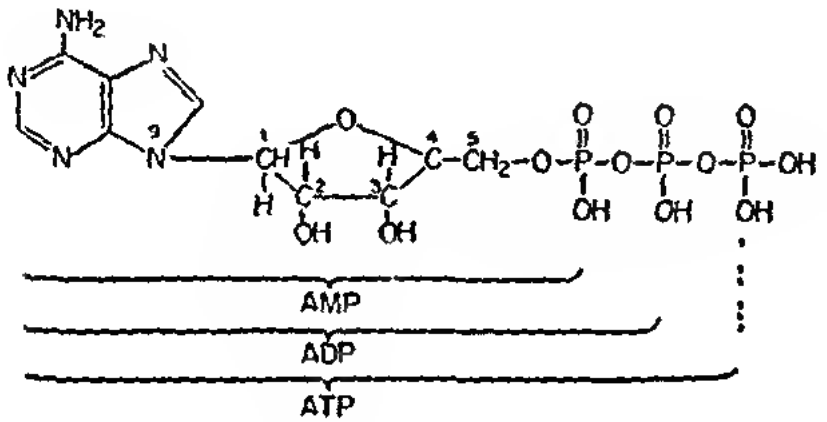


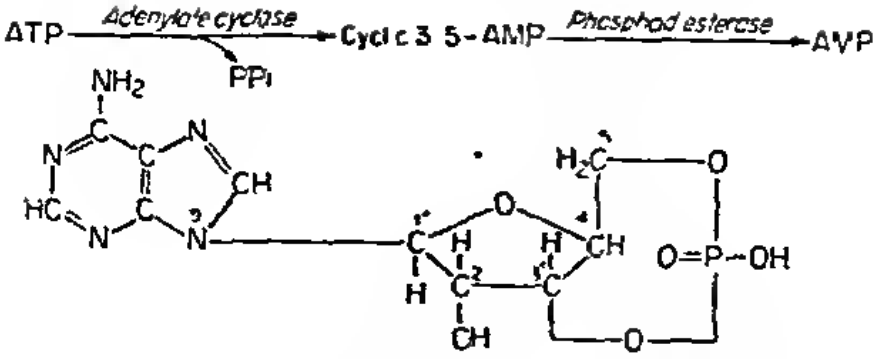
Fig 7 9

NATURALLY OCCURRING NUCLEOTIDES

Free nucleotides which are not a part of nucleic acids are also found in tissues Many have important roles Some are discussed below

1 ATP & ADP—(a) These are the important compounds as regards to their participation in oxidative phospborylation (b) ATP is the high-energy phosphate for energy requiring reactions in the cells (e) ATP is the most abundant intracellular free nucleotide (d) Its concentration in living mammalian cells is near 1 mM

2 Cyclic AMP (cAMP or 3 , 5 adenosine monophosphate)—
(a) This is present in most animal cells
(b) It is formed from ATP by the enzyme adenylate cyclase the activity of which is regulated practically by hormone receptors
(c) It mediates a series of reactions
(d) It is destroyed in tissues by its conversion to AMP by the enzyme cAMP phosphodiesterase
(e) The intracellular cAMP concentrations are near 1 μM

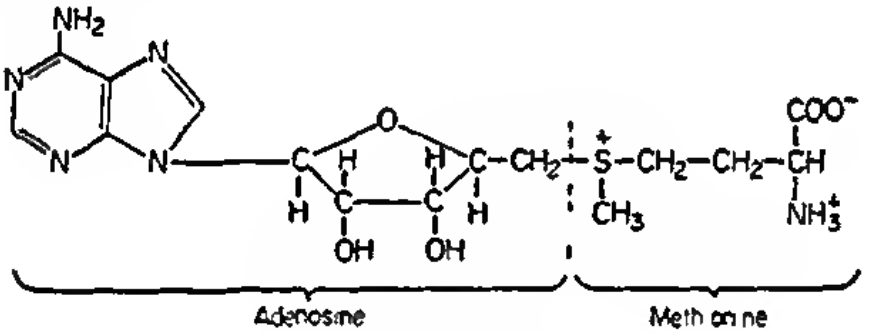


Structure of CAMP(3'5' adenosine monophosphate)

Fig 7 10

3 S adenosylmethionine—

(a) It serves as a form of "active" methionine
(b) It serves as a methyl donor in many methylation reactions
(c) It is a source of propylamine for the synthesis of polyamines

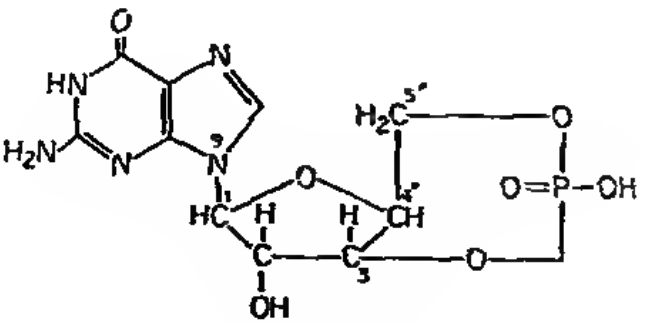


(Structure of S adenosylmethionine)

Fig 7 11

4 Cyclic GMP (cGMP or 3 , 5 -guanosine monophosphate)—

(a) It is formed from GTP by the enzyme guanylate cyclase
(b) It is catabolized by phosphodiesterase to produce 5 -monophosphate
(c) It is an important intracellular signal of extracellular events
(d) It acts antagonistically to cAMP



(Structure of Cyclic 3'5' guanosine monophosphate)

Fig 7 12

5 Inosine Monophosphate (IMP)—

(a) IMP can be formed by the deamination of AMP which takes place particularly in the muscle This IMP when reconverted to AMP results in the net production of ammonia from aspartate

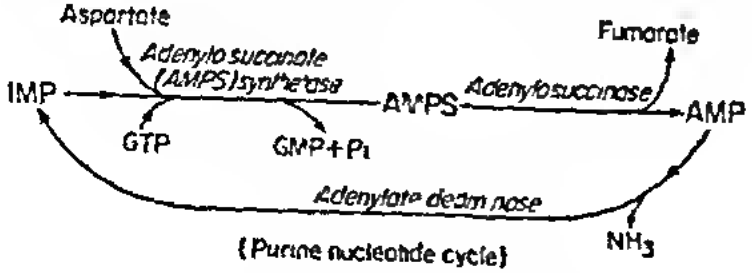


Fig 7 13

(b) Inosine triphosphate (ITP) and Inosine diphosphate (IDP) play an important role in the formation of oxaloacetate from pyruvate and also in energy trapping reactions during oxidation of α-Ketoglutarate

6 Uridine nucleotide derivatives (UDP & UTP)—

(a) These are the important coenzymes required in the metabolism of galactose to form lactose in the mammary gland and the polymerization of glucose to form glycogen In these reactions the substrates are UDP Glucose and UDP Galactose

(b) Another coenzyme uridine diphosphoglucuronic acid (UDP Glc UA) serves as the "active" glucuronide for conjugation reactions such as the formation of bilirubin glucuronide in the liver

(c) They are involved in the epimerization of galactose to glucose and vice versa

(d) They also participate in the formation of high energy phosphate compounds

(e) UTP is the precursor for the polymerization of uridine nucleotides into RNA

7 Cytosine derivatives (CMP, CDP & CTP)—

(a) These derivatives are the high energy phosphate compounds

(b) CTP acts as the precursor for the polymerization of CMP into nucleic acids

(c) CTP is required for the biosynthesis of phosphoglycerides in animal tissue

(d) Ceramide and CDP-choline are responsible for the formation of sphingomyelin and other substituted sphingosines

8 Vitamin nucleotides—Described in vitamin chapter

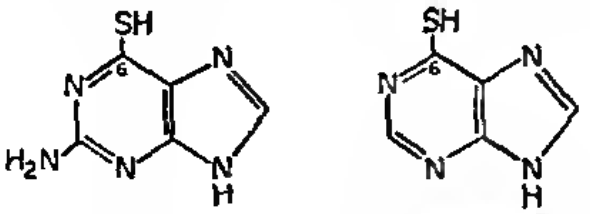
SYNTHETIC DERIVATIVES

Synthetic nucleobases, nucleosides and nucleotides are widely used in the medical sciences and clinical medicine

The pharmacologic view is that either the heterocyclic ring structure or the sugar moiety is changed in such a way as to induce toxic effects when they

are incorporated into various cellular constituents resulting in the inhibition of enzyme activities

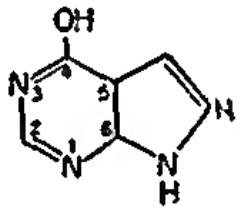
6-thioguanine and 6-mercaptopurine, in which the hydroxyl groups are replaced with thiol groups at the 6 position are widely used clinically



6 Thioguanine 6 Mercaptopurine

Fig 7 14

4 hydroxypyrazole pyrimidine (allopurinol) is marketed as an inhibitor of xanthine oxidase Hence, it is used for the treatment of hyperuricemia and gout



Allopurinol
(Lactim)

Fig 7 15

Cytarabine (arabinosyl cytosine) and vidarabine (arabinosyl adenine) are used in the chemotherapy of cancer and viral infections

Azathioprine is useful in organ transplantation

More recently, both aminophylline and theophylline are used clinically to inhibit the catabolism of intracellular cAMP

Exercise

1 Give a brief account of the pyrimidine nucleotides (R U 72A)

CHAPTER 8

NUCLEIC ACIDS AND CHROMATIN

Nucleic acids are the polynucleotides There are two types of nucleic acids—DNA and RNA DNA is the chemical basis of heredity Avery, Macleod and McCarty in 1944 first demonstrated in a series of experiments that DNA contained the genetic information These authors referred DNA as "transforming factor"

Chemistry of DNA

1 The four types of monomeric units of DNA are adenylate, guanylate, cytidylate and thymidylate

2 The monomeric units form a single strand of DNA which are held in polymeric form by 3 , 5 -phosphodiester bridges

3 The informational content of DNA is available in the sequence in which these monomers are ordered

4 The polymer possesses a polarity , one end has a 5 hydroxyl or phosphate terminus while the other has a 3 -phosphate or hydroxyl moiety

5 Since the genetic information is available in the monomeric units within the polymers, there is existance of a mechanism of reproducing or replicating this specific information with a high degree of fidelity

6 In DNA molecules the concentration of Adenosine (A) nucleotides equals that of thymidine (T) nucleotides (A=T) The concentration of guanosine (G) nucleotides equals that of cytidine (C) nucleotides (G=C) This accelerated Watson, Crick and Wilkins in 1950 to propose n model of double stranded DNA molecule

7 The 2 strands of this double-stranded molecule are held together by hydrogen bonds between the purine and pyrimidine bases The pairings between the purine and pyrimidine nucleotides on the opposite strands are dependent upon hydrogen bonding of A with T and G with C

8 The 2 strands of the double helical molecule are anti parallel i e one strand runs in the 5 to 3 direction and the other in the 3 to 5 direction Since the information resides in the sequence of nucleotides on one strand, the opposite strand is considered as "antisense" i e the complement of the sense" strand

9 3 hydrogen bonds hold the guanosine nucleotide to the cytosine nucleotide and A—T pair is held together by 2 hydrogen bonds Therefore, they are represented as G≡C and A=T G—C bond is stronger by 50%

10 The B form has n pitch of 3 4 nm per turn and within the single turn 10 base pairs exist

11 The double stranded structure in solution can be melted by increasing temperature or decreasing salt concentration

12 The double-stranded DNA molecule shows the properties of a fibre nnd it is a viscous material in solution and loses its viscosity upon denaturation

13 In the major and minor grooves winding along the molecule parallel to the phosphodiester backbones specific proteins interact with DNA molecules

14 In some organisms such as bacteria, bacteriophage and many DNA-containing animal viruses the two ends of the DNA molecules are joined to create n closed circle with no terminus This does not destroy the polarity of the two molecules but it eliminates all 3 and 5 free hydroxyl and phosphoryl groups /

15 It contains 1600 to 9000 nucleotides The molecules are long and its length is 250 times greater than its breadth. Its structure is highly complex

16 Heat, acid and alkali denature DNA

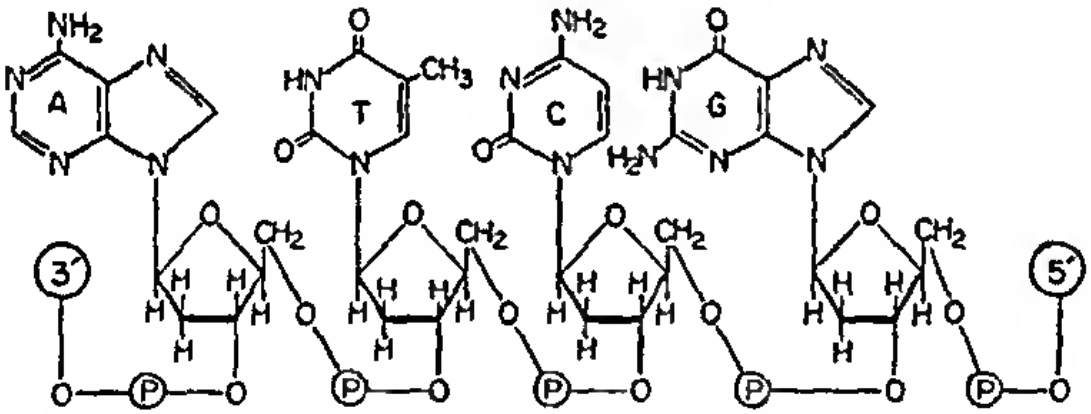


Fig 8 1 Segment of a structure of DNA molecule in which purine and pyrimidine bases Adenine (A) Thymine (T) Cytosine (C) and Guanine (G) are held together having 3 at one end and 5 at the other

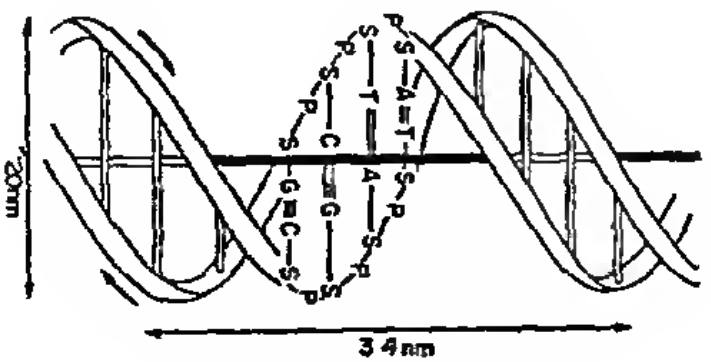


Fig 8 2 Double helical structure of DNA

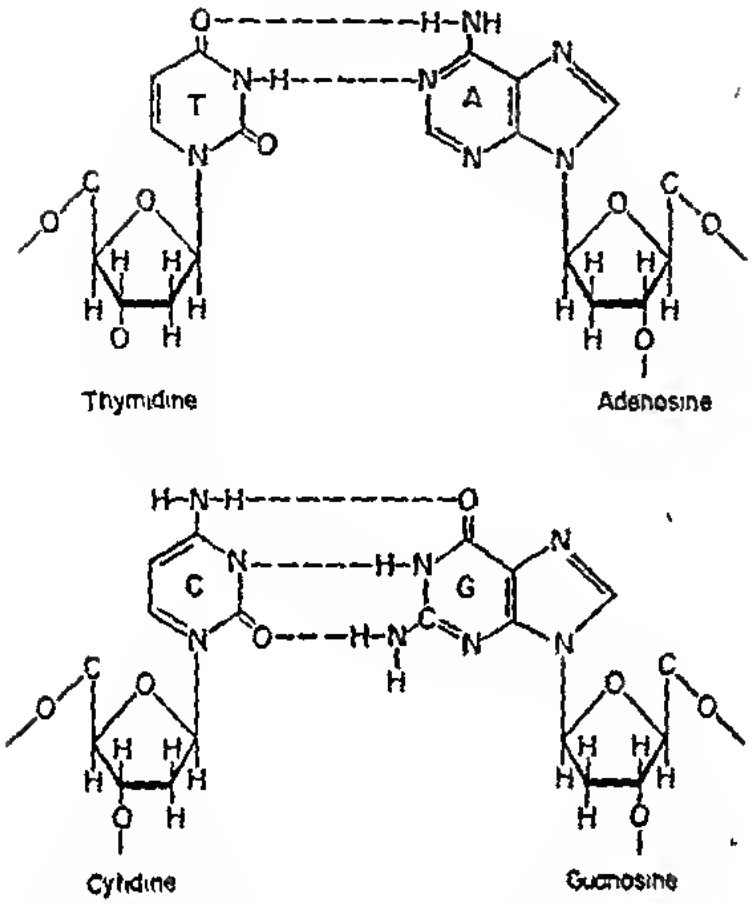


Fig. 8 3 Base pairing between adenosine and thymidine, cytidine and guanosine.

Chromatin :

1. Chromatin is the chromosomal material extracted from nuclei of cells.

2. It consists of a long double-stranded DNA molecules and a nearly equal mass of histones as well as smaller amount of nonhistone proteins and a small quantity of RNA

3. It contains a 10 nm repeating unit. The repeating units occur every 200 base pairs.

Chemistry of RNA :

1. RNA is a polymer of purine and pyrimidine ribonucleotides linked together by 3', 5'-phosphodiester bridges.

2. The sugar moiety in RNA is ribose.

3 It contains uracil instead of thymine in addition to adenine, guanine and cytosine.

4. It exists as a single-stranded molecule rather than as a double-stranded helical molecule.

5. Since it is a single-stranded molecule, its guanine content does not necessarily equal its cytosine content and its adenine content does not necessarily equal its uracil content.

6 It can be hydrolyzed by alkali to 2′, 3′ cyclic diesters of the mononucleotides An intermediate in this hydrolysis is the 2′, 3′, 5′-triester which can not be formed in DNA hydrolysis by alkali because of the absence of a 2′-hydroxyl group The alkali lability of RNA is useful diagnostically and analytically

7 RNA molecule does not hybridize with the "antisense" strand of the DNA of its gene Therefore, the sequence of RNA molecule (except U being replaced by T) is the same as that of the "antisense" strand of the gene Small amounts of double-stranded RNA have been detected from mammalian organisms including humans which may probably be associated with RNA viruses.

8 It contains 60 to 6000 nucleotides. The molecule is unbranched

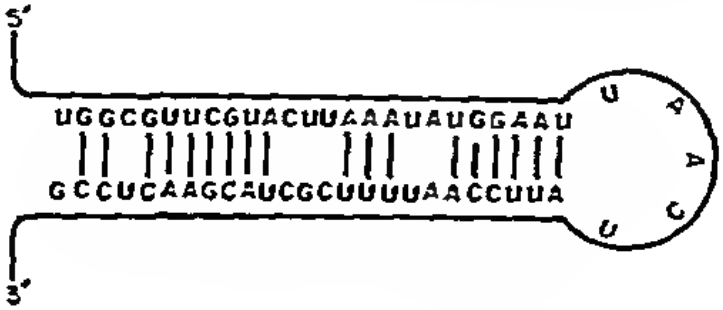


Fig 8 4 Hairpin structure of RNA

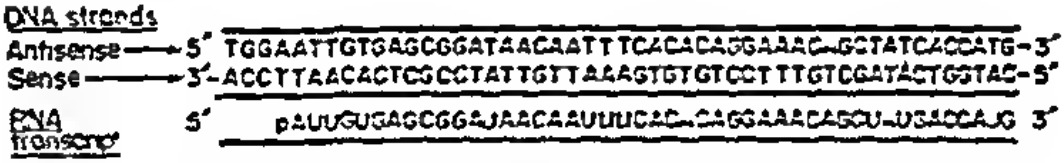


Fig 8.5 Relationship between the sequences of an RNA transcript and its gene in which the sense and antisense strands are shown with their polarities

Structural organization of RNA :

There are 3 main classes of RNA molecules exist in all prokaryotic and eukaryotic organisms These are

1 messenger RNA (mRNA)
2. transfer RNA (tRNA) or soluble RNA
3 ribosomal RNA (rRNA)

1 The messenger RNA (mRNA) ·

(i) The messenger RNA is single stranded and complementary to the sense strand of DNA.

(ii) The 5′ terminus of messenger RNA is 'capped" by a 7-methylguanosine triphosphate which is linked to an adjacent 2-0-methyl ribonucleoside at its 5′-hydroxyl throogb the 3 phosphates The protein-synthesizing machinery begins translating the mRNA into proteins at the 5′ or capped terminus The 3-hydroxyl terminus has attached a polymer of adenylate residues 20-250 nucleotides in length.

(iii) It is the most heterogeneous in size and stability

(iv) It passes from nucleus to cytoplasm conveying information in a gene to the protein synthesizing machinery where each serves as a template on which a specific sequence of amino acids is polymerized to form a specific protein molecule, the ultimate gene product

(v) It has a large molecular weight of 30,000 to 50,000 to have the coded information corresponding to long polypeptide chains

(vi) In mammalian nuclei, the immediate products of gene transcription are another class of RNA molecule which are quite large and heterogeneous in size These heterogeneous nuclear RNA (HnRNA) may exceed 10^7 daltons whereas the mRNA molecules are generally smaller than 2×10^6 daltons These HnRNA molecules are processed to generate the mRNA molecules which then enter the cytoplasm to serve as templates for protein synthesis

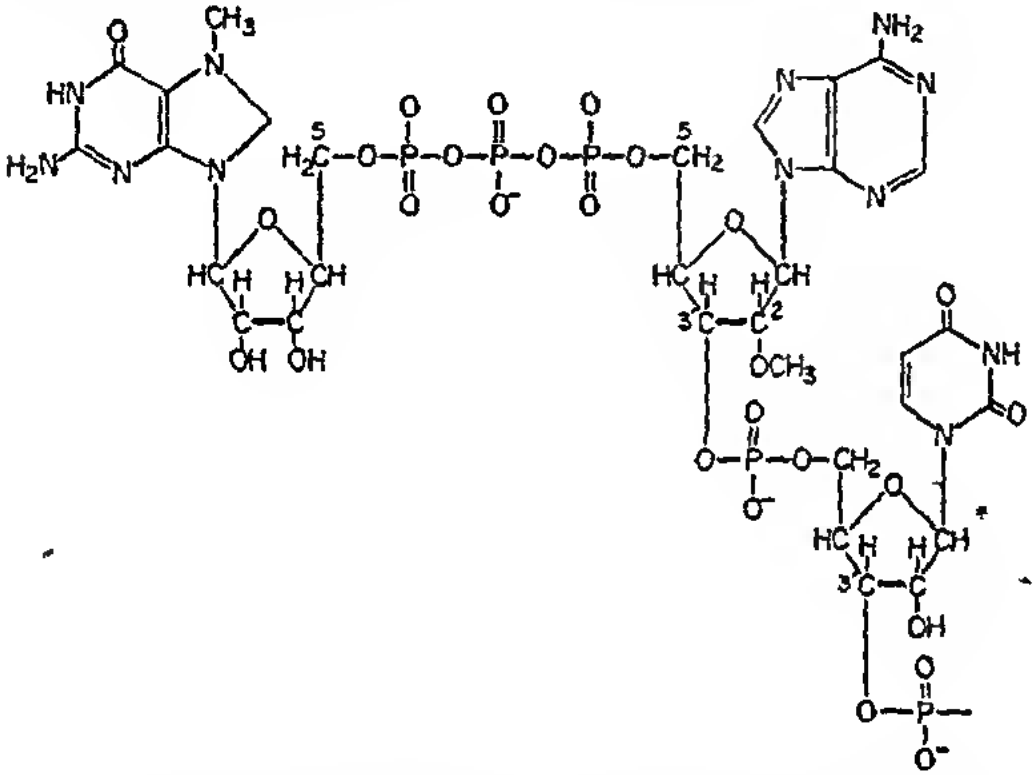


Fig 8 6 The expression of genetic information in DNA into the form of an mRNA transcript This is subsequently translated by ribosomes into specific protein molecule

2 The transfer RNA (tRNA) or soluble RNA (sRNA)

(i) The transfer RNA molecules amount to 10 to 20 Pc of the total cellular RNA molecules

(ii) They consist of about 75 nucleotides and have molecular weight of 25,000

(iii) There are at least 20 tRNA molecules in every cell, one corresponds to each of the 20 amino acids required for protein synthesis

(iv) They serve as adaptors for the translation of the information in the sequence of nucleotides of the mRNA into specific amino acids

(v) The primary structure allows extensive folding and intrastrand com-

plementarily to generate a significant secondary structure which appears like a cloverleaf as in the figure below

(vi) All tRNA molecules have a common CCA sequence at the 3′ termini The carboxyl groups of amino acids are attached to the 3′-hydroxyl group of the adenosyl moiety through an ester bond

(vii) The *anticodon loop* at the end of a base-paired stem recognizes the triplet nucleotide or codon of the template mRNA.

(viii) In nearly all tRNA molecules, there is a loop containing the nucleotides of ribothymine and pseudouridine and another loop containing the minor base dihydrouracil

(ix) They are quite stable in prokaryotes and less stable in eukaryotes.

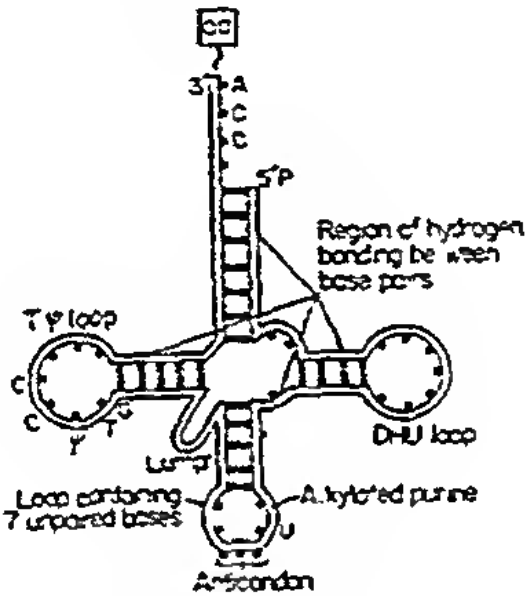


Fig 8.7 Structure of tRNA.

3 The Ribosomal RNA (rRNA)

(i) Ribosomes are nucleoprotein particles and reticular granules of 100-150Å, in diameter which act as the machinery for the synthesis of proteins from mRNA templates They contain 80 Pc. of the RNA within the cell

(ii) On the ribosomes, the mRNA and tRNA molecules interact to translate into a specific protein molecule the information transcribed from the gene.

(iii) Ribosomal particles are very complex The mammalian ribosome contains 2 major nucleoprotein subunits—a larger one (60 S) and a smaller one (40 S)

(iv) The 60 S subunits contains a 5 S ribosomal RNA (rRNA), a 5 8 S rRNA and a 28 S rRNA

(v) There are also more than 50 specific polypeptides The smaller (40 S) subunit contains a single 18 S rRNA and approximately 30 polypeptide chains.

(vi) The 5 S rRNA has its own precursor which is independently transcribed

(vii) In the cytoplasm the ribosomes remain quite stable and capable of many translations

DNA AND GENE

1 The most important constituent of chromosomes is DNA The double stranded helix of DNA is tightly coiled in each chromosomes thread

2 The unit of genetic information is the *gene or cistron*

3 Gene is a segment of the DNA molecule containing about 600 base pairs The genetic message is carried in the sequence of bases along the DNA strand

4 In the process of transcription, the genetic message is transferred to the messenger RNA which carries it to the ribosomes

5 The genes are arranged in orderly manner along the length of the DNA molecule in the chromosome

6 Each gene for any particular characteristic has its counterpart in the corresponding locus on the homologous chromosome and these two genes form an allelic pair When both loci of a pair carry genes with the same characteristics, say tallness, then the individual is said to be *homozygous* with respect to that character When one of the pair tallness and the other gene shortness, the individual is heterozygous

Comparison between DNA and RNA

	DNA		*RNA*
1	They are formed by 4 types of monomeric units such as adenylate, guanylate, cytidylate and thymidylate	1	They are also formed by 4 types of monomeric units such as adenylate, guanylate, cytidylate uracidylate
2	The sugar moiety is deoxyribose	2	The sugar moiety is D ribose
3	They are present in nucleus, probably in the chromosomes	3	They are present in cytoplasm and nucleolus
4	They carry "genetic information" from one generation of cells to the next and undergo mutation	4	They carry no "genetic information" and undergo no mutation
5	They contain 1600 to 9000 nucleotides The molecules are long and thread like having a length of about 250 times greater than their breadth Their structure is highly complex	5	They contain 60 to 6000 nucleotides The molecules are unbranched Their structure is less rigid
6	They are concerned with protein syothesis	6	They are also concerned with protein synthesis
7	They have 2 strands of antiparallel double helical structure	7	They exist as a single stranded molecule
8	Heat, acid and alkali denature DNA molecules	8	The alkali hydrolysis is useful diagnostically and analytically
9	They have 'sense' and 'antisense' strands	9	The sequence of RNA molecule is the same as that of the 'antisense' strand of DNA

Biological importance of nucleic acids

1 Nucleic acids are able to reproduce their kind or to store or express and transmit genetic information

2 They undergo mutation

3 In cell division the nucleic acid chain is duplicated preserving in each daughter cell the information contained in the parent cell So the double helix unravels and each of the two original strands then serves as a template for the synthesis of another complementary chain

4 According to the "pairing rule" in DNA, adenine can only combine with thymine and guanine only with cytosine The newly synthesized strand will be exactly constituted in its nucleotide sequence as was the original complementary strand of the parent strand The result is the synthesis of two pairs of strands.

5 DNA produces a messenger RNA (mRNA) which helps in placing amino acids in the code for protein synthesis

6 RNA functions primarily in the cytoplasm of the cell as a template in connection with the synthesis of proteins as well as in the ribosomes The formation of RNA template is directed by nuclear DNA

7 Ribosomal RNA (rRNA) and transfer RNA (tRNA) are also involved in protein synthesis

8 RNA can be synthesized by RNA polymerase which is dependent on the presence of DNA acting as a template

9 Adenylic acid in combination with two molecules of phosphate is the biochemical unit of energy exchange in all cells which is said to be ATP

10 Biological oxidation—reduction involves the transport of hydrogen atoms or electrons through organized systems of substances called hydrogen acceptors or electron transport agents The hydrogen acceptors are nucleotides such as NAD, FAD etc

NUCLEOPROTEINS

Nucleoproteins are conjugated proteins containing nucleic acids and a basic protein like protamines or histones

In nucleoproteins there are salt like combination between positively charged basic proteins and negatively charged nucleic acids. Cytoplasm also contains ribonucleoprotein which is found particularly in ribosomes All living cells contain nucleoproteins and some of the simplest system such as viruses consist entirely of nucleoproteins

Protamines—

1 These basic proteins have low molecular weight and these are found in association with DNA chiefly in fish sperms

2 They produce large quantities of arginine and less amounts of neutral amino acids on hydrolysis

3 Their isoelectric P^H is around 12

4 The polypeptide chains of protamines are wrapped around DNA molecules in such a way that the positively charged arginine form salt linkages with the phosphates of the nucleic acids

5 The best examples are salmin (salmon sperm) and clupeine (herring sperm)

Histones

1 They are more complex proteins than protamines
2 They have larger molecular weights and exist in combination with DNA
3 They produce a variety of amino acids on hydrolysis
4 Nucleohistones mainly occur in animal sperms birds, erythrocytes

Deoxyribonucleoproteins

1 They are viscous material which are insoluble in 0 15 M NaCl but soluble in 1 M NaCl solution
2 They have high molecular weights of 10 million to several hundred millioos
3 They are highly elongated and thread like in shape

Ribonucleoproteins

1 They are present in cytoplasm of the cells and ribosomes
2 They are composed of proteins and ribonucleic acids
3 Ribosomes of E coli consists entirely of ribonucleoproteins
4 Ribosomes from mammalian and plant sources contain about 40 50 per cent RNA, the remainder being proteins

Exercise

1 Write in brief the chemistry of Nucleic Acids (R U 70A M U 74S , P U 68S , Mith 62A, 67A)

2 What are Nucleic Acids ? Describe the main points of difference between DNA and RNA Mention the biological importance of nucleic acids (Mith 60A , M U 72A)

3 Describe what you know of DNA and its biological role (Bh U 75S , R U 70S Mith 63S)

4 Describe the chemistry of Hereditary Unit (P U 74A , Bh U 76S)

5. Write short notes on

(i) Nucleic acid (M U 75A)
(ii) DNA (M U 72A 74A , P U 68A R U 70A 72A Mith 74A)
(iii) RNA (R U 68S 76A , Mith 74A)
(iv) Transfer RNA (P U 76A , Bh U 76S)
(v) Codon (M U 76A)
(vi) Messenger RNA (Maz 74A Bh U 74S)
(vii) Adenine (C.U 1981)
(viii) Adenylic acid (C U 1982)
(ix) DNA as a carrier of heredity (C.U 1982)

6 Give the structural formula of nucleotide (C U 1981)

7 Write True or False

(i) The process of protein synthesis on m RNA linked ribosome is called transcription— (C U 1981)
(ii) Uracil on methylation forms thymine (C U 1983)

8 Write structure of ATP and its importance (C.U 1981)

CHAPTER 9

HEMOGLOBIN, PORPHYRINS AND BILE PIGMENTS

Hemoglobin is the red colouring matter of blood which is present in the red blood cells It is a conjugated protein consisting of *heme* and the protein *globin* It has a molecular weight of 64, 450 It can combine with oxygen and acts as the transport mechanism for oxygen within the blood It contains 4 gram atoms of iron per mole in the ferrous (Fe^{++}) state

Heme—It is the iron porphyrins The porphyrins are cyclic compounds formed by the linkages of 4 pyrrole rings through methylene bridges Iron in the ferrous state is bound to the nitrogen atom of the pyrrole rings

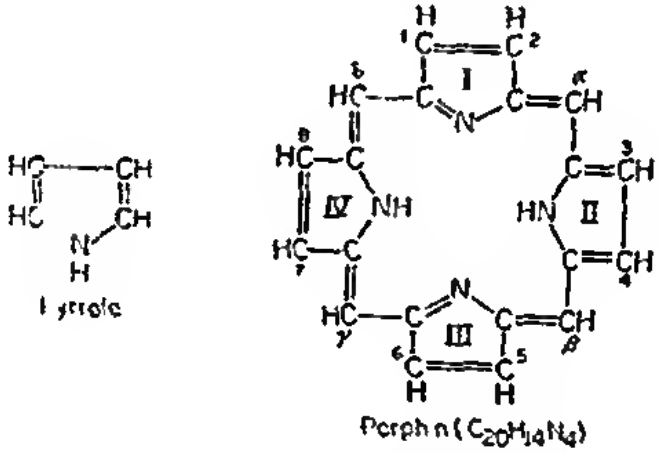


Fig 9 1

The porphyrins are found in nature in which the various side chains are substituted for the 8 hydrogen atoms as numbered in the porphin nucleus The arrangement of the A and P substituents in the uroporphyrin shown here is asymmetric (in ring IV the expected order of the acetate and propionate substituents is reversed) This type of asymmetric substitution is classified as a type III porphyrin A porphyrin with a completely symmetrical arrangement of the substituents is classified as a type I porphyrin Only types I and III are found in nature and the type III series is more abundant.

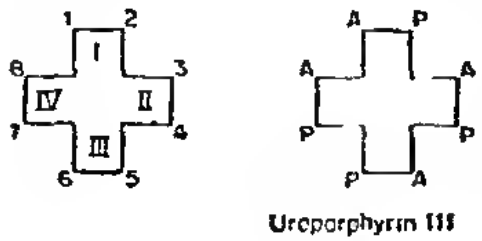


Fig 9 2

Biosynthesis of Porphyrins

Chlorophyll (magnesium containing porphyrin), the photosynthetic pigment of plants and *heme* (the iron containing porphyrin) of hemoglobin in animals are synthesized in living cells by a common pathway.

(i) The starting materials are 'active succinate' (succinyl CoA) derived from the citric acid cycle and glycine. Pyridoxal phosphate (B_6 Po_4) is necessary to activate glycine The product of the condensation reaction is α amino-β ketoadipic acid which is catalyzed by the enzyme AmLev synthetase.

(ii) α amino β ketoadipic acid is rapidly decarboxylated by the same enzyme AmLev synthetase producing δ aminolevulinic acid (AmLev) Synthesis of aminolevulinic acid occurs in the mitochondria The anemia has been observed in the deficiency of vitamin B_6 or pantothenic acid

(iii) 2 mols of AmLev condense to form porphobilinogen (the first precursor of pyrrole) which is catalyzed by the enzyme δ aminolevulinase (AmLev dehydrase)

(iv) 3 mols of porphobilinogen condense first to form a tripyrrylmethane which then breaks down into a dipyrrylmethane and a monopyrrole The dipyrryl compounds are of two types A and B The formation of tetrapyrrole occurs by condensation of two dipyrrylmethanes If two of the (A) components condense, a type I porphyrin results , if one (A) and one (B) condense a type III results

(v) The uroporphyrinogens I and III are converted to coproporphyrinogens I and III by decarboxylation being catalyzed by uroporphyrinogen decarboxylase

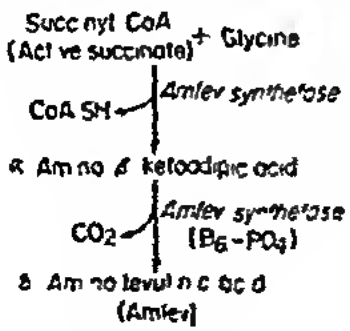
(vi) The coproporphyrinogen III then enters the mitochondria where it is converted to protoporphyrinogen III and then to protoporphyrin III The enzyme coproporphyrinogen oxidase catalyzes the formation of protoporphyrinogen III The oxidation of protoporphyrinogen to protoporphyrin is catalyzed by the enzyme protoporphyrinogen oxidase The enzyme coproporphyrinogen oxidase is able to act on type III coproporphyrinogen only for which type I protoporphyrin has not been identified in natural materials In mammalian liver the reaction of conversion of coproporphyrinogen to protoporphyrin requires molecular oxygen

(vii) In the final step of heme synthesis ferrous ion (Fe^{++}) is incorporated into protoporphyrin III which is catalyzed by heme synthetase or ferrochelatase The reaction takes place readily in the absence of enzymes but becomes rapid in presence of enzymes

A summary of the steps is given below

Note 1 The porphyrinogens are the reduced porphyrins containing 6 extra hydrogen atoms The oxidized porphyrins cannot be used for heme or chlorophyll synthesis

2 The porphyrinogens are readily auto-oxidized to the respective porphyrins in presence of light



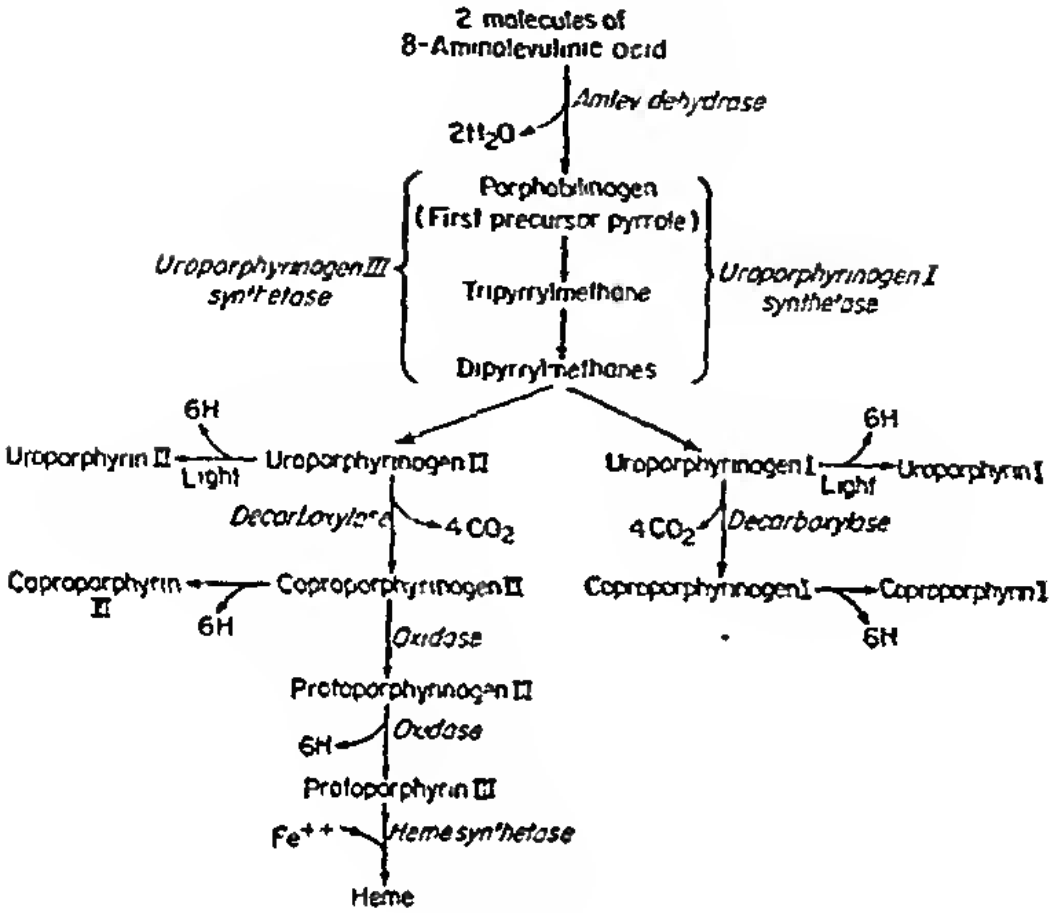


Fig 93

Chemical nature of globin :

(i) The globin of the hemoglobin is a protein which is composed of 4 parallel layers of closely packed polypeptide chains

(ii) Two of the chains (α-chains) have identical amino acid composition of 141 amino acids The two other chains may be two of the 4 polypeptide chains designated as β, γ, δ and ε (epsilon) Each is having 146 amino acids.

(iii) The total number of amino acids in globin is 574.

(iv) α-chains have Val·Leu-Ser in N-terminal residues and Lys-tyr-Aug in C-terminal residues

β- „ „ Val·His-Leu „ „ „ and Lys-tyr-His in C-terminal residues.

γ- „ „ Gly-His-Phe „ „ „ and Arg-Ty-His. in C-terminal residues

Properties of hemoglobin

1 *Oxyhemoglobin*—It forms oxyhemoglobin in combination with oxygen When hemoglobin is exposed to air, it takes up two atoms of oxygen for each atom of ferrous ion (Fe^{++}) present. Thus, hemoglobin will take up 4 molecules of oxygen In low oxygen tension, oxyhemoglobin gives up O_2 readily. By this way, blood carries O_2 to different parts of the body.

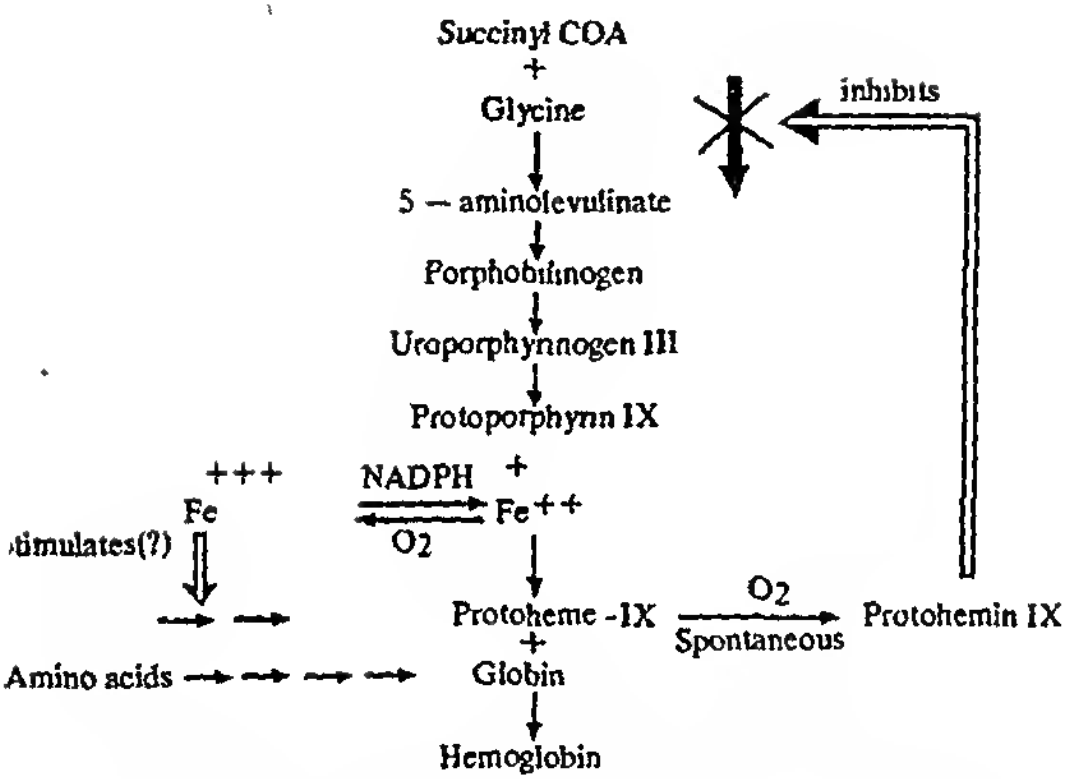


Fig 9 4 : Regulation of hemoglobin synthesis according to Current Concepts

2 *Formation of carbamino compound*—It reacts with CO_2 forming carbamino compounds

$$Hb-NH_2+CO_2 \longrightarrow Hb-NH\,COOH$$

3 *Reaction with carbon monoxide*—It forms carboxy hemoglobin after reacting with carbon monoxide (CO) Carboxy hemoglobin is stable and prevents the formation of oxyhemoglobin So inhalation of even small amounts of carbon monoxide is highly dangerous

4 *Buffering action*—One mol of hemoglobin contains 35 histidine residues Histidine exerts its buffering action through its basic imidazole ring Hence, hemoglobin plays an important role in regulating the acid—base balance of blood

5 *Formation of methemoglobin*—Methemoglobin is formed as a result of the oxidation of hemoglobin by the mild oxidizing agent, potassium ferricyanide The ferrous ion (Fe^{++}) is oxidized to the ferric ion (Fe^{+++}) Methemoglobin cannot carry oxygen in blood It is also formed by the action of some drugs This is found in the blood of some individuals owing to inborn errors of metabolism This can be reduced to hemoglobin by vitamin C which is used in the treatment of methemoglobinemia

6 *Sulphemoglobin* —It is formed by the administration of certain drugs It continues to remain in the blood and cannot be reconverted into hemoglobin

7 *Cyanomethemoglobin*—It is formed by the addition of cyanide to methemoglobin It has a bright red colour

8 *Absorption spectra*—The different hemoglobin derivatives can be easily identified by this characteristic absorption spectra

(a) *Oxyhemoglobin*—Two bands—one narrow and the other wide in the green region

(b) *Reduced hemoglobin*—One single broad band in the green region

(c) *Carboxy hemoglobin*—Two bands in the green region

(d) *Methemoglobin*—Three bands—one in red and two in the green regions

(e) *Sulphemoglobin*—Three bands similar to methemoglobin

Biosynthesis of hemoglobin

1 The biosyothesis of hemoglobin takes place in the bone marrow in the erythroid cell during its development to erythrocyte

2 It starts appearing at stage II (early normoblast) and the synthesis is complete when the cell reaches stage IV (late normoblast)

3 Iron in the ferrous state is incorporated into protoporphyrin to form heme

4 The heme gets attached to the newly synthesized globin to form hemoglobin

5 The iron of heme is coordinated to 2 imidazole nitrogen of histidine at positions 38 and 87 in α chains and 63 & 92 in β chains

In nature, the other metalloporphyrins which are compounds of importance in biologic processes are mentioned below

1 Erythrocruorins (a) They are iron porphyrinoproteins occurring in blood and tissue fluids of some invertebrates (b) Their function is corresponding to hemoglobin

2 Myoglobins (a) They are the respiratory pigments occurring in the muscle cells of vertebrates and invertebrates (b) The purified one has a molecular weight of about 17,000 (c) They contain only 1 gram atom of iron per mole

3 Catalases (a) They are iron porphyrin enzymes (b) They have been obtained in crystalline form (c) Their molecular weight is about 225,000 (d) They contain 4 gram atoms of iron per mol (e) In plants, their activity is minimal

4 Tryptophan pyrrolase —

(a) It is an iron porphyrin protein

(b) It catalyzes the oxidation of tryptophan to formyl kynurenine

5 Cytochromes —

(a) Cytochromes means the cellular pigments because these pigments are widely distributed not only in the tissues of higher animals and plants but also in yeast and bacteria

(b) At first cytochromes a b and c were identified and they had been shown to exist in oxidized and reduced forms and their fundamental role is in cellular respiration At present, some thirty cytochromes are known to exist and according to original cytochromes they are designated as a_1 a_2 a_3, c_1, c_2, c_3 c_4, c_5, b_2 b_3, b_4 etc

(c) They are iron porphyrins and acts as electron transfer agents in oxidation-reduction reactions

(d) The important example is cytochrome C which has been obtained in the purified form

(e) Cytochrome C has a molecular weight of about 13,000 and contains 0 43% iron

(f) The iron porphyrin group of cytochrome C is attached to protein more firmly than in the hemoglobin

(g) Cytochrome C is quite stable to heat and acids

(h) The reduced form of cytochrome C is not autooxidizable

(i) At physiological P^H ferrocytochrome C does not combine with O_2 or Co as does hemoglobin

(j) The peptide chain of human heart cytochrome C contains 104 amino acids Acetylglycine is the N terminal amino acid and glutamic acid the C-terminal amino acid The two cysteine residues are located at positions 14 and 17 in the peptide chain The linkage of iron in heme occurs through the imidazole nitrogen of a histidine residue at position 18 in the peptide chain

(k) The degree of difference in primary structure among the 13 cytochrome C might be related to the degree of phytogenetic relationship between the species Eg The cytochrome C of man as compared to that of rhesus monkey differs by only one amino acid of the 104 amino acids Human cytochrome C differs from that of the dog in 11 amino acid residues, from that of the horse in 12

(l) The enzymes that catalyze the reactions of molecular oxygen are known as oxidases Cytochrome a_3 which is found in heart muscle and other animal tissues is called cytochrome oxidase These oxidases catalyze many reactions in addition to terminal oxidation at the electron transport chain They can carry three general types of reactions e g oxygen transfer, mixed function oxidation electron transfer

Varieties of human hemoglobin

Normal adult hemoglobin or hemoglobin A has a molecular weight of 64,455 and contains two pairs of peptide chains (α & β) of which α chain contains 141 and β chain contains 146 amino acids

Fetal hemoglobin (F) is present in very small amounts

The normal human hemoglobins all possess a common half molecule, i.e. a pair of peptide chains (α chains), the other half consists of a pair of different types of peptide chains, one type for each hemoglobin. Hemoglobin A_2 has two δ chains and hemoglobin F has two γ chains, both types of chains contain 136 amino acids and thus are of the same length as the β chain. Hemoglobin A is represented as $\alpha_2^A \beta_2^A$, hemoglobin A_2 as $\alpha_2^A \delta_2^A$ and hemoglobin F as $\alpha_2^A \gamma_2^F$ for describing abnormal hemoglobin. In early embryonic life, a fourth hemoglobin $\alpha_2^A \varepsilon_2$ exists

Fetal hemoglobin

1 Fetal hemoglobin (F) comprises 50 to 90 per cent of the total hemoglobin in the new born

2. It takes up oxygen more readily at low oxygen tensions and releases carbon dioxide more readily than adult hemoglobin (A)

3 It is more resistant to denaturation by alkali and is more susceptible to conversion to methemoglobin by nitrites (contaminated water)

4 Hemoglobin F is gradually replaced by hemoglobin A during the first 6 months of extrauterine life

5 High concentration of hemoglobin F after two years of age occur in various types of anemia e g sickle cell anemia and thalassemia.

Abnormal hemoglobins

Over one hundred different types of abnormal hemoglobins have been described. Some of which are easily differennated by their electrophoretic mobilities and have given rise to the concept of "molecular disease", which explains that a defective gene (mutant) may direct the formation of a molecule similar to a normal molecule but differing from it in shape, composition and electrical charge One amino acid of the normal hemoglobin is replaced by another amino acid, i.e. acidic amino acid is replaced by a basic or a neutral amino acid for the formation of abnormal hemoglobin. The abnormal hemoglobins are named in alphabetic order as C, D, E, F, G, H, K, L, M, N, O, P, Q, S etc.

Some Amino acid distribution in abnormal hemoglobin.

Type of abnormal hemoglobin	*Replacement of the position of amino acid*	*Amino acid present in the normal hemoglobin at that position*	*Amino acid present in abnormal hemoglobin at that position*
C	6	Glutamic acid	Lysine
D (Ibdan)	87	Threonine	Lysine
E	26	Glutamic acid	Lysine
M (Boston)	58	Histidine	Tyrosine
N	95	Lysine	Glutamic acid
S	6	Glutamic acid	Valine

A Hemoglobin C—This occurs in the blood of some Negroes in West Africa The abnormality is found in the β chain at position 6, the amino acid glutamic acid is replaced by Lysine It is characterized by the mild anemia with a tendency to infarction

B Hemoglobin S—This appears among the Negroes of Africa The abnormality occurs in β chain, glutamic acid at position 6 is replaced by valine Sickle cell anemia develops and the RBC becomes long and boat-shaped The blood becomes more viscous which results in reduced blood flow

C Hemoglobin F—HbF is present in fetus and is replaced by adult hemoglobin as the child grows It is present only in traces in normal adult hemoglobin If HbF is present in large amounts in the blood of adults, it gets hemolysed rapidly producing a severe anemia called 'Thalassemia major'

D Hemoglobin M—There are two types of HbM—HbM (Boston) and HbM (Iwate) which are of clinical interest The abnormality is found in the α chain, the histidine residues in 58 and 87 positions are replaced by tyrosine Abnormal amounts of methemoglobin are found in the blood of persons affected by this condition This methemoglobin is not reduced to hemoglobin by reducing agents

E Hemoglobin D—This occurs rarely It exists in two forms—Dα and Dβ The persons having HbD do not show any clinical signs and symptoms

Technique for identification of hemoglobins

Finger print technique Ingram developed a technique by which the peptide chains in hemoglobin could be broken down into several smaller peptide fragments by digestion with trypsin Trypsin splits the peptides only at points where only lysine and arginine occur A mixture of smaller peptides were obtained He then separated this mixture using paper electrophoresis technique and paper chromatography The peptides appeared as spots when ninhydrin was sprayed Thus peptide maps had been prepared for different hemoglobins

ABNORMAL HEMOGLOBIN DERIVATIVES

In various disease states, abnormal amounts of certain derivatives of hemoglobin are present in the blood which are of clinical importance They generally result from abnormal exposure to toxic chemical agents

A *Methemoglobin* (i) *This is an oxidized hemoglobin* (ferrous iron is oxidized to ferric iron) Oxygen is in the firm combination

(ii) It is incapable of functioning as an oxygen carrier Cyanosis usually develops when the concentration of it reaches 3 gram per 100 ml of blood

(iii) Methemoglobin is present in normal erythrocytes 0 4 per cent of the total hemoglobin With very high concentration, the cells may be injured, destroyed and it may be liberated into the plasma along with hemoglobin and excreted in the urine

Causes of methemoglobin

(a) Drugs and other toxic agents—Nitrites, chlorates etc
(b) Other hemolytic agents
(c) Enterogenous cyanosis—Due to excessive production and absorption of nitrites from the intestine
(d) Congenital—One form is characterized by the abnormality in the molecular structure of the globin component In another type, there is the deficiency of diaphorase enzyme system which catalyzes reduction of methemoglobin to hemoglobin

B Sulfhemoglobin

(i) Reduced hemoglobin combines with hydrogen sulphide to form sulfhemoglobin

(ii) Sulfhemoglobinemia is an unusual condition which occurs chiefly as a result of the action of nitrites and coal tar preparations in the presence of excessive amounts of sulfur

(iii) It is also observed in subjects with marked constipation in the presence of nitrite producing bacteria in the intestine

(iv) Cyanosis usually occurs when the concentration of sulfhemoglobin reaches 3 to 5 gram per 100 ml of blood

C Carboxyhemoglobin

(i) It is formed as a result of the combination of carbon monoxide with the iron in the hemoglobin molecule

(ii) It has a bright cherry red colour

(iii) It is formed by the excessive exposure to artificial illuminating gas and to automobile exhaust gases in closed or poorly ventilated rooms

PORPHYRIAS

It is the condition in which the excretion of both coproporphyrin and uroporphyrin is increased

Porphyrias are of two types

1 Hereditary and familial (congenital)

2 Acquired

1 Congenital porphyrias

A Erythropoietic porphyria

B Erythropoietic protoporphyria

C Erythropoietic coproporphyria

D Hepatic porphyria

(a) Acute intermittent porphyria

(b) Porphyria variegata

(c) Hereditary coproporphyria

(d) Porphyria cutanea tarda

A Erythropoietic porphyria

(i) Highly increased excretion of uroporphyrin I and to a lesser extent, coproporphyrin I in both urine and feces Both these porphyrins are present in higher concentration in the circulating erythrocytes

(ii) Photosensitivity and severe skin lesions from infancy leading to severe deformities of the fingers, nose ears and eyes

(iii) Excess hair on the face and limbs

(iv) Teeth and bones may be brownish or pink due to porphyrin deposition

(v) Tendency to hemolysis and defective erythropoiesis

(vi) The disease is rare

B Erythropoietic protoporphyria

(i) Increased protoporphyrin and uroporphyrin in the circulating erythrocytes, the plasma and the feces

(ii) Photosensitivity is the major sign

(iii) Chronic skin lesions may develop

(iv) The condition is much more commonly seen

C Erythropoietic coproporphyria

(i) Large amounts of coproporphyrin III containing increased amounts of protoporphyrins and uroporphyrins are present in the erythrocytes

(ii) Swelling and itching of skin

(iii) The condition is very rare

D Hepatic porphyria

(a) Acute intermittent porphyria

(i) Excess of ALA and porphobilinogen in the urine

(ii) Increase in serum protein-bound iodine (PBI), some degree of hypercholestemia and a diabetic type of glucose tolerance curve The condition is due to a marked increase of hepatic ALA synthase

(iii) Periodic attacks of abdominal pain which is associated with fever and leukocytosis

(iv) There may be constipation and severe neurological and psychiatric disturbances

(v) Freshly passed urine is often normal in colour but on standing in sunlight turns to red wine or black

(vi) The disease is extremely rare before the age of 15 years and after the age of 60 It is slightly more common in females

(b) Porphyria variegata

(i) Increased excretion of porphobilinogen in the urine during acute neurological manifestations and turns to normal in remission

(ii) Elevated urinary copro- and uroporphyrins and also fecal proto porphyrin

(iii) Frequent uremia and electrolyte disturbances

(iv) Increased hepatic ALA synthase

(v) Photosensitivity and cutaneous lesions

(c) Hereditary coproporphyria

(i) Increased urinary output of porphobilinogen and ALA during acute attacks

(ii) Marked increased excretion of coproporphyrin III mainly in the feces and this does not always occur in the urine

(iii) No increase in erythrocyte porphyrins

(d) Porphyria cutanea tarda

(i) Increased urinary uro- and coproporphyrins This also occurs in feces which may also contain protoporphyrin

(ii) Increased hepatic ALA synthase

(iii) Frequent rise in serum iron

(iv) Skin lesions in response to minor trauma

(v) No pink fluorescence in teeth by ultraviolet light

(vi) Abdominal pain with fever

2 Acquired porphyrias

This disease is caused by

(a) Severe liver diseases

(b) Ingestion of certain toxins

Alcoholic cirrhosis often leads to the development of cutaneous hepatic porphyria. This form is common in Southern African Countries In Turkey in 1955, the consumption of wheat contaminated with hexachlorobenzene caused an outbreak of this type of porphyria

Important biochemical defects

(i) Increased excretion of uroporphyrin in urine

(ii) Fecal porphyrin level is normal

(iii) ALA and PBG are not found in urine

Effects of chemical substances on induction of porphyria

(i) Drugs barbiturates sulfonamides chloroquine and sexhormones

(ii) Chemical agents—Hexachlorobenzene, Di and tri-chlorophenols, phenobarbital, sulfonmethane chloropropamide, and alcohols , orally administered contraceptives

CATABOLISM OF HEMOGLOBIN

The catabolism of hemoglobin can be represented under two main heads

1 Conversion of hemoglobin to bile pigments

2 Metabolism of bile pigments

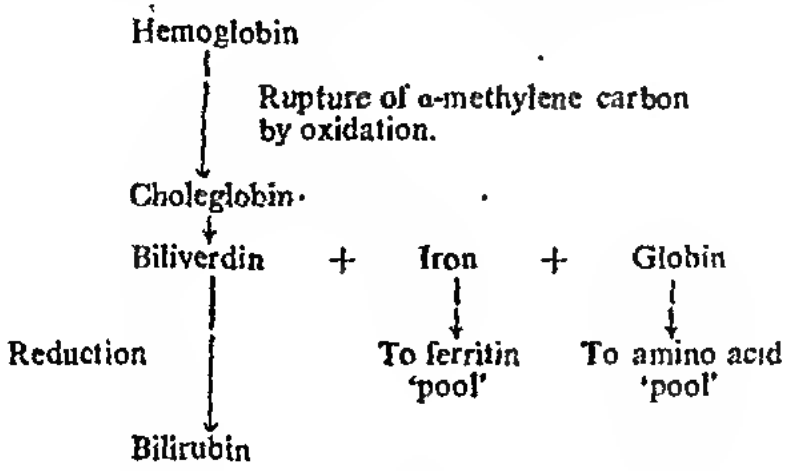
1 Conversion of hemoglobin to bile pigments

About 8 grams of hemoglobin is broken down each day in the normal adult and this amount contains 27 mg of iron The protoporphyrin yields about 300 mg of bilirubin

When hemoglobin is catabolized in the body, the protein portion globin, may be reutilized either as such or in the form of its constituent amino acids, and he iron enters ferritin Pool for reuse The porphyrin portion is broken down mainly in the reticulnendothelial cells of the liver, spleen and bone marrow First, it involves the opening of the porphyrin ring between pyrrole residue I and II and elimination of the alpha methylene carbon as carbon monoxide (Co) The iron is still present Such a green conjugated protein formed by the oxidation of hemoglobin by oxygen in presence of ascorbic acid is said to be *cholcglobin* The first of the bile pigment biliverdin, is then formed after removal of iron and cleavage of the porphyrin ring of heme It is the chief pigment of the bile in *birds* Biliverdin is easily reduced to bilirubin which is the chief pigment in human bile by the enzyme *bilirubin reductase* requiring the reduced coenzymes NAD or NADP

Normally, there are only slight traces of biliverdin in human bile but the colour is very intense Watson (1969) states that biliverdin jaundice is largely

limited to (i) biliary obstruction as a result of carcinoma. (ii) Severe parenchymal liver disease as in advanced cirrhosis of the liver. The amounts of biliverdin in the serum are very small in obstructive jaundice caused by the presence of a stone in the common bile duct. No biliverdin occurs in the serum in hemolytic jaundice.



(Conversion of hemoglobin to bile pigment)

Fig. 9.5

2. Metabolism of bile pigments :

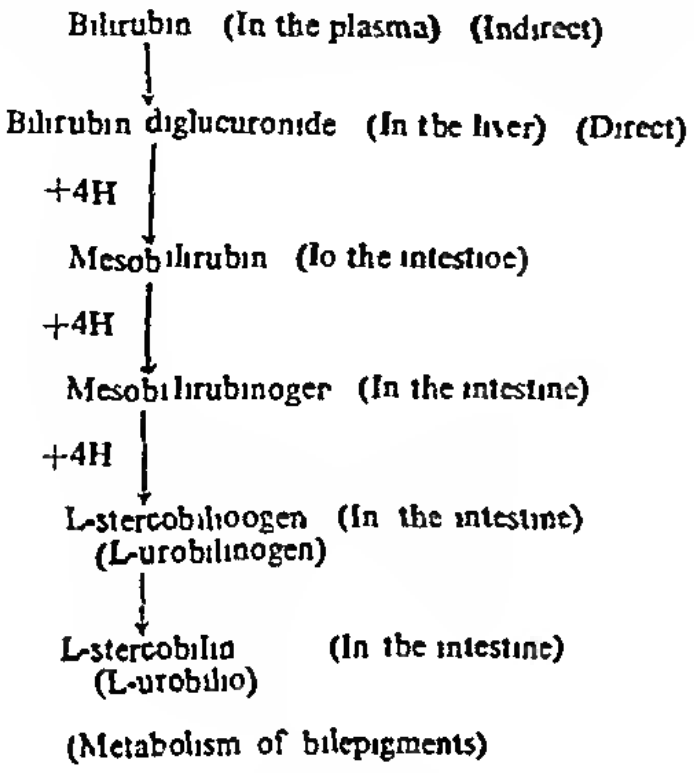
The normal concentration of bilirubin in human plasma is 0 1—1.5 mg per 100 ml. One gram of hemoglobin yields 35 mg of bilirubin which is carried in loose association with protein (mainly albumin) of the plasma This is carried to the liver where it is conjugated with glucuronic acid. The soluble bilirubin conjugate (direct bilirubin) is readily excreted into the intestine with the bile. Bilirubin +2UDP glucuronate→bilirubin diglucuronide +2UDP.

In the lower portions of the intestinal tract, the cecum and the colon, the bilirubin is released from the conjugate and is then reduced by enzyme systems present in the intestinal tract, mainly derived from anaerobic bacteria in the cecum. If the intestinal flora is diminished by the administration antibiotic agents capable of producing partial sterilization of the intestinal tract, bilirubin may not be further reduced rather auto-oxidized to biliverdin in contact with air. Under this condition the feces acquire a green tinge.

Progressive hydrogenation takes place to produce a series of intermediary compounds, beginning with mesobilirubinogen which may be oxtdized with the loss of hydrogen, to coloured compounds. The end product is colourless L-stercobilinogen (L-urobilinogen). Auto-oxidation in the presence of air produces stercobilin (L-urobilin), an orange-yellow pigment which gives the normal colour of the feces. Stercobilin is strongly levorntatory.

A portion of the urobilinogen is absorbed from the intestine into the blood. Some of this is excreted by the kidney and appears in the urine in the

concentration of 0-4 mg/day The remainder is re excreted in the bile The unabsorbed urobilinogen is excreted in the stool (40-280 mg/day) On exposure to oxygen urobilinogen is oxidized to urobilin which gives the darkening of stools



(Metabolism of bilepigments)

Fig 96

JAUNDICE

When the concentration of the bilepigment in the blood is more, it diffuses into the tissues producing a yellow pigmentation This condition is said to be *jaundice* or *icterus*

Causes

1 Production of more bile pigment than the normal liver can excrete or the failure of a damaged liver to excrete the bilirubin produced in normal amounts

2 Obstruction of the excretory ducts of the liver by preventing the excretion of bilirubin

Types

A Hemolytic jaundice —

If the concentration of bilirubin in the serum rises above normal due to the more formation as a result of increased erythrocyte destruction hemolytic jaundice results

The rate of destruction of red blood cells is increased.

1 In hemolytic anemia
2 By the action of some drugs
3 By malarial or viral infections
4 By incompatible blood transfusion

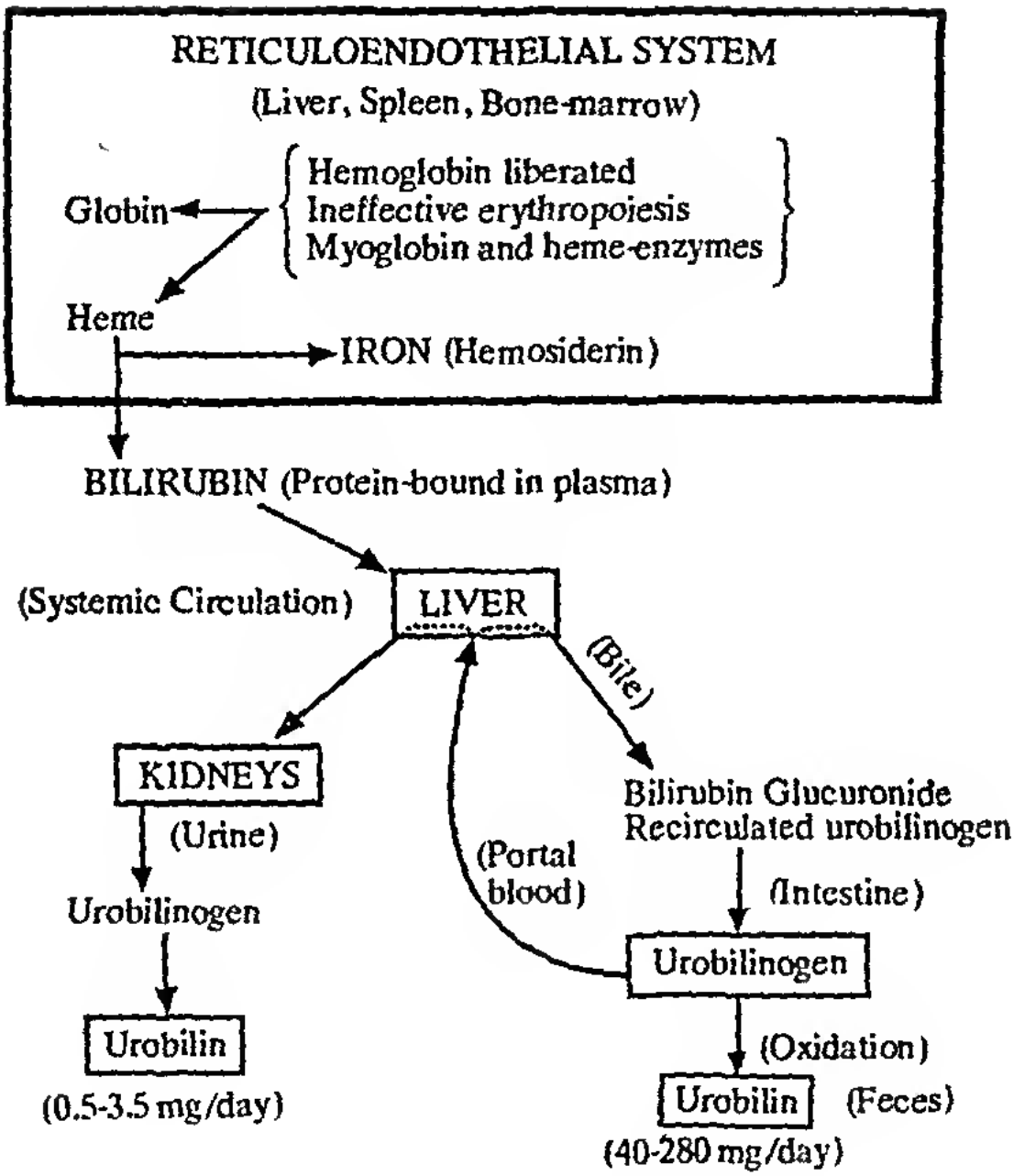


Fig. 9.7 . Bile pigment metabolism

Findings in bemolytie jaundiee :

(a) Increased level of unconjugated or indireet bilirubin in serum.

(b) Increased excretion of urobilinogen in urine.

(c) Dark brown colour of the faeces.

B. **Obstructive (Regurgitation) jaundice :**

This condition occurs from blockage of the hepatic or common bile ducts. The bile pigment passes from blood to the liver cells as usual. However, failing to be excreted by the bile capillaries, it is absorbed into the hepatic veins and lymphatics.

Tbe usual *causes* of obstruction are

1. Blocking of the bile passage by gall stones.
2. Obstruction caused by enlarged glands, tumour of tbe head of the pancreas.
3. Narrowing of tbe bile duet as a result of surgery.

The important findings in obstructive jaundice :

(a) Increased amount of conjugated or direct bilirubin in serum.

(b) Highly coloured urine due to excretion of increased amounts of bilirubin.

(c) Clay coloured stools due to absence of stercobilinogen.

(d) Increased serum alkaline phosphatase activity.

C. **Hepatie jaundice :**

This type of jaundice is caused by liver dysfunction as a result of the damage to the parenchymal cells by infection, toxins and liver poisons.

At a certain stage, the inflammation and damage to liver cells become severe leading to partial obstruction to the flow of bile. This results in the absorption of conjugated bilirubin and bile into the general circulation.

Tbe important findings :

(a) Increased amounts of botb conjugated and unconjugated bilirubin in serum.

(b) Highly coloured urine due to the presence of conjugated bilirubin and urobilinogen.

(c) Increased SGPT actuvity

(d) Highly positive flocculation tests.

	Urine	*Fecal*
Normal:	Urobilinogen 0-4 mg/24 hours. Bilirubin absent.	Uribilinogen 40-280 mg/24 hours.
Hepatic :	Urobilinogen increased. Bilirubin present.	Urobilinogen decreased
Hemolytic :	Urobilinogen increased. Bilirubin absent.	Urobilinogen increased.
Obstructive :	Intermittent stone— Urobilinogen fluetuates. Complete block— Urobilinogen absent.—	Urobilinogen trace to absent.

On the basis of the type of plasma bilirubin i e. Unconjuguted bilirubin or onjugated bilirubin, the hyperbilirubinemia may be classified as *retention* hyper-ilirubinemia or *regurgitation* hyperbilirubinemia.

A Crigler-Najjar Syndrome, Type I; Congenital Nonhemolytic jaundice:

1. It is a rare recessive disorder of humans owing to a primary metabolic defect n the conjugation of bilirubin which is involved in the inherited absence of bilirubin UDP—Glucuronyltransferase activity in hepatic tissues.

2. The disease is usually alarming within the first fifteen months of life. But a few teenagers did not develop difficulties until puberty. These children have been treated with phototherapy with some reduction in plasma bilirubin levels.

3. Phenobarbital and other drugs have no effect on the formation of bilirubin glucuronides in patients with this syndrome.

4. Serum bilirubin usually exceeds 20 mg./dL. on untreatment.

B. Crigler-Najjar syndrome, Type II:

1. This rare inherited disorder is caused by a milder defect in the bilirubin conjugated system.

2. The serum bilirubin level does not exceed 20 mg./dL. All of the bilirubin accumulated is of unconjugated type. The bile in these patients contains bilirubin monoglucuronide.

3. Patients with this syndrome respond to large doses of Phenobarbital.

4. This syndrome is the homozygous state of the defect present in heterozygous form in the mild chronic hyperbilirubinemia of Gilbert.

C. Gilbert's Disease:

1. It is a beterozygous group of diseases many of which are due to a compensated hemolysis associated with unconjugated hyperbilirubinemia.

2. There is defect in the hepatic clearance of bilirubin due to a defect in the uptake of bilirubin by the liver parenchymal cells

3. The bilirubin UDP-Glucuronyltransferase activities in the liver are found to be reduced.

D. Toxic hyperbilirubinemia:

1. Unconjugated hyperbilirubinemia results from liver dysfunction caused by chloroform, carbon tetrachloride, hepatitis virus, and cirrhosis.

2. There is a component of obstruction of the billary trec within the liver which results in the presence of some conjugated hyperbilirubinemia.

CHAPTER 10

ENZYMES AND COENZYMES

Introduction

Enzymes were first divided into two classes "unorganised" and "organised ferments The notion of ' organised ferments" was thought to be due to "vital activity This view was encouraged by the "rediscovery' of bacteria by Pasteur and Koch in the middle of the last century The "vital ' concept of enzyme was finally discredited by Buchner in 1897 He separated active enzymes from the living cells of yeast and later of bacteria.

Enzymes promote and control the conversion of the complex carbohydrates fats and proteins of our body into simple substances which the intestines can absorb and also the various reactions by which these simple substances are used in the body for building up new tissues or producing energy The enzymes are not broken down or changed in the process They are as potent at the end of the reaction as at the beginning and very small amounts can effect the conversion of large quantity of material They are really the true catalysts

Hydrolysis of a protein takes at least a day by the action of strong acid at 100°C. The same change takes place in a few hours in the alimentary canal at 37°C. The acid in the laboratory can cause the hydrolysis of various substances but each enzyme attacks a special class of substances or even a single substance

Many enzymes have been purified and found to be active Willstatter and Pollinger purified peroxidase and found to be the most active Even the enzymes are active although they are not purified The total number of molecules of a particular enzyme in a cell is very small although the number of different enzymes in a cell is very large

Definition

Enzymes are soluble, colloidal organic catalysts formed by living cells, specific in action protein in nature, inactive at 0°C and destroyed by moist heat at 100°C

Intracellular Enzymes—Enzymes which are used in the cells which make them are said to be intracellular enzymes These enzymes correspond to the old "organised ferments'

Extracellular Enzymes—Enzymes which are produced by other cells and are secreted to other parts of the body (e g digestive juice) are called extracellular enzymes These enzymes correspond to the old ' unorganised ferments".

Zymase secretion—An extracellular enzyme which is secreted ready for action is called a zymase secretion Example Amylase of saliva.

Zymogen secretion—An enzyme which is secreted in inactive form and ultimately activated by an agent secreted by other cell is said to be zymogen secretion Example Trypsinogen (in pancreatic juice) activated by enterokinase (in intestinal mucosa) to give active trypsin, prothrombin (in blood) activated by thromboplastin (in tissues) to give active thrombin.

Zymogen secretion is probably a protective mechanism to prevent digestion of cell walls and ducts, since it is most frequently found with protein splitting enzymes

Substrate—The substance on which an enzyme acts is called the substrate

Example Maltose is the substrate on which the enzyme maltase acts to form glucose

Names

Except the enzymes ptyalin, pepsin, trypsin and erepsin, enzymes are usually named by adding the suffix—ase to the main part of the name of the substrate on which they act

Examples

Maltase acts on maltose

Lactase acts on lactose

Lipases act on lipids

Carbohydrases act on carbohydrates

Proteases act on proteins

Amylases act on starch (Amylum)

But there are many substances which are acted on by some enzymes in different ways A dipeptide can be attacked by three enzymes These enzymes are named by their function

Example

A dipeptide can be hydrolysed by dipeptidase into amino acids The free amino group of the amino acid is removed by another enzyme and the free carboxyl group is also removed by another enzyme So the names of these three enzymes acting on a dipeptide are a dipeptidase, a deaminase and a decarboxylase

Other enzymes are named by their functions only Examples transferases, dehydrogenases, hydrolases, oxidases and reductases

Many enzymes with the same function act on one substance it is therefore, better to specify the enzyme by its source Examples Pancreatic amylase bone phosphatase, liver esterase

Some enzymes acting on the substrates are freely described by the adjectives Examples Amylolytic lipolytic proteolytic

CLASSIFICATION

The 6 major classes of enzymes with some examples are given below

1 **Oxidoreductases** Enzymes catalyzing oxidoreductions between two substrates A and B

$$A_{reduced} + B_{oxidized} \rightarrow A_{oxidized} + B_{reduced}$$

These enzymes can be grouped in many different ways Three main groups can be explained in order to get the simpler expression

Oxidases—The enzymes which use oxygen as hydrogen acceptor Examples tyrosinase, cytochrome oxidase uricase

Anaerobic dehydrogenases—The enzymes which use some other substance as hydrogen acceptor Examples Malate dehydrogenase succinate dehydrogenase, lactate dehydrogenase

Hydroperoxidases—The enzymes which use hydrogen peroxide as substrate. Examples : Peroxidase, catalase.

Aerobic dehydrogenases—The enzymes which use either oxygen or another substance as hydrogen acceptor. Examples : D- and L-amino acid oxidases, xanthine oxidase, aldehyde oxidase.

Two other groups are :

Oxygenases—The enzymes which act on single hydrogen donors with incorporation of oxygen.

Example : Tryptophan oxygenase.

Hydroxylases—The enzymes which act on paired donors with incorporation of oxygen into one donor. Examples : Steroid hydroxylases, phenylalanine-4-hydroxylase

2. Transferases (Transferring enzymes) :

They catalyse the transfer of some group or radical, R, from one molecule, A, to another molecule, B.

$$A \cdot R + B \rightleftharpoons A + B \cdot R$$

Fig 10.1

The group includes

(a) *Transphosphorylases*—Examples : Hexokinase, phosphoglucomutases, phosphoglycerate kinase.

Hexokinase

ATP + D-hexose = ADP + D-hexose-6-phosphate.

(b) *Transglycosidases*—Examples : Phosphorylase.

(c) *Transaminases*—Examples : Glutamate-pyruvate transaminase, aspartate amino transferase

(d) *Transacylase*—Examples : Choline acetyl transferase, acetoacetate transacetylase, amino acid transacetylase.

$$\text{Acetyl-CoA} + \text{Choline} \xrightarrow{\text{Choline acetyl transferase}} \text{CoA} + \text{Acetylcholine}$$

Fig 10.2

(e) *Transmethylase*.

3. Hydrolases Enzymes catalyzing hydrolysis of ester, ether, peptide, glycosyl, acid anhydride by the addition of water.

The group includes the extracellular digestive enzymes and many intracellular enzymes.

(a) Enzymes acting on glycosyl compounds, e g β-galactosidase.

β-D-galactoside + H_2O = An alcohol + D-galactose.

(b) Enzymes acting on peptide bonds, e.g. pepsin, rennin, chymotrypsin.

(c) Esterases, e g lipases, phosphatases, sulphatases

(d) Amidases, e g urease, arginase, glutaminase

(e) Hydrolytic deaminases, e g guanine deaminase

4 Isomerases Enzymes catalyzing interconversion of optical, geometric, or positional isomers

(a) Racemases and epimerases, e g alanine racemase

L-alanine=D-alanine

(b) Cis trans isomerases, e g retinene isomerase

All transretinene=II cis-retinene

(c) Enzymes catalyzing interconversion of aldoses and ketoses, e g triosephosphate isomerase

D-glyceraldehyde-3-phosphate=Dihydroxyacetone phosphate

5 Lyases—Enzymes that catalyze removal of groups from substrates by mechanisms other than hydrolysis leaving double bonds

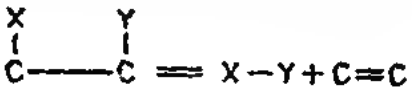


Fig 10 3

(a) Aldehyde-lyases, e g aldolase

Ketose-1-phosphate=Dihydroxyacetonephosphate+an aldehyde

(b) Carbon-oxygen lyases, e g fumerase

L-malate=Fumerate+H_2O

6 Ligases (ligare=to bind)—Enzymes catalyzing the linking together of two compounds couple to the breaking of a pyrophosphate bond in ATP or a similar compound

(a) Enzymes catalyzing formation of C-S bonds, e g succinate thiokinase.

GTP+succinate+CoA=GDP+P_i+succinyl—CoA

(b) Enzymes catalyzing formation of C-N bonds, e g glutamine synthetase

ATP+L-glutamate+NH_4^+=ADP+orthophosphate+L-glutamine

(c) Enzymes catalyzing formation of C C bonds, e g Acetyl CoA carboxylase

ATP+acetyl-CoA+CO_2=ADP+P_i+malonyl—CoA

PROPERTIES OF ENZYMES

1 Enzymes are proteins So the amino, carboxyl and sulfhydryl groups of the side chains of amino acids are available for linkage between polypeptide chains

2 The above mentioned groups together with others such as the imidazole ring and the alcoholic group of serine are also responsible for the union between substrate and enzyme These groups are termed "active groups" and the region of the protein surface at which they are located is termed "active centre"

3 The enzyme-substrate complex theory assumes combination of enzymes and substrate and then liberation of enzyme and the reaction product Thus,

Enzyme+Substrate⇌Enzyme—Substrate Complex

Enzyme—Substrate Complex⇌Enzyme+Reaction Product(s)

4 In many cases, a series of reactions may be involved in addition to the above one This may be indicated thus,

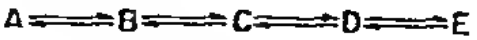


Fig 10 4

5 Alkaline phosphatase hydrolyses a number of phosphate esters to produce orthophosphate and an alcohol such as

Glucose 6-phosphate→Glucose+Orthophosphate

6 From an energy standpoint enzyme reactions are divided into three classes

(a) Exergonic reaction means the system undergoes a loss of free energy, e g lipase, catalase, urease

(b) There is little change in free energy and the reactions come to equilibrium so that product molecules and the substrate molecules are present in constant amounts e g glycogen+inorganic phosphate =glucose 1 phosphate under the influence of phosphorylase

(c) Endergonic reaction means the supply of energy in order to proceed the reaction

7 Many enzymes are conjugated proteins The prosthetic groups (coenzymes) are readily detached These enzymes have special role in metabolic processes The enzymes catalase and peroxidase have cofactors but no coenzymes

ENZYME SPECIFICITY

1 Enzymes, the organic catalysts differ from inorganic catalysts in their extraordinary specificity

2 An enzyme catalyzes only a very few reactions (frequently only one) As for example, arginase, catalase and urease only attack arginine hydrogen peroxide and urea respectively

3 Most enzymes can catalyze the same type of reaction (phosphate transfer, oxidation reduction etc) with several structurally related substrates Frequently reactions with alternate substrates take place if they are present in high concentrations Reactions may occur in the living organism depending in part on the relative concentration of alternate substrates in the cell and their relative affinities for an enzyme

4 Optical specificity

(a) Enzymes show absolute optical specificity for at least a portion of substrate molecule Thus, maltase catalyzes the hydrolysis of α but not β glycosides while enzymes of the Embden Meyerhof and direct oxidative pathway catalyze the interconversion of D but not L phosphosugars. With some exceptions such as the D amino acid oxidase of kidney, the majority of the mammalian enzymes act on the L-amino acids

(b) The glycosidases which catalyze hydrolysis of glycosidic bonds between sugars and alcohols are highly specific for the sugar portion and for the linkage (α or β) but relatively non specific for the alcohol portion or aglycone

(c) Many substrates form 3 bonds with enzymes This 3 point attachment can thus confer asymmetry on the symmetric molecule

5 Group specificity ·

(a) A particular enzyme acts only on particular chemical groupings, e g glycosidases on glycosides, alcohol dehydrogenase on alcohols, pepsin and trypsin on peptide bonds and esterases on ester linkages

(b) Certain enzymes exhibit a higher order of group specificity Chymotrypsin hydrolyzes peptide bonds in which the carboxyl group is contributed by the aromatic amino acids phenylalanine, tyrosine or tryptophan Carboxypeptidases split off carboxyl group and aminopeptidases split off amino group from the polypeptide chain

(c) Although some oxidoreductases function equally with either NAD or NADP as electron acceptor, most use one or the other

Oxidoreductases function in biosynthetic processes in mammalian system (e g fatty acid synthesis) tend to use NADPH as reductant but those function in degradative processes tend to use NAD as oxidant

(d) In liver, about 90% of the NADP specific enzyme occurs extra mitochondrially This may be concerned with biosynthetic processes, as the NAD specific enzyme of mitochondria is specifically activated by ADP

6 The less specific enzymes are the proteases, lipases and esterases These relatively non specific enzymes are really the mixtures of several specific enzymes Thus, trypsin of pancreatic juice is a mixture of trypsin, chymotrypsin A and chymotrypsin B

Preparation and Isolation of Enzymes

1 Tissues are extracted with saline or glycerol Such extracts do not keep for any length of time

2 These extracts are carefully dried at low temperature

3 The dried substance is then grinded to a fine powder which keeps well

4 Then these powders are extracted with suitable solvents

5 More active preparations are made by various processes of purification involving dialysis to remove inorganic impurities, adsorption in suitable material or precipitation with suitable reagents These are done at low temperature

6 In this way much inactive materials can be removed and the enzymes are only present in tissues in very small amounts They have the high potency Over 100 enzymes have been obtained as crystalline proteins which are regarded as the pure enzymes

Recognition of Enzymes

If by adding a neutral solution to some starch at 37°C we obtain sugar, we can conclude that a catalyst is present

If the neutral solution after boiling fails to produce sugar under similar conditions, we may then conclude that the catalyst is an enzyme and not an inorganic catalyst

Therefore, in the experiments on enzymes it is necessary to arrange a control with the boiled enzyme solution along with the test

The conditions of action of enzymes

or,

Factors influencing action of enzymes

1 Contact between Enzyme and Substrate :

(a) Enzyme and substrate must be in contact to form the enzyme—substrate complex

(b) The enzyme and substrate should be well mixed for efficient reaction to proceed

(c) Even the insoluble substrates must be made soluble by the help of hydrotropic substances whenever required for good mixing with enzymes

(d) The digestion of fats which are not in solution is greatly facilitated by fine emulsification of the fat giving a greater area of contact for the lipases

(e) Enzymes which are not soluble but are bound to membranes their contact with substrate must be maintained by setting up suitable concentration gradient.

2 Concentration of Enzyme and Substrate :

(a) The rate of enzyme action is influenced by the concentration of substrate, the amount of enzyme and the time

(b) In case, the concentration of substrate is low in comparison to the concentration of enzyme, not all the active groups of the enzyme molecule will be utilized for the formation of enzyme substrate complex. The rate of reaction will be low

(c) In case, the concentration of substrate increases, more and more active sites of enzyme molecule will be used for the formation of enzyme-substrate complex The rate of reaction will increase.

(d) If the substrate concentration is such that all the active groups of the enzyme are utilized, the rate of reaction will be proportional to the substrate concentration.

(e) Above this concentration mentioned in (d), no matter how high, the rate is maximum and remains constant unless the substrate inhibits the reaction. If the substrate inhibits, the reaction rate declines

The effect of substrate concentration on the initial rate of an enzyme reaction is shown below by a graphical representation which is known as a Michaelis curve

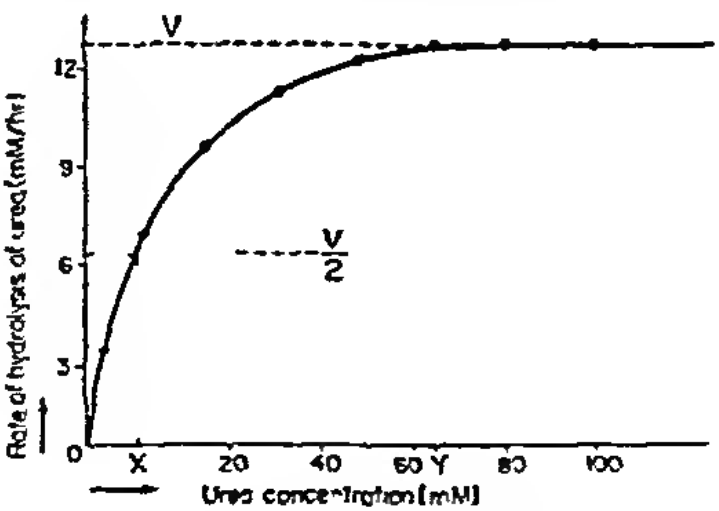


Fig 10.5 Michaelis curve.

It is seen that at low substrate concentration (upto Y in the above figure) the rate increases with increasing substrate concentration At substrate concentration above Y the rate of the reaction has a constant maximum value, V. This is called the maximum velocity of the reaction. The kinetic equation can be expressed mathematically by Michaelis-Menten equation

$$v=\frac{V[S]}{Km+[S]}$$

v is the velocity, V the maximum velocity, S the substrate concentration, Km Michaelis constant

The substrate concentration that produces half-maximal velocity is called Michaelis constant or Km value.

$$v=\frac{V}{2}. \quad \text{where} \quad Km=[S]$$

3 **Temperature :**

(a) The rise in temperature acceferates an enzyme reaction but at the same time causes inactivation of the enzymes due to denaturation of the protein

(b) At a certain temperature known as the optimum temperature the activity is maximum.

(c) The optimum temperature for man is in the region 37°C and for most animals in the region of 40°C The optimum temperature of plant urease is 60°C. The optimum temperature for enzymes from microorganisms adapted to growth in natural hot springs is close to the boiling point of water.

(d) If the temperature is lowered, the rate of an enzyme reaction is dimmished At the temperature 0°C, most enzymes are practically inactive.

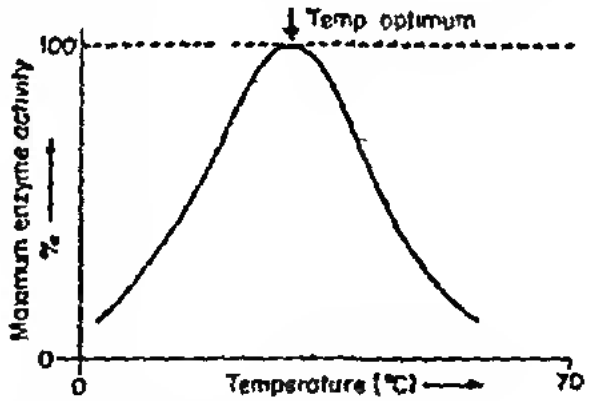


Fig. 10 6 Effect of temperature on enzyme activity

4 **Hydrogen ion concentration or P^H**

(a) A small change in P^H may inhibit enzyme activity.

(b) Pepsin works only in acid medium and is inactivated by making the medium alkaline

(c) Trypsin rather works in alkaline solution and cannot digest protein in acid solution

(d) The maximum activity of the enzyme is at the optimum P^H This value is generally between 5 0 and 9 0

(e) (i) At the optimum P^H, the enzyme (E^-) will react with substrate (S^+) as

$$E^- + S^+ \longrightarrow ES$$

(ii) At low P^H values E^- will be protonated and lose its negative charge

$$E^- + H^+ \longrightarrow E$$

(iii) At the very high P^H values S^+ will lose its positive charge

$$S^+ \longrightarrow S - H^+$$

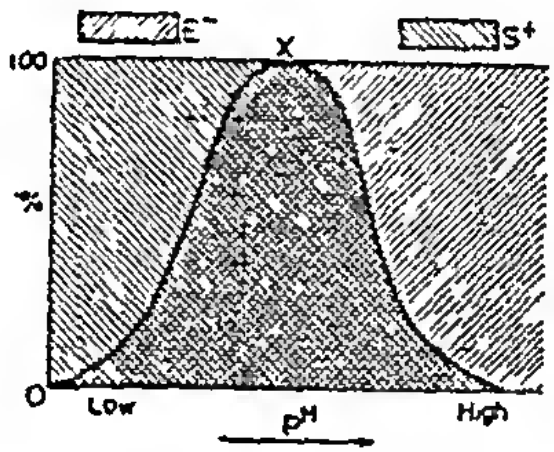


Fig 10 7 Effect of P^H on enzyme activity

Enzymes	*Optimum* P^H
Pepsin	1 5
Salivary amylase	6 8
Trypsin	8 0
Intestinal lipase	7 8

5 Oxidation

(a) The sulfhydryl (SH) groups of many enzymes are essential for enzyme activity

(b) Oxidation of these (SH) groups by many oxidizing agents including the

oxygen of air forms the disulfide (S—S) linkages and results in loss of enzyme activity.

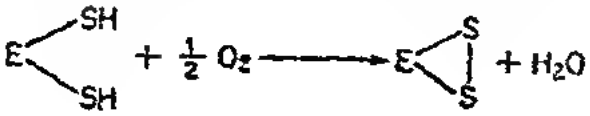


Fig 108

(c) Full activity may be regained by reduced sulfhydryl compounds such as cysteine or glutathione (R—SH)

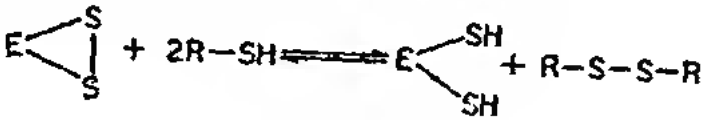


Fig 109

6 **Radiation :**

(a) Enzymes are highly sensitive to short wave length (high-energy) radiation such as x , β^- or γ^- rays

(b) High energy radiation forms peroxides which causes oxidation of the enzyme resulting loss in enzyme activity

(c) The enzyme activity is lost by irradiation which may support indirect effects on the DNA of genes

7 **Coenzymes and Activators :**

(a) Enzymes (excepting the enzymes of G I T) work efficiently in presence of some other substances These substances may be organic and inorganic known as coenzymes and activators respectively

(b) In absence of coenzymes and activators the enzymes may be inactive or sluggish

(c) The activators (Cl^-, Mg^{++}, Ca^{++}, Mn^{++} etc) may take part in the formation of enzyme-substrate complex

(d) Mn^{++} in the action of some peptidases may prevent the inactivation of the enzyme by inhibitors

(e) Some enzymes which are activators are called kinases, e g enterokinase converts trypsinogen to trypsin by removing hexapeptide from trypsinogen

8 **Inhibiting agents :**

(a) Many enzymes are inhibited by the salts of mercury, silver, gold and salts of heavy metals or fluorides

(b) Oxidases are generally inhibited by cyanides

(c) Certain preservatives such as chloroform, glycerol and thymol inhibit some enzymes

(d) Toluene has no action on enzymes but is the best preservative for enzyme solutions.

(e) Formaldehyde destroys enzymes.

(f) The inhibitors present in an enzyme solution occupy the active sites of the enzyme leaving free active sites for substrate to combine.

For example, many enzymes (known as "—SH enzymes") depend for their activity on the presence of free —SH groups They can be inactivated by mercuric chloride which reacts with free —SH groups, thus .

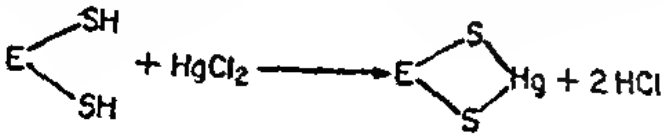


Fig 10 10

This type of inhibition is *non-specific*

(g) The specific inhibitor which is structurally so similar to the substrate molecule that the inhibitor can combine with the enzyme in place of the substrate decreasing the enzyme-substrate union The inhibitor competes with the substrate for the active group This is known as "competitive inhibition"

The followings are the examples of competitive inhibition

(i) Succinic acid (HOOC CH_2 CH_2 COOH) is converted to fumaric acid when combines with succinic dehydrogenase and the fumaric acid is released from the enzyme complex leaving the enzyme free to unite with more succinic acid

Fig 10 11

Malonic acid (HOOC CH_2 COOH) can also combine with the enzyme but does not undergo any change and is not readily released from the enzyme complex So the enzyme is not available for union with succinic acid In such a way malonic acid inhibits succinic dehydrogenase

(ii) Sulphanilamide competes P-aminobenzoic acid.

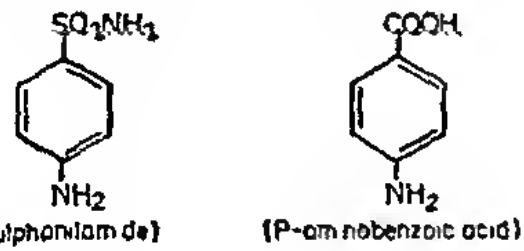


Fig 10 12

P-aminobenzoic acid is essential for the synthesis of folic acid by the enzyme action of the enzyme in the bacteria The union of sulphanilamide with the enzyme prevents the union of the enzyme with the P aminobenzoic acid Synthesis of folic acid is thus completely prevented

(h) Some inhibitors are attached not to the active group but to some other group of enzymes. Under such conditions the activity of the unoccupied active

group is affected , so that union with the substrate occurs less readily or not at all This type of inhibition is called "Non-competitive inhibition"

Enzymes which show "allosteric" inhibition have two sites—(i) Isosteric site, (ii) allosteric site

(i) *Isosteric site*—It can bind the substrate or other molecules structurally similar to it

(ii) *Allosteric site*—It can bind other substances

When the allosteric site binds a molecule, the isosteric site is changed in shape so that the substrate can no longer be bound and hence the enzyme is inhibited This is superficially similar to non-competitive inhibition But it is more complex because the substrate can also affect the shape of the allosteric site and for this reason allosteric inhibition is regarded as a distinct process

(i) *Reversal of inhibition* can be brought about by increasing the amount of substrate relative to inhibitor

(ii) Inhibition may also be reversed by removal of the inhibitor by the treatment with hydrogen sulphide (H_2S)

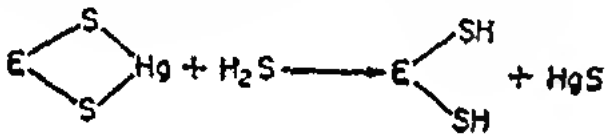


Fig 10 13

9 Anti-enzymes

(a) If certain enzymes are repeatedly injected into an animal, a substance will be produced in the animal's serum which can prevent the normal action of the enzymes injected This substance is called an anti enzyme Examples Antipepsin, anti trypsin anti rennin, anti urease

(b) Some people believe that the mucus membrane contains suitable anti-enzymes for which the alimentary canal is not digested by its own secretions

COENZYMES

Many reactions of substrates are catalyzed by enzymes only in the presence of a specific non protein organic molecule called the coenzyme Coenzymes combine with the apoenzyme (the protein part) to form holoenzyme The coenzymes are also regarded as cosubstrates

Definition

Coenzymes are heat stable, dialyzable non-protein organic molecules and the prosthetic groups of enzymes

Classification

1 Based on chemical characteristics

A Containing an aromatic hetero ring

(i) ATP & its relatives
(ii) NAD, NADP
(iii) FMN, TPP, B_6—PO_4

B Containing a non aromatic hetero ring Biotin, lipoic acid

C No hetero ring
Sugar phosphate, coenzyme Q

II. Based on functional characteristics

A **Group transferring coenzymes**

(i) ATP and its relatives
(ii) Sugar phosphates
(iii) Thiamine pyrophosphate (TPP)
(iv) CoA
(v) Pyridoxal phosphate (B_6—PO_4)
(vi) Biotin

B **Hydrogen transferring coenzymes**

(i) Nicotinamide adenine dinucleotide (NAD) and Nicotinamide adenine dinucleotide phosphate (NADP)
(ii) Flavin adenine dinucleotide (FAD) and flavin mono nucleotide (FMN)
(iii) Coenzyme Q

III Based on nutritional characteristics

(a) **Containing B vitamins**

(i) CoA. (ii) TPP (iii) NAD & NADP (iv) B_6—PO_4 (v) FMN, FAD (vi) Folic acid coenzyme (vii) B_{12} coenzyme (viii) Biotin

Functions

1 Their function is usually to accept atoms or groups from a substrate and to transfer them to other molecules

2 They are less specific than are enzymes and the same coenzyme can act as such in a number of different reactions

3 The coenzymes are also attached to the protein at a different but adjacent site so as to be in a position to accept the atoms or groups which are removed from the substrate

4 NAD and NADP coenzymes function as hydrogen acceptors in dehydrogenation reactioos

5 The chief function of CoA is to carry acyl groups and they are used in the oxidative decarboxylation of pyruvic acid and synthesis of fatty acids and acetylation

6 The function of TPP (co carboxylase) is to carry 'active aldehyde (R CH(OH)⁻) group

7 The chief function of pyridoxal phosphate (B_6—PO_4) is involved in transamination reactions

8 The chief function of tetrahydrofolic acid is as a carrier of formate and it is used in the synthesis of purines and pyrimidines

LYSOSOMES

1. Lysosomes are one type of organelles in the cytoplasm which can he regarded as a type of 'ground substance'.

2. They are similar in size to mitochondria and consist of sacs containing a solution of hydrolytic enzymes which are active at an acid P^H.

3. Protein, carbohydrate or fat molecules are brought to the lysosome where they are hydrolysed, so that lysosomes constitute an intracellular digestive system.

4. They are responsible for post-mortem autolysis.

5. Congenial disease such as glycogen storage disease is due to the absence of specific lysosomal enzymes.

6. The enzyme systems present in lysosomes are for hydrolytic processes, e.g. phosphatases, glycosidases, glucuronidases.

7. The enzymes within the lysosomes are unable to act on substances within the cytoplasm so long the lipoprotein membrane of the lysosome remains intact. Once the membrane is ruptured, there will be the release of lysosomal enzymes.

LYSOZYME

1. Lysozyme is an enzyme present in tears, nasal mucus, sputum, tissues, gastric secretions, milk and eggwhite.

2. It catalyzes the hydrolysis of β-1, 4 linkages of N-acetylneuraminic acid in mucopolysaccharides or mucopeptides.

3. It destroys the cell walls of many airborne gram-positive bacteria in tears and nasal mucus.

4. Its molecular weight is about 15,000.

5. It consists of a single polypeptide chain of 129 amino acid residues having no coenzymes or metal ion cofactors.

6. As the lysozyme molecule may readily be unfolded and refolded, catalysis and specificity as well as 3-dimensional structure are determined solely by these residues.

7. There are little α-helix and large regions without secondary structure.

8. It has a central catalytic site with 6 subsites which hind various substrates or inhibitors.

9. The residues responsible for bond cleavage lie between sites D and E, close to the carboxyl groups of ASP. 52 and Glu. 35,

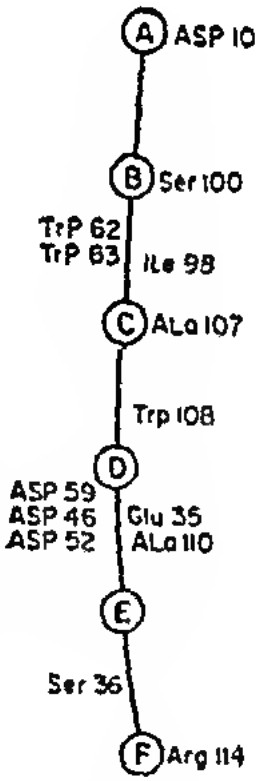


Fig. 10.14

ISOENZYMES or ISOZYMES

Definition

The enzymes that occur in a number of different forms and differ each other chemically, immunologically and electrophoretically are called "Isoenzymes or Isozymes"

Occurrence

Isozymes are present in the serum and tissues of mammals, amphibians, birds, insects, plants and unicellular organisms

Examples Isozymes of numerous dehydrogenases, and several oxidases, transaminases, phosphatases, transphosphorylases, proteolytic enzymes, aldolases

Characteristics

1 They catalyze the same reaction but they can be distinguished by physical methods such as electrophoresis or by immunological methods

2 The difference between some isozymes are due to differences in the quarternary structure of the enzymes, e g lactate dehydrogenase exists in five isozymic forms

3 The isozymic forms of lactate dehydrogenase are tetramers, each is made up from two types of units H and M The molecular weight of active lactate dehydrogenase is 1,30,000 Only the tetrameric molecule possesses catalytic activity The subunits are expressed in the following 5 ways

HHHH
HHHM
HHMM
HMMM
MMMM

4 Splitting and reconstitution of lactate dehydrogenase-I_1 or lactate dehydrogenase I_5 produces no new isozymes Therefore, each consists of a single subunit

But when a mixture of purified lactate dehydrogenase I_1 and lactate dehydrogenase I_5 is subjected to splitting and reconstitution, lactate dehydrogenase I_2, -I_3 and -I_4 are also produced The approximate proportions of the isozymes result if the relationships are

Lactate dehydrogenase isozyme	*Subunits*
I_1	HHHH
I_2	HHHM
I_3	HHMM
I_4	HMMM
I_5	MMMM

Synthesis of H and M subunits are controlled by distinct genetic loci.

5 Lactate dehydrogenase (LDH) catalyzes the transfer of two electrons and one hydrogen ion from lactate to NAD

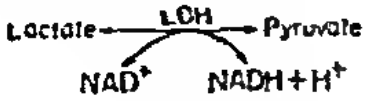


Fig 10 15

6 The medical discovery in 1957 had shown that the relative proportions of several lactate dehydrogenase isozymes of human serum were changed significantly in some pathologic conditions

Method of assay

1 Serum sample is subjected to electrophoresis at PH 8 6 using starch, agar medium

2 The isozymes have different charges at this PH and migrate to 5 regions of the electrophoretogram

3 Isozymes are then localized by means of their ability to catalyze reduction of a colourless dye to a coloured form

Diagnostic importance

(i) In certain solid tumors there is an increase in serum LD_1 and LD_2 These isoenzymes are also present in the blood of patients with acute leukemia

(ii) LD_3 is usually the predominant isozyme in the tumors

(iii) Serum isozymes levels are elevated in acute leukemia

(iv) Alkaline phosphatase isozymes can distinguish liver lesions from bone lesions in metastatic carcinoma

KINETIC THEORY OF REACTION

The kinetic or collision theory states that the molecules to react must collide and must possess sufficient energy to overcome the energy barrier for reaction

If the molecules have sufficient kinetic energy to react, factors that increase the frequency of collision between molecules will increase their rate of reaction Factors that decrease the frequency of collision or the kinetic energy will decrease the rate of reaction

If some molecules have insufficient energy to react, increased temperature which increases their kinetic energy will increase the rate of the reaction These concepts are illustrated diagramatically below In A none, in B a portion and in C all of the molecules have sufficient kinetic energy to overcome the energy barrier for reaction

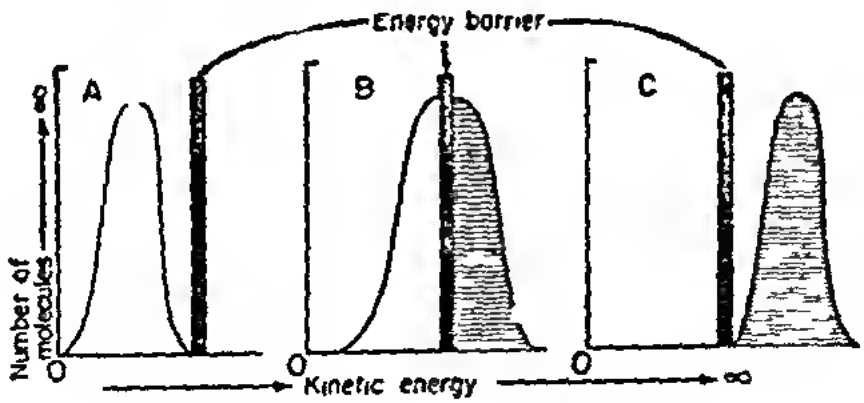


Fig 10 16 The energy barries for chemical reactions

Molecules are in motion at all temperatures above absolute zero (−273°C) at which all molecular motion ceases With the increasing temperature, the rate of diffusion increases

In the absence of enzymic catalysis, many chemical reactions proceed slowly at the temperature of living cells At this temperature even the molecules are in active motion and are undergoing collision They fail to react rapidly because most of them possess insufficient kinetic energy to overcome the energy barrier for reaction At the lower temperature the reaction is spontaneous but slow , at the higher temperature the reaction is spontaneous and fast

The overall energy changes in chemical reactions are independent of the path or mechanism of the reaction

THE CATALYTIC SITE OF ENZYME

Some restricted region of the enzyme which was concerned with the process of catalysis was termed the active site In the beginning, the biochemists were puzzled why enzymes were so large, when only a portion of their structure was involved in substrate binding and catalysis Today, it is clear from 3-dimensional models of enzymes that a far greater portion of the protein interacts with the substrate

Rigid model of the catalytic site

The lock and key or rigid template model is still useful for understanding certain properties of enzymes

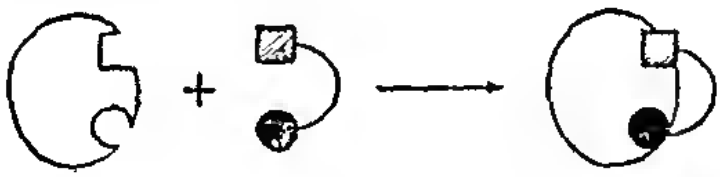


Fig 10 17 Representation of formation of enzyme complex

Flexible model of the catalytic site

In the Fischer Model, the catalytic is presumed to be pre shaped to fit the substrate

In the induced fit model, the substrate induces a conformational change in the enzyme This aligns amino acid residues or other groups on the enzyme in the correct spatial orientation for substrate binding, catalysis, or both At the same time, other amino acid residues may be buried in the interior of the molecule

Hydrophobic groups (hatched portion) and charged groups (dots) both are involved in substrate binding A phosphoserine (—P) and the —SH of a cysteine are involved in catalysis Other residues involved in neither process are represented by the side chains of *lysine* and *methionine*

In the absence of substrate, the catalytic and the substrate-binding groups are several bond distances removed from one another Approach of the substrate induces a conformational change in the enzyme At the same time, the

spatial orientations of other regions are also altered, the lysine and methionine are now closer together.

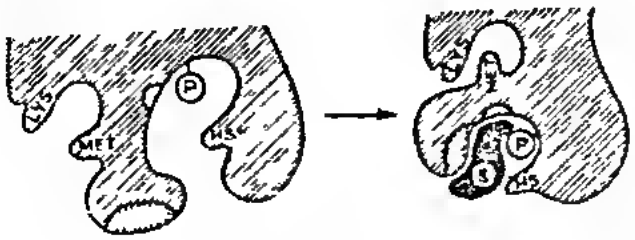


Fig 10 18 Representation of an induced fit by a conformational change in the protein structure

In the representation of a catalytic site shown in the figure below, several regions of a polypeptide chain contribute amino acid residues to the site. Three types of amino acid residues are distinguished in enzymes.

(1) *Contact residue*—An amino acid residue with one bond distance (0.2 nm) of the substrate.

(2) *Specificity residue*—An amino acid residue is involved in substrate binding as well as in catalytic process

(3) *Catalytic residue*—An amino acid residue is directly involved in covalent bond changes during enzyme action

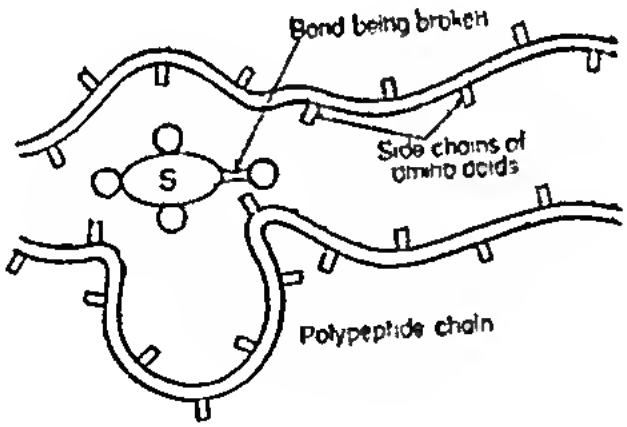


Fig 10 19 The catalytic site

Substrate analogs may causes some of the conformational changes (Fig. No. 10 20). On attachment of the true substrate (A), all groups (shown as closed circles) are brought into correct alignment. Attachment of a substrate analog that is too "bulky" (Fig. 10 20B) or too "slim" (Fig. 10.20C) induces incorrect alignment.

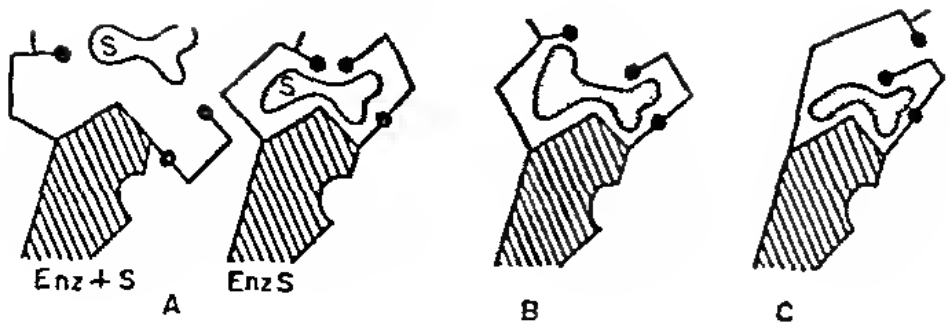


Fig 10 20 Representation of a conformational changes in an enzyme.

The exact sequence of events in a substrate induced conformational change remains to be established. Several possibilities stand (Fig 10 21)

It is still difficult to decide exactly which residues constitute the catalytic site even after knowing the complete primary structure of an enzyme.

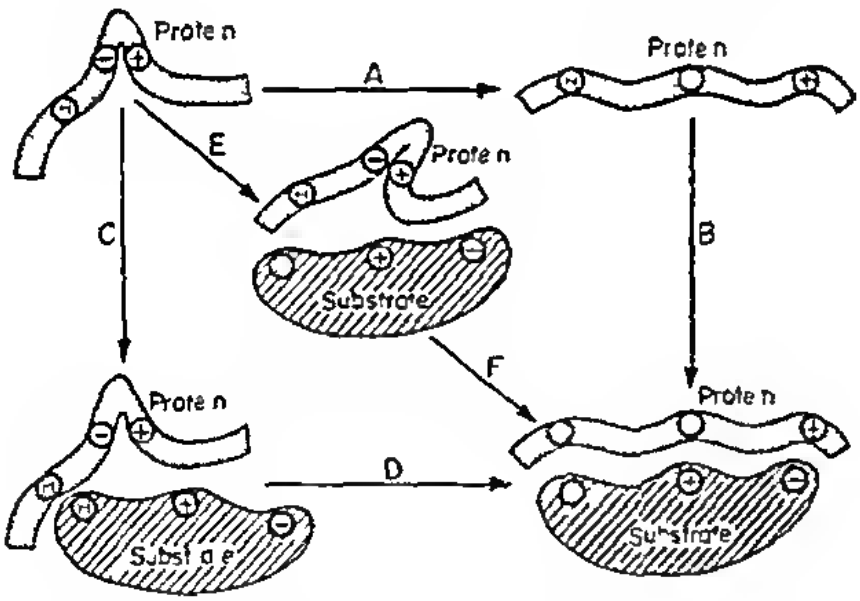


Fig.10 21 Alternative reaction paths for a substrate-induced conformational change.

Modifiers of enzyme activity

Like all mammalian proteins, enzymes are degraded to amino acids Although this mechanism reduces enzyme concentration and hence catalytic activity still they are slow, wasteful of carbon and energy and rather they are like turning out a light by smashing the bulb, then inserting a new one when light is needed

The catalytic activity of certain key enzymes can be reversibly decreased or increased by small molecules Small molecule *modifiers* which decrease catalytic activity are termed negative modifiers and those which increase or stimulate activity are called positive modifiers.

ORDERED AND RANDON BINDING OF SUBSTRATES

Many enzymes catalyze a reaction between two or more substrates producing one or more products But for some enzymes, all substrates must be present simultaneously for the reaction to take place For others, the enzyme first changes one substrate and then catalyzes its reaction with a second substrate The order in which an enzyme binds its substrates may be random or ordered (Fig 10 22)

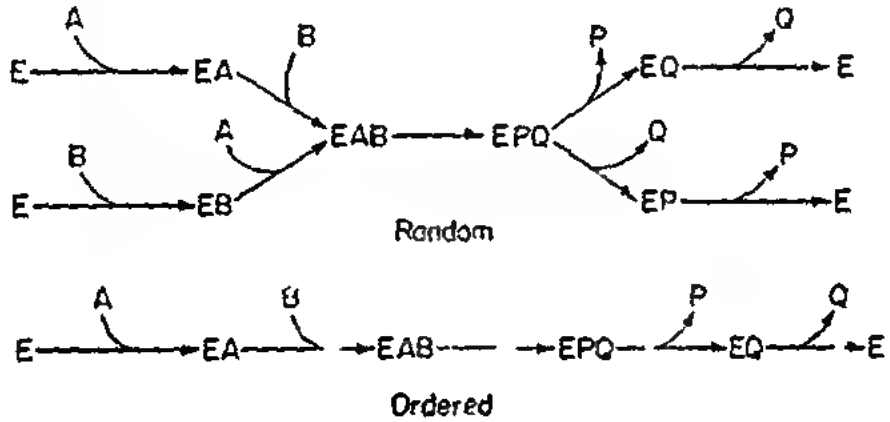


Fig 10 22 Random and ordered addition of substrates A and B and dissociation of products P and Q from an enzyme E

Many reactions which need coenzymes proceed by "ping pong" mechanisms (Since the enzyme alternates between forms E and E' (Fig10 23)

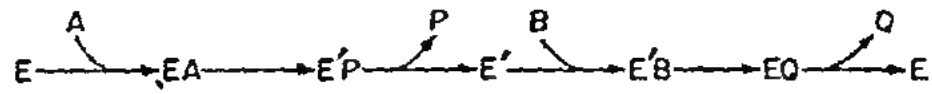


Fig 10 23-Generalized "Ping Pong" mechanism for enzymic catalysis

ENZYMES AS GENERAL ACID OR BASE CATALYSTS

Reactions whose rates vary as regards to changes in H^+ or H_3O^+ concentration but are independent of the concentrations of other acids or bases present

in the solution are said to be *specific acid* or *specific base catalysis*.

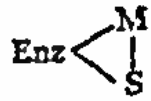
Reactions whose rates are responsive to all acids or bases present in solution are said to be *general acid* or *general base catalysis*. Mutarotation of glucose is one reaction subject to general acid-base catalysis.

Role of Metal Ions

More than 25 per cent of the enzymes contain tightly-bound metal ions for their activity. The functions of these metal ions may be studied by X-ray crystallography, nuclear magnetic resonance (NMR) and electron spin resonance (ESR).

Metalloenzymes and metal-activated enzymes: A definite quantity of functional metal ion is present in metalloenzymes and that metal ion is also retained throughout purification. Metal-activated enzymes bind metals less tightly but require added metals. The mechanism of action in both cases appears to be similar.

Ternary Enzyme—Metal—Substrate Complexes: Four schemes (shown below) are possible for ternary (3-component) complexes of the catalytic site (Enz), a metal ion (M), and substrate (S) that exhibit 1 : 1 : 1 stoichiometry.

Enz—S—M	M—Enz—S
Substrate-bridge complex	Enzyme-bridge complex
Enz—M—S	
Simple metal-bridge complex	Cyclic metal-bridge complex

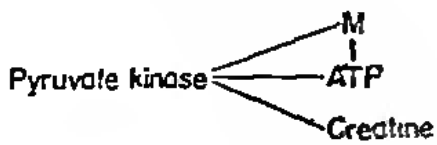
The above all four schemes are possible for metal-activated enzymes. Metalloenzymes cannot form the Enz-S-M complex, because they retain the metal throughout purification. Three stages can be stated:

(i) Most kinases form substrate-bridge complexes of the type Enz-nucleotide-M.

(ii) Phosphotransferases using pyruvate or phosphoenolpyruvate as substrate can form metal-bridge complexes.

(iii) A given enzyme may form one type of bridge complex with one substrate and a different type with the other.

Enzyme-Bridge Complexes (M-Enz-S): The metals in enzyme-bridge complexes perform structural roles maintaining an active conformation (e.g. glutamine synthase) or form a metal-bridge to a substrate (e.g. pyruvate kinase) The metal ion in pyruvate Kinase also holds one substrate (ATP) to activate it.



Substrate-Bridge Complexes (Enz-S-M): This complex with ATP involves the displacement of water from the co-ordination sphere of the metal by ATP.

$$ATP^{4-} + M(H_2O)_6^{2+} \rightleftharpoons ATP{-}M(H_2O)_3^{2-} + 3H_2O$$

$$ATP{-}M(H_2O)_3^{2-} + Enz \rightleftharpoons Enz{-}ATP{-}M(H_2O)_3^{2-}$$

Metal-Bridge Complexes (Enz$\begin{smallmatrix} M \\ | \\ S \end{smallmatrix}$: Activation by metal ions for many peptidases is a slow process which requires many hours. The slow reaction is probably conformational re-arrangement of the binary Enz-M complex to an active conformation.

However, for metalloenzymes, the ternary metal-bridge complex is formed by combination of the substrate (S) with the binary Enz-M complex.

$$Enz{-}M + S \rightleftharpoons Enz{-}M{-}S \text{ or } Enz\begin{smallmatrix} M \\ | \\ S \end{smallmatrix}$$

Role of Metal Ions in Catalysis:

Metal ions may take part in each of the four mechanisms by which enzymes can accelerate the rates of chemical reactions:

(i) General acid-base catalysis, (ii) Covalent catalysis, (iii) Approximation of reactants, (iv) Induction of strain in the enzyme or substrate.

Metal ions like protons can share an electron pair forming a sigma bond. They may also be considered "super acids" and may form pi bonds. Unlike protons, metals can serve as 3-dimensional templates for orientation of basic groups on the enzyme or substrate.

Metal ions can also accept electrons via sigma or pi bonds to activate electrophiles or nucleophiles (general acid-base catalysis). They can also activate nucleophiles or act as nucleophiles themselves by donating electrons.

The coordination sphere of a metal may bring together enzyme and substrate or form chelate-producing destortion in either the enzyme or substrate (strain). A metal ion may also "mask" a nucleophile and thus prevent side-reaction.

FEEDBACK INHIBITION

The changes in the concentration of substrates, coenzymes, activators or inhibitors affect the catalytic efficiency of an enzyme Feedback inhibition inhibits the activity of an enzyme early in the biosynthetic pathway. In the biosynthetic reaction sequence leading from A to D catalyzed by enzymes Enz_1 through Enz_3

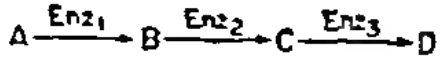


Fig 10 24.

A high concentration of D will inhibit conversion of A to B. D is specifically able to bind to and inhibit Enz_1 D thus acts as a *negative allosteric effector* or *feedback inhibitor of* Enz_1 This feedback inhibition on an early enzyme by an end product of its own biosynthesis, achieves regulation of synthesis of D.

Frequently, the feedback inhibitor is the last small molecule before a macromolecule, e g amino acid before proteins or nucleotides before nucleic acids.

Control of enzyme synthesis : Induction :

For a molecule to be metabolized or for an inducer to act, it first must enter the cell In some cases, a specific transport system or *permease* is needed. Permeases have many properties in common with enzymes and perform functions like cytochromes in electron transport.

Escherichia coli grown on glucose will not ferment lactose due to the absence of specific permease for a β galactoside (Lactose) and of β-galactosidase. If lactose is added to the medium, both the permease and the β-galactosidases are induced and the culture will now ferment lactose The inducer (lactose) is a substrate for the induced proteins, the permease and the β-galactosidase. Compounds structurally similar to the substrate may be inducer but not substrates. These are termed *gratuitous inducers.*

Enzymes whose concentration in a cell is independent of an added inducer are termed *constitutive enzymes.*

The structural genes which specify a group of catabolic enzymes comprise an operon All the enzymes of that operon are induced by a single inducer. This process is known as *coordinate induction*

Control of enzyme synthesis : Repression and Derepression :

In bacteria the presence of the synthesized particular amino acid in the culture medium prevents new synthesis of that amino acid via *repression.* A small molecule such as histidine or leucine, acting as a corepressor, can ultimately block the synthesis of the enzymes involved in its own synthesis.

The genetic information coding for the biosynthesis of enzymes is again expressed after the removal or exhaustion of an essential biosynthetic intermediate from the medium This is termed as derepression

LOCATION OF ENZYMES IN THE CELL

Subcellular Fraction	*Enzyme systems present for*
Cytoplasm	(i) Glycolysis (ii) Glycogenolysis (iii) Glycogenesis (iv) Synthesis of fatty acid
Mitochondria	(i) Electron transport chain (ii) Citric acid cycle (iii) β-oxidation (iv) Urea cycle (v) Oxidative phosphorylation
Microsomes	(i) Protein synthesis (ii) Hydroxylation
Lysosomes	(i) Hydrolytic processes, e g cathepsin, glycosidases, glucuronidases, phosphatases
Nuclei	(i) Synthesis of NAD (ii) „ „ RNA (iii) „ „ Histone

DIAGNOSTIC VALUE OF SERUM ENZYMES

Very small amounts of enzymes which are involved in the reactions in the tissues are present in blood under normal conditions The concentrations of these enzymes are significantly increased due to their more liberation by the affected tissues to the blood stream under certain clinical conditions

Increase in the enzyme activities in cerebrospinal fluid is not reflected in the blood Changes may occur in the cerebrospinal fluid enzymes in certain diseases of the central nervous system Lactate dehydrogenase activity in cerebrospinal fluid is increased frequently in meningitis, cerebral thrombosis and hemorrhage Glucose phosphate isomerase concentration in the cerebrospinal fluid is also elevated in malignant tumors of the brain and frequently in meningitis and cerebral thrombosis

The determination of the activity of the following enzymes can give valuable confirmatory or suggestive diagnostic evidence to the physicians.

	Serum Enzymes	*Concentration increased in*	*Concentration decreased in*
1	Lipase	Acute pancreatitis, pancreatic carcinoma	Liver disease, vitamin A deficiency, diabetes mellitus
2	Amylase	High intestinal obstruction, acute pancreatitis parotitis, diabetes	Liver disease
3	Trypsin	Acute disease of the pancreas	—
4	Cholinesterase	Nephrotic syndrome	Liver disease, malnutrition, acute infectious diseases
5	Alkaline phosphatase	Rickets Paget's disease hyperparathyroidism obstructive jaundice, metastatic carcinoma osteoblastic sarcoma, kidney diseases Alkaline phosphatase isozymes can distinguish liver lesions from bone lesions in cases of metastatic carcinoma	—
6	Acid phosphatase	Metastatic prostatic carcinoma	—
7	SGOT	Myocardial infarction Slightly elevated in acute liver diseases	—
8	SGPT	Acute liver diseases Slightly elevated in cardiac necrosis	—
9	Lactate dehydrogenase	Myocardial infarction, acute hepatitis renal tubular necrosis In certain solid tumors LD_1 and LD_5 are increased	—
10	Isocitrate dehydrogenase	Liver diseases	—
11	Creatine phosphokinase (CK or CPK)	Muscular dystrophy, myocardial infarction	—
12	Glucose-6-phosphate dehydrogenase	Myocardial infarction	Congenital deficiency causes hemolytic anemia

	Serum Enzymes	Concentration increased in	Concentration decreased in
13	Ceruloplasmin (Ferroxidase activity)	Cirrhosis bacterial infection, pregnancy	Wilson's disease (hepatolenticular degeneration)
14	Aldolase	Muscular dystrophy diabetes mellitus acute infective hepatitis, leukemia, infectious diseases hemolytic anemia etc	
15	Oxytocinase	Normal pregnancy from fourth month Increasing level shows good fetal prognosis	Intrauterine fetal death

NORMAL VALUES OF SERUM ENZYMES

	Serum Enzymes	Normal values (Values vary with procedure used)	SI Units (Systeme International d' unites)
1	Amylase	80—180 somogyl units/dl or 0 8—32 IU/liter	2 48—5 58 μ kat/l
2	Lipase	0 2—1 5 units/dl	0 93—6 96 μ kat/l
3	Lactic dehydrogenase	90—200 IU/liter	1 50—3 34 μ kat/l
4	Acid phosphatase	1—5 units (King Armstrong)	4 4o—17 94 μ kat/l
5	Alkaline phosphatase	5—13 units (King Armstrong)	59—153 4 μ kat/l
6	SGOT	5—40 Units/dl or, 6—25 IU/liter	40 1—320 8 nKat/l
7	SGPT	5—35 units/dl or, 3—26 IU/liter	40 1—280 7 nkat/l
8	Aldolase	2—9 5 units/dl or, 7 5—7 0 IU/liter	

COENZYME A (CoA)

Chemistry .

1 It is composed of adenosine triphosphate (ATP), pantothenic acid and β-mercaptoethylamine So it is the coenzyme form of pantothenic acid, a vitamin

2 It is a group transferring coenzyme

3 The reaction gronp is the sulfhydryl (—SH) group

4 The acyl group is accepted by the sulfhydryl group to form acetyl coenzyme A (CH_3CoS CoA) The acyl coenzyme derivatives are the high energy compounds

Functions

1 Carrier of acyl groups, e g acetyl, succinyl, benzoyl

2 Some of the pantothenic acid is bound to protein in the form of "acyl carrier protein" This can be regarded as coenzyme A in which the adenine dinucleotide is replaced by protein "Acyl carrier protein" chiefly functioos in the synthetic processes, e g of fatty acids and cholesterol

3 It is required in the oxidative decarboxylation of pyruvic acid aod a ketoglutaric acid, in the breakdown aod syothesis of fatty acids aod in the synthesis of cholesterol which is involved in bile acids, bilesalts, steroid hormones and vitamin D formation

4 It is used for conjugation with amino compounds to form N-acetyl compounds and in the formation of hippuric acid (Benzoyl glycioe)

5 It is involved in the formation of ketone bodies.

6 It is used in the formation of acetyl choline

7 It is finally oxidized to CO_2, H_2O and ATP via citric acid cycle.

LACTIC DEHYDROGENASES

It is a glycolytic enzyme involved in the conversion of lactic acid to pyruvic acid and vice versa by the help of the coenzyme NAD

Fig 10.25

It exists in five different isozyme forms, e g LDH_1, LDH_2, LDH_3 LDH_4 and LDH_5

Source Found in heart muscle as well as in many other tissues It is liberated to the blood stream during myocardial injury

Concentration The normal level of serum LDH is 200—425 units/100 ml or 90—200 I U /liter

Diagnostic importance ·

1 The rise of serum LDH is not so prompt as that of SGOT and the peak is not reached until the fourth or fifth day but the level may remain abnormally high for 6 to 12 days

2 In case of suspected myocardial infarction, the absence of elevation of serum LDH is more significant in ruling out the disease

3 In hepatitis, there is an insignificant rise in LDH , but a great rise in LDH is seen in untreated pernicious anemia

4 Analysis of an overnight eight-hour urine specimen for lactic dehydrogenase activity is of great value in the diagnosis of serious diseases of kidney and bladder Elevated findings are found particularly in carcinoma of these organs

5 An occasional high value is found in non carcinomatous lesions of the kidney and bladder

TRANSAMINASES

The enzymes which are involved in transamination reactions in the living organisms are said to be *transaminases*

Examples are Glutamate oxaloacetate transaminase (GOT)

Glutamate aspartate transaminase (GPT)

The transaminases are widely distributed in nature

They also exist in isozyme forms

Source GOT is present in greatest amounts in heart muscle, skeletal muscle, brain, liver, and kidney Traces of these enzymes normally escapes into the blood stream

Concentration . The normal level of SGOT is 5—40 units/dl or 6—25 I U /liter

" " " " SGPT is 5—35 units/dl or 3—26 I U /liter

Diagnostic importance :

1 When even a small portion of heart muscle is actually injured by occlusion of a branch of the coronary arteries an appreciable amount of GOT escapes into the blood stream and can be recognized by a sharp rise in the serum concentration

2 In myocardial injury, a rise in SGOT concentration may occur in a few hours after onset of symptoms The peak level is as high as 160 units within 48 hours and returns to the normal level within 3 to 5 days

3 In myocardial infarction, the rise in SGPT is not so high as that of SGOT But in liver disease SGPT is greatly increased

4 In viral hepatitis SGPT is always higher than SGOT , but in cirrhosis and hemolytic jaundice SGOT is higher than SGPT

CREATINE KINASE (CK or CPK)

This enzyme is involved in the formation of phosphocreatine from creatine and ATP (adenosine triphosphate) It is frequently referred to as creatine phosphokinase (CPK) It exists in isozyme forms

Source .

(1) It is found in greatest amount in skeletal muscle

(ii) It is present in appreciable amounts in cardiac muscle and in brain

(iii) Small amounts are present in the lung, thyroid and adrenals

(iv) It is apparently not present in liver and kidney and is not detectable in erythrocyte Hence, serum levels are not affected by hemolysis

Concentration

The normal level in serum is 12 to 99 U/l for males
" " " " 10 to 66 U/l for females } by Rosalki method.

Diagnostic significance

1 There is a marked increase in serum creatine kinase levels, upto 50 times normal in patients suffering from the Duchenne type of muscular dystrophy There is a small elevation in this enzyme activity in patients suffering from certain other forms of muscular dystrophy like limb-girdle and myotonic types.

2 Enzyme levels are normal in muscular atrophy resulting from neurological disorders or associated with hyperthyroidism

3 Increased levels are also found in muscle trauma, in polymyositis, in MC Ardle's syndrome and after severe exercise

4 The activity of this enzyme is increased within 12 hours after the onset of an acute myocardial infarction The level returns to normal in 3 or 4 days.

5 Increased levels of it can also occur in individuals with hypothyroidism, in patients with diabetic ketoacidosis, after pulmonary infarction, in the presence of convulsive disorders and in malignant hyperthermia

CARBONIC ANHYDRASE

This is an enzyme which is associated with the hemoglobin in the red-cells (never in the plasma) It has been isolated in highly purified form and shown to be a zinc protein complex

Source (i) Small amounts of carbonic anhydrase are found in muscle tissue, in the pancreas and in spermatozoa

(ii) Much larger quantities occur in the parietal cells of the stomach

Function

1 It specifically catalyzes the removal of CO_2 from H_2CO_3 The reaction is reversible

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

2. In the stomach it is involved in the secretion of hydrochloric acid

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

3 Since it occurs in the tubules of the kidney it is also involved in hydrogen ion secretion

MECHANISM OF ENZYME ACTION

1 Fisher in 1894 first proposed that enzyme is like key which fits into lock

2 In 1913, Michaelis and Menton proposed that enzyme (E) combines with the substrate (S) to form enzyme-substrate complex (ES) This complex finally gives rise to the reaction product(s) (P) and the enzymes becomes free for further reaction.

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

Fig 10.26.

During the complex formation the substrate molecules are attached to the specific groups on the enzyme molecules These specific groups are said to be 'active groups' and the regions on which these groups are located are said to be "active sites" The active groups are sulfhydryl (—SH) group of cysteine, phenolic group of tyrosine (as in pepsin), alcoholic group of serine (as in trypsin and chymotrypsin) and imidazole group of histidine

3 In 1963, Koshland proposed "induced fit mechanism" for mode of enzyme action According to this, the substrate fits the active sites effectively by inducing a configurational change in the enzyme Thus, the complexes are formed by multiple bonding (covalent, hydrogen or electrostatic) The functional groups of the active sites are arranged in a definite spatial configuration for which d or l isomer of a substance can act as a substrate for an enzyme

Exercise

1 Define Enzyme What is the mechanism of their action ? Discuss the factors which influence enzyme action (R U 76A , Muz 72 March , Mith 67S 74A , R U 72S)

2 How are enzymes classified ? Describe the general properties of enzymes and mention their mechanism of action (M U 75S)

3 Describe the mechanism of enzyme action and mention the role of coenzyme if required (Bn U 76A)

4 Most of the vitamins are involved in Enzyme catalyzed reactions—Elucidate with at least two examples (P U 69A)

5 Discuss the factors that regulate enzyme catalyzed reactions in the body (P U 72S)

6 Short notes on

(a) Mechanism of enzyme action (Bh U 75S , R U 73A)
(b) Specificity of enzyme action (Mith 62A)
(c) Competitive inhibition of enzymes (R U 66A, 70A Mith 73A)
(d) Enzyme inhibitor (Bh U 75S)
(e) Isoenzymes (Bh U 76S , P U 71A)
(f) Coenzymes (Muz 75S R U 64A 70S Mith 61S 68A)
(g) Lactic dehydrogenase (R U 69A)
(h) Coenzyme A (Bh U 76A P U 72A)
(i) Allosteric enzymes (P U 76A)
(j) Carbonic anhydrase (P U 74A)
(k) Transaminases (M U 76A)
(l) Creatine kinase (P U 72S)
(m) Lysozyme (B U 76A)
(n) Lysosome (B U 77S)
(O) Apoenzyme holoenzyme and coenzyme (C.U 1983)

7 Write in short the effects of the following factors on the activity of an enzyme Explain each effect along with graph (C.U 1982)

(a) Temperature

(b) Concentration of substrate

(c) Competitive inhibitors

8 Fill up the gaps with correct word

Activation of enzymes can be brought about by — and — (C.U 1981)

9 Enumerate coenzymes of nicotinic acid Write one reaction for each of them to illustrate their actions (C.U 1981)

CHAPTER 11

BIOLOGIC OXIDATION

Oxidation is chemically defined as the removal of electrons and reduction is the gain of electrons

Fig 11 1

Substrate molecules are oxidized by removal of hydrogen by dehydrogenases The reduced dehydrogenases are then reoxidized by a group of respiratory catalysts known as cytochrome system The substrate is thus oxidized by both processes The reducing equivalents ultimately react with molecular oxygen in the presence of cytochrome oxidase, the last member of the cytochrome system

Respiratory Chain The sequence of enzymes and carriers responsible for the transport of reducing equivalents from substrates to molecular oxygen is known as respiratory chain.

The respiratory chain is localized within the mitochondria Formation of ATP in the mitochondria is the active area of research

Redox Potential In oxidation and reduction reactions, the free energy exchange is proportionate to the tendency of reactants to donate or accept electrons This is expressed as an *oxidation reduction or redox potential*

Enzymes and coenzymes involved in oxidation and reduction

In 1961 the International Union of Biochemistry has designated all enzymes concerned in oxidative processes as oxidoreductases These are classified into 5 groups

1 Oxidases

(a) Enzymes that catalyze the removal of hydrogen from a substrate but use only oxygen as a hydrogen acceptor to form water as a reaction product (with the exception of uricase and monoamine oxidase which form H_2O_2)

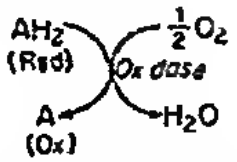


Fig 11.2 Oxidation of a metabolite by oxidase

(b) They are conjugated proteins containing copper as prosthetic groups

(i) Cytochrome oxidase

(a) Cytochrome oxidase is a hemoprotein widely distributed in plants and animal tissues

(b) It is the terminal component of respiratory chain found in mitochondria

(c) It is poisoned by cyanide and hydrogen sulfide

(d) More recent studies show that 2 cytochromes are combined with the same protein and the complex is known as cytochrome aa_3

(e) Cytochrome aa_3 contains 2 molecules of heme A each having one Fe atom 2 atoms of Cu are also present which are associated with the cytochrome oxidase activity

(ii) Phenolase (tyrosinase, polyphenol oxidase, catechol oxidase)

(a) It is a copper containing enzyme
(b) It converts monophenol to O quinones

(iii) Laccase

(a) It is widely distributed in plants and animals
(b) It converts P hydroquinones to P quinones
(c) It also contains copper

(iv) Ascorbic oxidase

(a) It contains copper
(b) It is found only in plants

(v) Uricase

(a) It also contains copper
(b) It catalyzes the oxidation of uric acid to allantoin

(vi) Monoamine oxidase

(a) It is found in the mitochondria of several tissues
(b) It oxidizes epinephrine and tyramine

2 Aerobic dehydrogenases

(a) They catalyze the removal of hydrogen from a substrate and use either oxygen or artificial substances such as methylene blue as hydrogen acceptor

(b) H_2O_2 is formed as a product

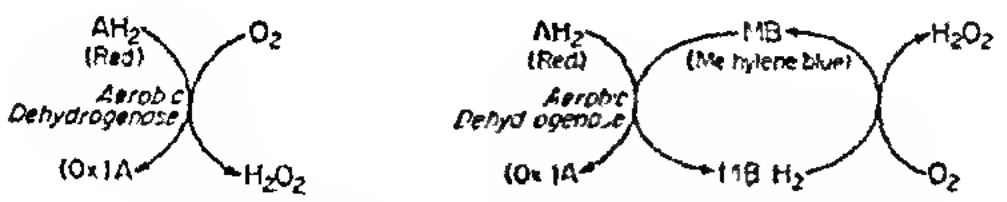


Fig 11 3 Oxidation of a metabolite by an aerobic dehydrogenase

(c) They are flavoprotein enzymes having FMN (flavin mononucleotide) or FAD (flavin adenine dinucleotide) as prosthetic groups

(d) Many of the flavoprotein enzymes contain a *metal* for which they are known as *metalloflavoprotein* enzymes

(i) D amino acid dehydrogenase (D-amino acid oxidase)

(a) It is an FAD-linked enzyme

(b) It is found particularly in liver and kidney

(c) It catalyzes the oxidative deamination of the unnatural (D-) forms of amino acids

(ii) L-amino acid dehydrogenase (L-amino acid oxidase)

(a) It is an FMN linked enzyme

(b) It is found in kidney

(c) It catalyzes the oxidative deamination of naturally occurring L amino acids

(iii) Xanthine dehydrogenase (Xanthine oxidase)

(a) It occurs in milk and in liver

(b) In the liver it converts purine bases to uric acid

(c) It contains FAD as the prosthetic group

(d) It is highly significant in the liver and kidneys of birds which excrete uric acid as the end product of purine metabolism and also of protein and amino acid catabolism

(e) It is a metalloflavoprotein containing nonheme iron and molybdenum

(f) It also oxidizes all aldehydes

(iv) Aldehyde dehydrogenase (aldehyde oxidase)

(a) It is an FAD linked enzyme

(b) It is present in pig and other mammalian livers

(c) It is also a metalloflavoprotein containing nonheme iron and molybdenum.

(d) It oxidizes aldehydes

(v) Glucose oxidase

(a) It is an FAD linked enzyme

(b) It is prepared from fungi

(c) It is used in estimating glucose

3. Anaerobic dehydrogenases

(a) They catalyze the removal of hydrogen from a substrate but not able to use oxygen as hydrogen acceptor

(b) They transfer hydrogen from one substrate to another by oxidation reduction reaction not involving a respiratory chain (shown in Fig. 11.4)

(c) They perform oxidation of a metabolite utilizing several components of a respiratory chain (shown in Fig. 11.5)

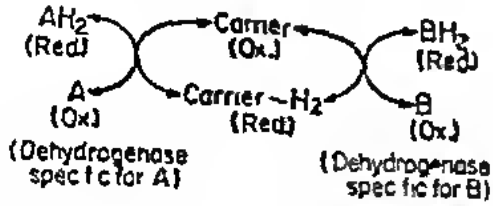


Fig 11 4 *Oxidation of a metabolite by anaerobic dehydrogenases not involving a respiratory chain.*

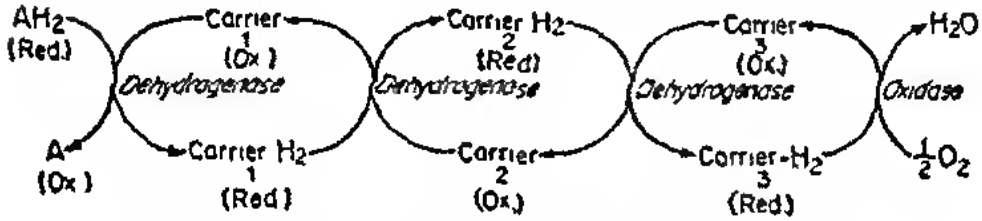


Fig 11 5 Oxidation of a metabolite by aerobic dehydrogenases by several components of a respiratory chain

(i) **Dehydrogenases dependent on Nicotinamide Coenzymes ·**

(a) They are linked as coenzymes either to NAD (Nicotinamide adenine dinucleotide) or to NADP (Nicotinamide adenine dinucleotide phosphate)

(b) The coenzymes are reduced by the particular substrate of the dehydrogenase and reoxidized by a suitable electron acceptor and synthesized from the vitamin niacin (nicotinic acid and nicotinamide)

(c) NAD linked dehydrogenases catalyze oxidoreduction reactions in glycolysis, the citric acid cycle and in the respiratory chain of mitochondria

(d) NADP-linked dehydrogenases are found in fatty acid and steroid synthesis in the extramitochondria They are also found in hexose monophosphate shunt

(e) Some nicotinamide coenzyme dependent dehydrogenases contain *Zinc*, particularly alcohol dehydrogenase from liver and glyceraldehyde-3 phosphate dehydrogennse from skeletal muscle The zinc ions do not take part in the oxidation and reduction

(ii) **Dehydrogenases dependent on Riboflavin Prosthetic Groups ·**

(a) Most of the riboflavin linked anaerobic dehydrogenases are concerned with electron transport in the respiratory chain

(b) Succinate dehydrogenase, acyl—CoA dehydrogenase and mitochondrial glycerol-3-phosphate dehydrogenase transfer electrons directly from the substrate to the respiratory chain

(c) In the dehydrogenation of reduced lipoate, an intermediate in the oxidative decarboxylation of pyruvate and α-Ketoglutarate, the flavoprotein (FAD) due to the low redox potential acts as a carrier of electrons from reduced lipoate to NAD^+. The electron transferring flavoprotein is an intermediary carrier of electrons between acyl—CoA dehydrogenase and the respiratory chain

(iii) The Cytochromes .

(a) The cytochromes excepting cytochrome oxidase are anaerobic dehydrogenases They are involved as carriers of electrons from flavoproteins to cytochrome oxidase in the respiratory chain

(b) They are iron-containing hemoproteins in which iron becomes Fe^{+++} and Fe^{++} during oxidation and reduction The cytochromes in the respiratory chain are b, c_1, c, a and a_3

(c) Cytochromes are also found in the endoplasmic reticulum (cytochromes P-450 and b_5), plant cells, bacteria and yeast

4 Hydroperoxidases

They utilize hydrogen peroxide as a substrate Two enzymes fall into this category (i) Peroxidase, (ii) Catalase

(i) Peroxidase :

(a) It is found in milk and leukocytes and the prosthetic group is protoheme

(b) It catalyzes the reduction of hydrogen peroxide by the help of ascorbic acid, quinones and cytochrome C which act as electron acceptors The reaction is complex but the overall reaction is as follows

$$H_2O_2 + AH_2 \xrightarrow{Peroxidase} 2H_2O + A$$

Fig 11 6

(ii) Catalase

(a) It is a hemoprotein and found in blood and liver

(b) It uses one molecule of H_2O_2 as a substrate electron donor and another molecule as electron acceptor

$$2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2$$

Fig 11 7

(c) Its function is to destroy H_2O_2 formed by the action of aerobic dehydrogenases

5 Oxygenases :

They catalyze the incorporation of oxygen into a substrate molecule

(i) Dioxygenases (Oxygentransferases, true oxygenases)

(a) They catalyze the incorporation of two atoms of oxygen (O_2) into the substrate

$$A + O_2 \longrightarrow AO_2$$

Fig 11.8

(b) Enzymes containing iron as a prosthetic group e g homogentisate dioxygenase, 3-hydroxyxanthranilate dioxygenase and enzymes utilizing heme as a prosthetic group such as L-tryptophan dioxygenase (tryptophan pyrrolase) from the liver.

(ii) Mono-oxygenase (Mixed function oxidases, Hydroxylases) :

(a) They catalyze the incorporation of only one atom of the oxygen molecule into a substrate The other oxygen atom is reduced to water A cosubstrate is necessary for this purpose.

$$A{-}H + O_2 + BH_2 \longrightarrow A{-}OH + H_2O + B$$

Fig 11 9

(b) Many of the enzymes involved in steroid synthesis are mono-oxygenase using NADPH as a cosubstrate They are found mainly in the endoplasmic reticulum (microsomes) of the liver and in mitochondria and the microsomes of the adrenal glands

(c) They are also involved in the metabolism of many drugs by hydroxylation They are found in the microsomes of the liver together with cytochrome P-450 and cytochrome b_5 The drugs metabolized by this system are benzpyrene, aminopyrine, aniline, morphine and benzphetamine But phenobarbital induce the formation of microsomal enzymes and of cytochrome P-450

$$\underset{\text{(P-450C}}{DRUG{-}H + O_2 + 2Fe^{++} + 2H^+} \xrightarrow{Hydroxylase} \underset{\text{(P 450I}}{DRUG{-}OH + H_2O + 2Fe^{+++}}$$

Fig 11 10

(d) They are concerned with the synthesis or degradation of many different types of metabolites

SUPEROXIDE METABOLISM

1. Recently, Friedovich (1975) has suggested that the toxicity of oxygen is due to its conversion to *superoxide* Superoxide is formed when reduced flavins, e g xanthine dehydrogenases are reoxidized by molecular oxygen in the respiratory chain.

$$Enz\ H_2 + O_2 \longrightarrow Enz\ H + O_2^- + H^+$$

Fig 11 11

2 Superoxide can reduce oxidized cytochrome C

$$O_2^- + Fe^{+++} \longrightarrow O_2 + Fe^{++}$$

Fig 11 12

3 Superoxide can be removed by the specific enzyme *superoxide dismutase* in the presence of protons

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

Fig 11 13

Superoxide dismutase

(i) The cytosolic enzyme is composed of two similar subunits, each one contains Cu^{++} and Zn^{++}

(ii) The mitochondrial superoxide dismutase contains Mn^{++}

(iii) This enzyme is present in all tissues

(iv) It decomposes superoxide as soon as it is formed and thus protects the tissues against the harmful action of superoxide

(v) The enzyme is increased particularly in the lungs on the exposure of the animals to an atmosphere of 100% oxygen

THE RESPIRATORY CHAIN

The mitochondrion has been termed the "Power house" of the cell due to the following reasons :

1 Most of the useful energy derived from oxidation within the tissues is captured in the form of the high-energy intermediate, ATP

2 All the useful energy formed from the oxidation of fatty acids and amino acids and virtually all the energy formed from the oxidation of carbohydrates are available in the mitochondrion

3 For the production of this energy *the mitochondrion contains the series* of catalysts known as the respiratory chain which are concerned with the transport of reducing equivalents (hydrogen and electrons) and with their final reaction with oxygen to form water

4 Mitochondrion also contains the enzyme systems i e the eozymes of β-oxidation and of *the citric acid cycle* which are responsible for producing the reducing equivalents to the first place

These relationships are shown below .

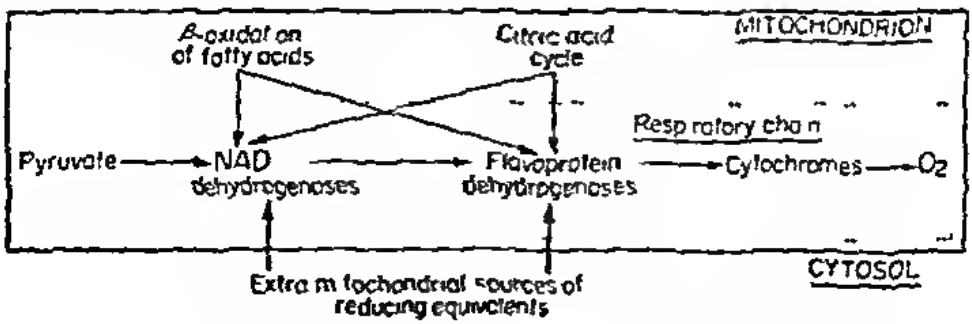


Fig 11 14 Relationship of electron transport in the respiratory chain

Organization of the Respiratory chain in Mitochondria

1 The major components of the respiratory chain are arranged in order of increasing redox potential shown below

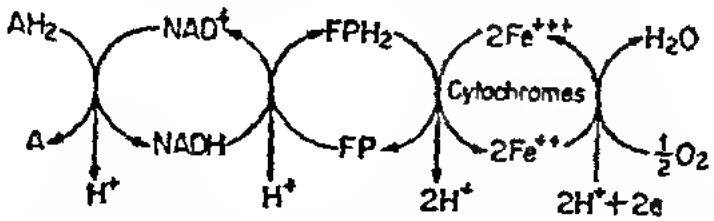


Fig 11 15 Transport of reducing equivalents through the respiratory chain

2 Electron flow through the chain from the more electronegative components to the more electropositive oxygen Thus the redox potential of a component of the chain gives the information regarding the position in the chain

3 The main respiratory chain in mitochondria starts from the NAD linked dehydrogen systems on the one hand through flavoproteins and cytochromes to molecular oxygen on the other The reducing equivalents are transported either as H^+ or as covalent hydrogen

4 All substrates are not linked through NAD specific dehydrogenases, some are linked directly to flavoprotein dehydrogenases because of their more positive redox potential and then linked to the cytochromes of the respiratory chain

5 Recently, it has been established that an additional carrier is present in between flavoproteins and cytochrome b Cytochrome b has the lowest redox potential among the cytochromes This additional carrier is said to be ubiquinone or coenzyme Q (CoQ)

6 An additional component, nonheme iron (NHI) is associated with the flavoproteins and with cytochrome b

7 At the electronegative end of the chain, dehydrogenase enzymes catalyze the transfer of electrons from the substrate to NAD of the chain Pyruvate and α-Ketoglutarate have complex dehydrogenase systems involving lipoate and FAD before the passage of electrons to NAD L(+) β hydroxyacyl—COA, D(−)-β hydroxybutyrate, glutamate malate and isocitrate dehydrogenases couple directly with NAD of the respiratory chain

8 The reduced NAD is oxidized by a metalloflavoprotein enzyme *NADH dehydrogenase* This enzyme contains nonheme iron and the prosthetic group FMN

9 Succinate, Glycerol 3 phosphate and acyl—CoA is linked directly to the respiratory chain through flavoprotein dehydrogenases The flavin moiety of these dehydrogenases is FAD and these enzymes contain nonheme iron (NHI) In the dehydrogenation of acyl—CoA, an additional flavoprotein, *electron transporting flavoprotien* (ETF), is essential for transferring of electrons to the respiratory chain

10 The cytochromes are arranged in order of increasing redox potential The terminal cytochrome a_3 (cytochrome oxidase) is responsible for the final combination of reducing equivalents with molecular oxygen to form H_2O This

enzyme allows the respiratory chain to function at the maximum rate until the tissue becomes virtually anoxic.

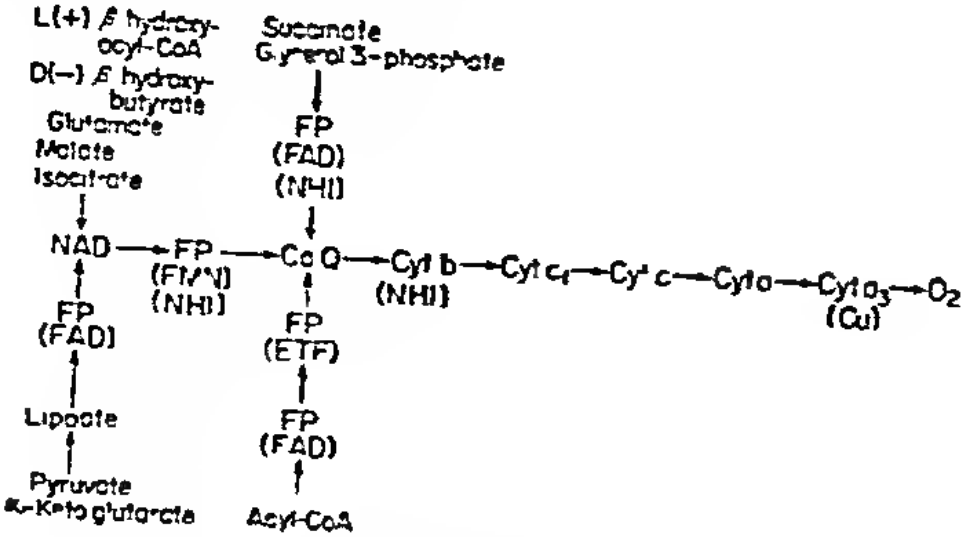


Fig 11 16 Components of the respiratory chain in mitochondria

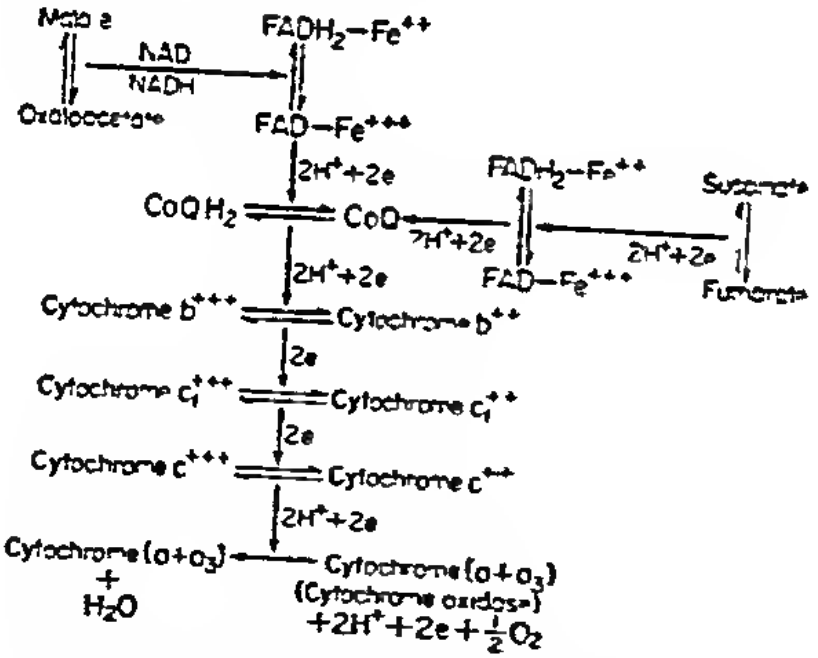


Fig 11 17 Electron transport oxidation of hydrogen removed from substrate to water

ENERGY CAPTURE IN THE RESPIRATORY CHAIN

1 When substrates are oxidized through an NAD-linked dehydrogenase, 3 mol inorganic phosphate are incorporated into 3 mol of ADP to form 3 mol of ATP per half mol of O_2 ($\frac{1}{2}O_2$) consumed, i e the P O ratio=3

2. When a substrate is oxidized through a flavoprotein-linked dehydrogenase, only 2 mol of ATP are formed, i e. P O ratio=2. These reactions are known as oxidative phosphorylation at the respiratory chain level

3 There must be a redox potential of about 0 2 volts or a free energy change of about 9 K Cal between components of the respiratory chain if that particular site is to support the coupled formation of 1 mol of ATP

Four sites fulfill these requirements in the respiratory chain—

(i) between NAD and flavoprotein
(ii) „ flavoprotein and cytochrome b.
(iii) „ cytochrome b and cytochrome c.
(iv) „ cytochrome a and oxygen

4 When ADP is deficient in the presence of excess substrate, 3 crossover points can be identified. These crossover points coincide with 3 of the possible sites on thermodynamic grounds. The 3 sites of phosphorylation have been designated as sites I, II and III respectively So P . O ratio of succinate is only 2 as site I is bypassed by the flavoprotein-linked succinate dehydrogenase

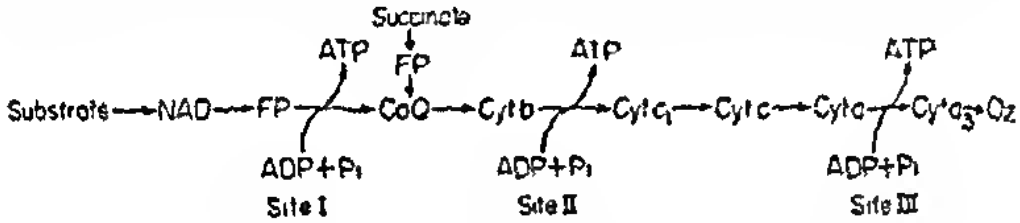


Fig 11.18. Sites of phosphorylation in the respiratory chain.

Inhibitors :

Site I—Inhibited by barbiturates such as amobarbital, by the antibiotic piericidin A, by the fish poison rotenone Some steroids and mercurials also affect this site

Site II—Inhibited by *Dimercaprol and antimycin* A.

Site III— „ „ „ „ „

Some redox potentials in mammalian oxidation systems .

System	*E'_o volts*
Oxygen/water . .	+0 82
Cytochrome a ; Fe^{+++}/Fe^{++}	+0 29
Cytochrome c ; Fe^{+++}/Fe^{++} ..	+0 22
CoQ ; ox /red.	+0 10
Cytochrome b , Fe^{+++}/Fe^{++}	+0 08
Flavoprotein ; ox./red.	—0 12
Fumarate/succinate	+0·03
$NAD^+/NADH$	—0 32
Oxalo acetate/Malate	—0 17
Lipoate ; ox./red.	—0 29

Free energy change

$\Delta G = -nF\ \Delta E_0$ coulomb joules

where,

n = number of electrons transferred

F = Faraday constant

ΔE_0 = Difference in redox potential between two systems

ΔG = Free energy

This can be converted into calories by dividing by the factor 4 18

$$\Delta G = \frac{nF \times \Delta E_0}{4\ 18} \text{calories}$$

Oxidative phosphorylation

The process by which ADP is phosphorylated by P_i to ATP in the respiratory chain is called oxidative phosphorylation

This process is limited to the mitochondria Various oxidations occur in other parts of the cell and liberate heat in stead of energy

Various mechanisms have been proposed for this process Chance and Williams have proposed the following mechanism in which I and X are intermediates of unknown nature B is the oxidized form of a carrier in the chain AH_2 is the reduced carrier which reduces B

$$B + I \rightleftharpoons BI$$

$$BI + AH_2 \rightleftharpoons BH_2 \sim I + A$$

$$BH_2 \sim I + X \rightleftharpoons X \sim I + BH_2$$

$$X \sim I + P_i \rightleftharpoons X \sim P_i + I$$

$$X \sim P_i + ADP \rightleftharpoons ATP + X$$

Uncoupling agents

A large number of substances uncouple oxidative phosphorylation in the respiratory chain i e they prevent the formation of ATP but permit oxidation to proceed with the generation of heat One of the uncoupling agents is 2, 4-dinitrophenol (DNP)

DNP uncouples phosphorylation by the hydrolysis of $X \sim I$ or $X \sim P_i$ It has been considered to increase the ATPase activity of mitochondria

$$X \sim I + DNP \rightleftharpoons X + DNP\ I$$

Other uncoupling agents are

(i) Methylene blue, (ii) Arsenite, (iii) Dicoumarol (iv) Aureomycin (v) Gramicidin

Thyroxine is an uncoupling agent and causes swelling of the mitochondria

Inhibitors

I Oligomycin completely blocks oxidation and phosphorylation in intact mitochondria

2 Atractyloside inhibits oxidative phosphorylation It inhibits the transporter of ADP into the mitochondrion and of ATP out of the mitochondrion

Coenzyme Q (Ubiquinone, CoQ)

1 It exists in mitochondria in the oxidized quinone form under aerobic conditions and in the reduced quinol form under anaerobic conditions

2 It has a structure very similar to vitamin K and vitamin E It is also similar to plastoquinone found in chloroplasts All of these possess poly isoprenoid side chain

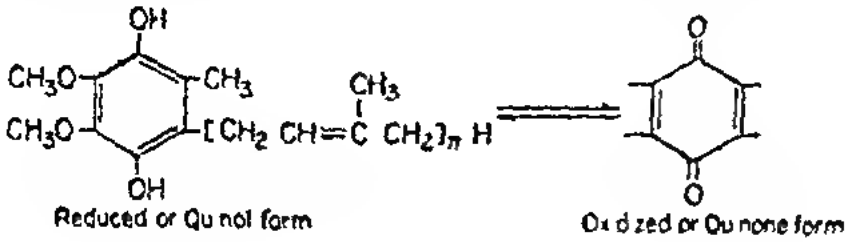


Fig 11 19

3 It is the additional carrier present in the respiratory chain linking the flavoprotein to cytochrome b

4 It is a constituent of the mitochondrial lipids

5 In mitochondria, there is a large stoichiometric excess of CoQ compared to other members of the respiratory chain

6 It is possible that there is more than one pool of CoQ and that some of it is not in the direct pathway of oxidation

Role of high energy phosphates

Low energy phosphates—These are the esterphosphates i e AMP found in the intermediates of glycolysis

High energy phosphates—The value is higher than that of ATP, e g ATP, ADP, phosphoenolpyruvate, creatine phosphate, arginine phosphate

High energy compounds—Acetyl CoA, active methionine (S adenosylmethionine) and UDPG (Uridine diphosphate glucose)

Lipmann introduced the symbol ~P indicating high energy phosphate bond ATP contains 2 high energy phosphate groups and ADP contains one

TRANSPORT OF SUBSTANCES INTO AND OUT OF MITOCHONDRIA

Oxidation of Extramitochondrial NADH

NADH is produced continuously in the cytosol by 3 phosphoglyceraldehyde dehydrogenase an enzyme in the Embden Meyerhof glycolysis, but it cannot penetrate the mitochondrial membrane Still then it is oxidized by the respiratory

chain in the mitochondria This is possible by the transfer of reducing equivalents through the mitochondrial membrane via substrate pairs linked by suitable dehydrogenases. The substrate pairs are lactate/pyruvate, dihydroxyacetone phosphate/glycerol-3-phosphate, and malate/oxaloacetate. The specific dehydrogenase is essential to be present on both sides of the mitochondrial membrane Lactate dehydrogenase is found only in the cytosol and glycerol-3-phosphate dehydrogenase is NAD-linked in the cytosol, whereas the enzyme is a flavoprotein enzyme found in the mitochondria The mechanism of transfer is shown in the figure 11.20. Since the mitochondrial enzyme is linked to the respiratory chain via a flavoprotein rather than NAD, only 2 mol of ATP are formed instead of 3 mol of ATP Rapid oxidation of NADH occurs only when aspartate-α-keto-glutarate transaminase and malate dehydrogenase together with glutamate, aspartate and malate are added to mitochondria The malate "shuttle" system is also shown in figure 11.21. The complexity of this system is due to the impermeability of the mitochondrial membrane to oxaloacetate

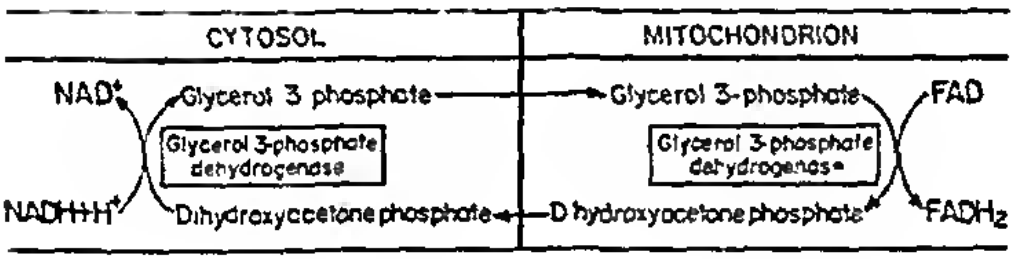


Fig 11.20 Glycerophosphate shuttle for transfer of reducing equivalents from the cytosol into the mitochondrion

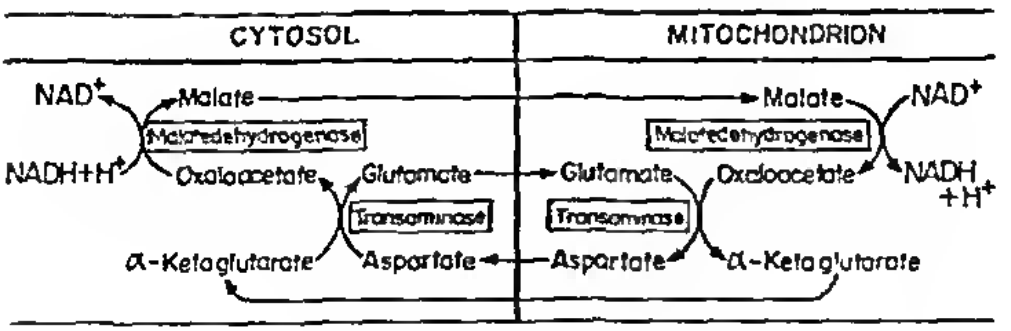


Fig 11.21 Malate shuttle for transfer of reducing equivalents from the cytosol into the mitochondrion

Energy-linked ion transport in Mitochondria

Actively respiring mitochondria maintain or accumulate cations such as K^+, Na^+, Ca^{++}, and Mg^{++}. Loss of ions from the mitochondria is due to the uncoupling with dinitrophenol. The ion uptake is not inhibited by oligomycin on the fact that the energy need not be supplied by phosphorylation of ADP.

Mitochondrial Transporter Systems

Oxygen, water, CO_2, β-hydroxybutyrate, acetoacetate and acetate are freely permeable to the inner mitochondrial membrane. Long chain fatty acids are transported into the mitochondria by the help of carnitine system. There is a special carrier for pyruvate also. Specific transporter or carrier systems are required for the transport of amino acids, dicarboxylate and tricarboxylate anions across the membrane. Monocarboxylate anions are more readily penetrated owing to the lesser degree of dissociation.

The transport of di- and tricarboxylate anions is closely linked to that of inorganic phosphate which penetrates readily as the $H_2PO_4^-$ ion in exchange for OH^-. The uptake of malate by the dicarboxylate transporter requires inorganic phosphate for exchange in the opposite direction. The uptake of citrate, isocitrate or CiS-aconitate by the tricarboxylate transporter requires malate in exchange. α-ketoglutarate transport also requires an exchange with malate. Thus, osmotic balance is maintained by the use of exchange mechanisms. Citrate transport across the mitochondrial membrane depends not only on malate transport but on the transport of inorganic phosphate also. The adenine nucleotide transporter allowed the exchange of ATP and ADP but not AMP. Na^+ can be exchanged for H^+. Active uptake of Ca^{++} by mitochondria is facilitated by the membrane potential rather than by exchange with an ion of opposite charge. Phosphate transporter is inhibited by N-Ethylmaleimide and Adenine nucleotide transporter is inhibited by Atractyloside.

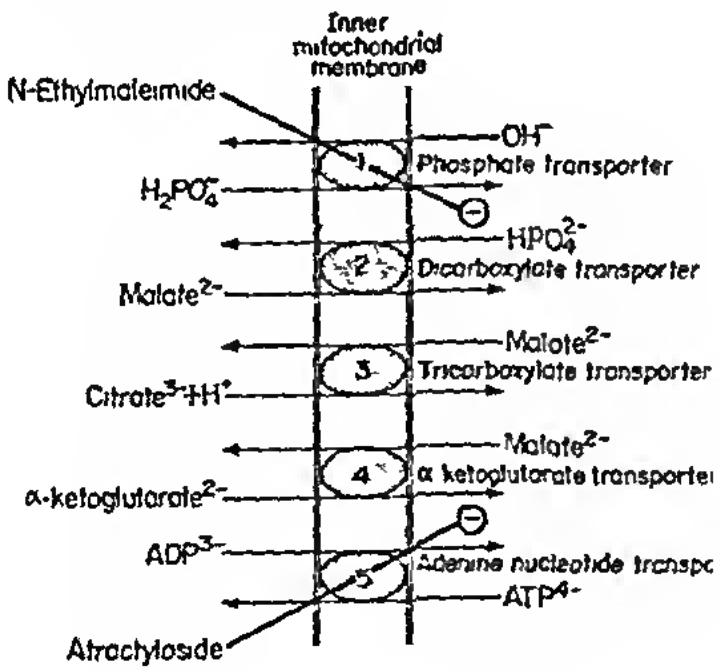


Fig. 11.22. Transporter systems in the mitochondrial membrane

ANATOMY AND FUNCTIONS OF THE MITOCHONDRIAL MEMBRANES

Anatomy

1 The outer membrane of mitochondria is permeable to most metabolites, but the inner membrane is selectively permeable.

2 The inner membrane has a matrix and the inner membrane is thrown into folds or cristae.

3 The presence of monoamine oxidase and a few other enzymes such as acyl CoA synthetase, phospholipase A_2, glycerophosphate acyltransferase, monoacyl glycerophosphate acyltransferase is found in the outer membrane and the outer membrane may be removed by the treatment with digitonin

4 Adenylate kinase is found in the intermembrane space.

5 Cardiolipin is concentrated in the inner membrane where most of the lipid is phospholipid

Functions

1 The phosphorylating subunits responsible for the production of ATP are scattered over the surface of the inner membrane. These subunits consist of several proteins collectively known as an F_1 subunit which project into the matrix. These F_1 subunits also contain the ATP synthetase. These subunits are attached, probably by a stalk, to a membrane protein subunit known as F_0 which extends through the membrane

2 One ATP molecule is formed from ADP and P_i when every proton pair passes through the F_0—F_1 complex Similar phosphorylating units are found inside the plasma membrane of bacteria but outside the membrane of chloroplasts.

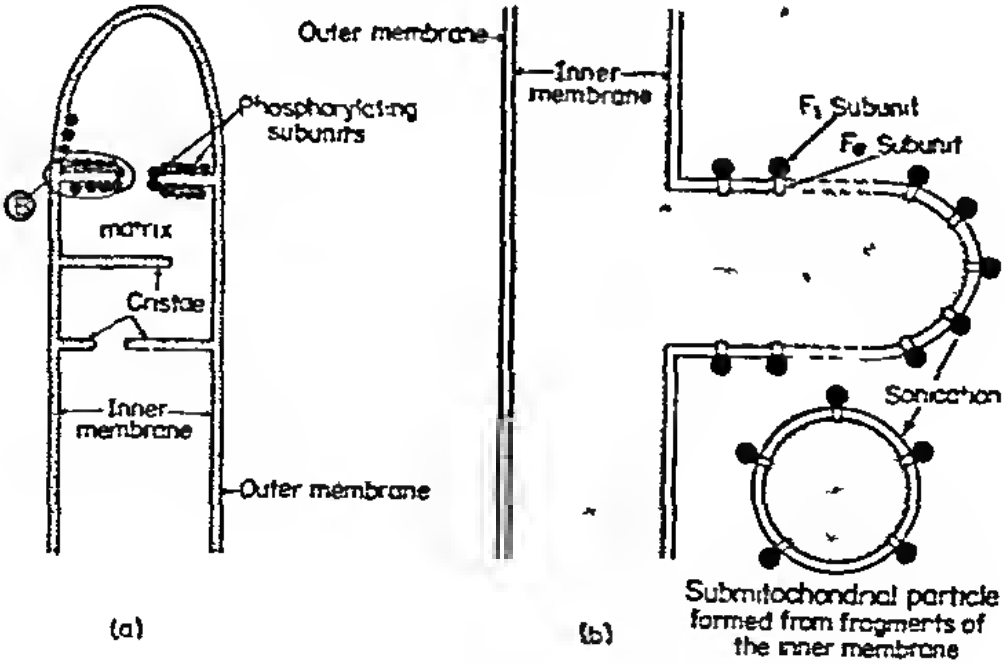


Fig 11.23 Structure of the mitochondrial membranes

3 Vesicles (submitochondrial particles) that are "inside-out" are formed on the inner mitochondrial membrane and therefore, the phosphorylating units are located on the outside of the membrane

4 The enzymes of the TCA cycle and β-oxidation of fatty acids are found in the matrix to associate mechanisms for transporting ions and fatty and other organic acids as well as nucleotides across the inner membrane

5 Succinate dehydrogenase is found on the inner surface of the inner mitochondrial membrane and it transports reducing equivalents into the respiratory chain at ubiquinone (CoQ)

6 β hydroxybutyrate dehydrogenase is also bound to the matrix side of the inner mitochondrial membrane

The Chemiosmotic Theory

Mitchell explains that the primary event in oxidative phosphorylation is the translocation of Protons (H^+) to the exterior of a coupling membrane driven by oxidation in the respiratory chain The membrane is impermeable to protons which accumulate outside the membrane creating an *electrochemical potential difference across the membrane* This electrochemical potential difference is utilized to drive a *membrane located ATP Synthetase* which in the presence of P_i + ADP forms ATP (Fig 11 24)

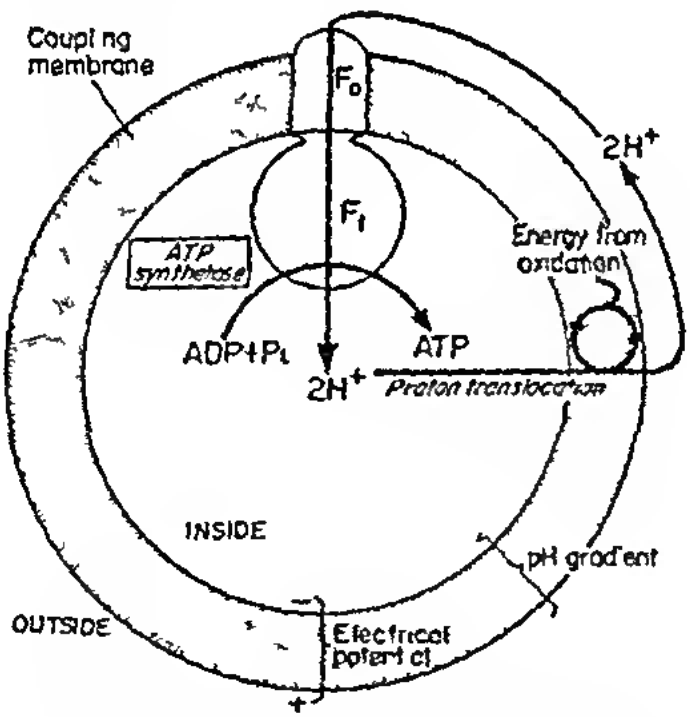


Fig 11 24 *Principles* of the chemiosmotic theory of oxidative phosphorylation F_1 F_o Protein subunits responsible for phosphorylation

It is suggested that the respiratory chain is folded into three oxidation/reduction (o/r) loops in the membrane, each loop functionally corresponds to site I, site II, and site III of the respiratory chain A single loop consists of a hydrogen carrier and an electron carrier shown in fig 11 25 A configuration of the respiratory chain folded into three functional o/r loops is shown in fig 11 26

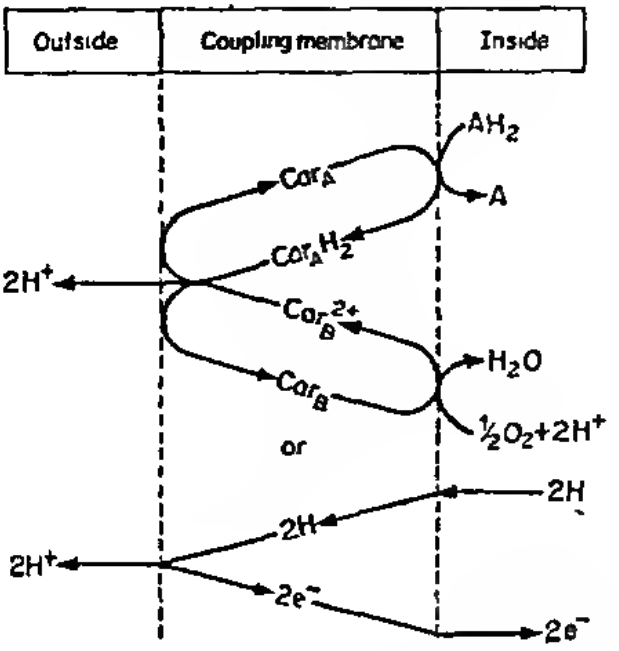


Fig. 11.25. Oxidation/reduction (O/r) loop (chemiosmotic theory)

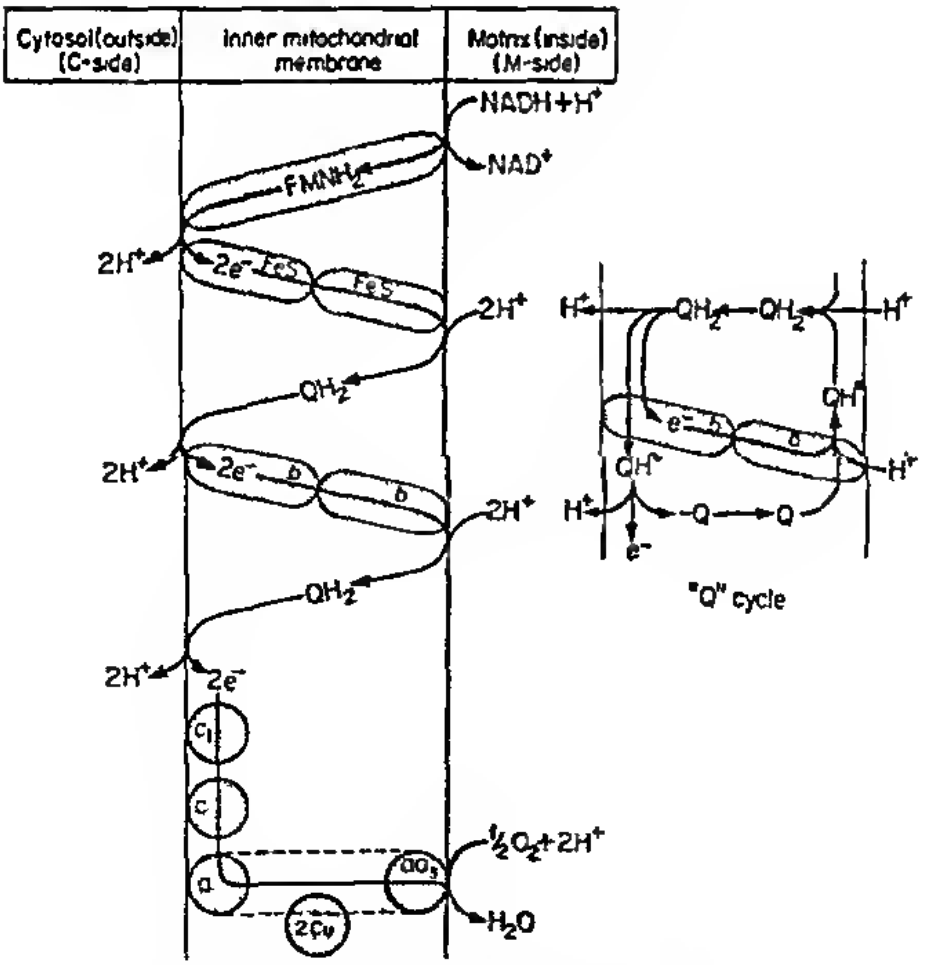


Fig. 11.26. Configuration of O/r loops in the respiratory chain (Chemiosmotic Theory)

Each electron pair transferred from NADH to oxygen causes six protons to be translocated from the inside to the outside of the mitochondrial membrane. NADH first donates one proton and two electrons, which, together with another proton from the internal medium, reduce FMN to $FMNH_2$. FMN is considered to extend the full width of the membrane and enables it to release two protons to the outside of the membrane and then to return two electrons to the inside surface via FeS proteins which is reduced. Each reduced FeS complex donates one electron to an ubiquinone (Q) molecule which upon taking up a proton from inside the membrane forms QH_2. QH_2, being lipid-soluble and a small molecule, is free to move to the outside of the membrane where it discharges a proton pair into the cytosol and donates two electrons to two molecules of the next carrier in the respiratory chain, cytochrome b. This electron carrier is thought to span the mitochondrial membrane, enabling the electrons to join another molecule of ubiquinone together with two more protons from the internal medium. The resulting QH_2 shuttles to the outer surface where two protons are liberated and two electrons passed to two molecules of cytochrome C. These electrons then pass through the remainder of the cytochrome chain, traversing the membrane to cytochrome aa_3 which lies on the inside of the membrane. At this site, two electrons combine with two H^+ from the internal medium and an oxygen atom to form water.

The phosphrylating subunits responsible for the production of ATP are scattered over the surface of the inner membrane. These consist of several proteins collectively known as an Fi subunit which project into the matrix and which contain the ATP synthetase (Fig. 11.24). These subunits are attached, possibly by a stalk, to a membrane Protein subunit known as F_o, which probably extends through the membrane. For every Proton pair passing through the F_o—F_1 complex, one ATP molecule is formed from ADP and Pi.

Mitchell suggested one model which is shown in fig. 11.27 below.

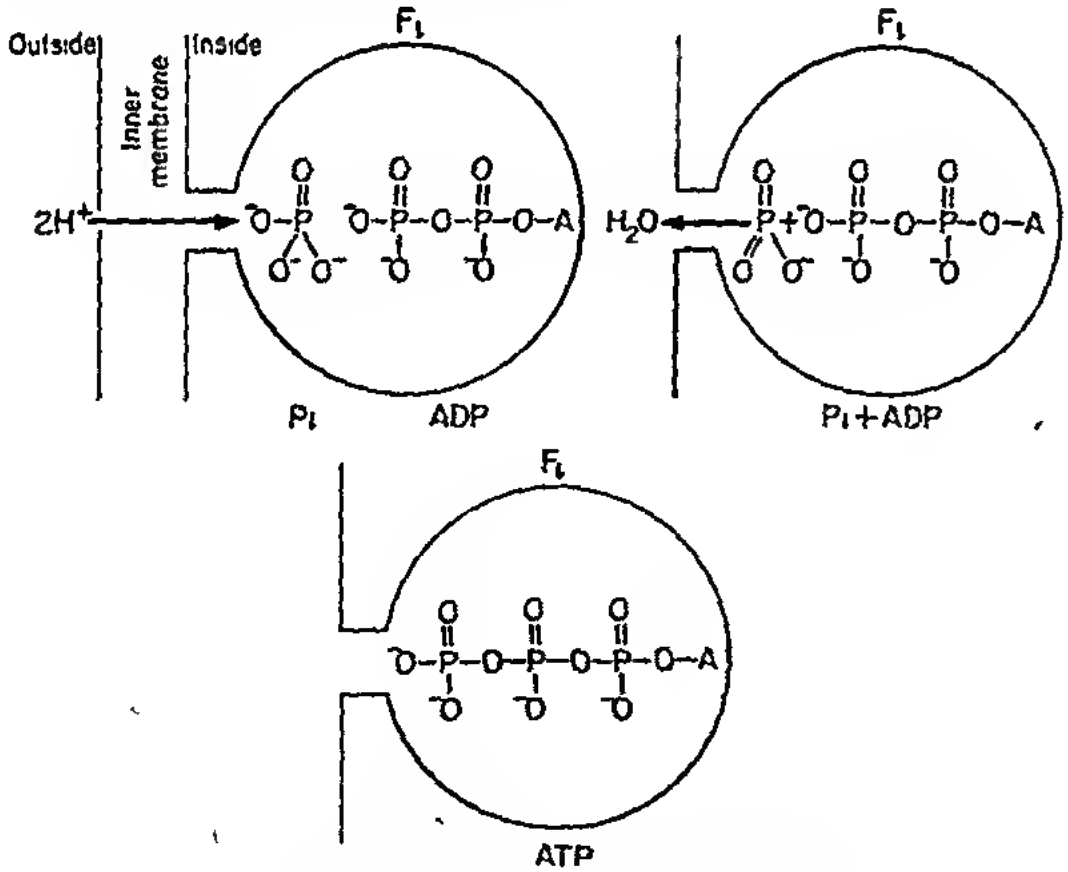


Fig. 11.27. Proton translocating ATP Synthetase (Mitchell)

A proton pair attacks one oxygen of P_i to form H_2O and an active form of P_i which immediately combines with ADP to form ATP Other studies have suggested that ATP synthesis is not the main energy requiring step rather it is the release of ATP from the active site This may involve conformational changes in the F_1 Subunit

The ion leakage would have to be balanced by extrusion of ions against the electric gradient to prevent swelling and lysis It was, therefore, postulated that the coupling membrane contains exchange diffusion systems for exchange of anions against OH^- ions and of cations against H^+ ions Such system would be necessary for uptake of ionized metabolites through the membrane.

The electrochemical Potential difference across the membrane would inhibit further transport of reducing equivalents through the o/r loops unless it was discharged by back translocation of protons across the membrane through the vectorial ATP synthetase system This depends on the availability of ADP and P_i

Several points arise from the chemiosmotic theory that have expermental support These are as follows

1 Mitochondria are generally impermeable to protons and other ions The specific transport systems enable ions to penetrate the inner mitochondrial membrane

2 Uncouplers like dinitrophenol increase the permeability of mitochondria to protons reducing the electrochemical potential for the generation of ATP

3 Addition of acid to the external medium leads to the generation of ATP

4 The P/H^+ (transported out) quotient of the ATP synthetase is $\frac{1}{2}$ and the H^+/O quotients for succinate and β hydroxybutyrate oxidation are 4 and 6 respectively, conforming with the expected P/O ratios of 2 and 3 respectively These ratios are compatible with the postulated existance of 3 o/r loops in the respiratory chain

5 Oxidative phosphorylation does not occur in soluble systems where there is no possibility of a vectorial ATP synthetase

Exercise

1 Discuss the oxidation and reduction in biological system Name some types of enzymes involved in the process (P U 69S)

2 Discuss the respiratory chain (P U 72A)

3 Write short notes on

(a) Coenzyme Q (R.U 70A)
(b) Oxidative phosphorylation (B U 74A)
(c) Superoxide dismutase (Bh U 73S)

4 What do you mean by biologic oxidation and oxidative phosphorylation? Describe in detail how the oxidation of a substrate take place through the respiratory chain in mitochondria mentioning the possible sites of ATP formation with reasons

Mention the names of some inhibitors of respiratory chain (C.U 1983)

CHAPTER 12

THE CHEMISTRY OF RESPIRATION

Definition :

Respiration is defined as the utilization of O_2 from inspired air by the body and elimination of Co_2 produced by the metabolites in the cells in the expired air.

Composition of Atmospheric and Expired Air :

	Atmospheric air (Inspired air)	*Expired air*
Oxygen	20·96%	15%
Carbon dioxide	0·04%	5%
Nitrogen	79%	79%

About $\frac{1}{4}$ of the oxygen of the inspired air has passed into the blood and has been replaced in the expired air by equal amount of Co_2.

Partial pressure of gases :

The atmospheric air contains nitrogen, oxygen, carbon dioxide and water vapour. The pressure exerted by a mixture of gases is equal to the sum of their partial pressures.

$$AP - P_{H_2O} = P_{N_2} + P_{Co_2} + P_{O_2}.$$

The partial pressure of the gases (Y) N_2, O_2 and Co_2 can be calculated as follows :

$$Py = \frac{(760 - P_{H_2O})X\%Y}{100}.$$

Diffusion of gases in the lungs :

1. The gases of the inspired air when come in contact with the alveolar membrane of the lung, the exchange of gas takes place following the usual laws of diffusion. Thus, the gas passes into the blood through the membrane.

2. The gas pressure in the blood is usually expressed as gas "tensions".

Examples : The oxygen tension (Po_2) is the pressure of the dry gas with which the dissolved oxygen in the blood is in equilibrium.

3. Oxygen tension in alveolar air : 107 mm. Hg.

Oxygen tension in venous blood : 40 mm. Hg.

Hence, a pressure difference of 67 mm. Hg. drives oxygen from the alveoli of the lung into the blood.

Co_2 tension in alveolar air : 36 mm. Hg.

Co_2 tension in venous blood : 46 mm. Hg.

The difference of 10 mm Hg is sufficient to drive Co_2 from the blood into the lung

In the resting state, a difference of 0 12 mm Hg in Co_2 tension causes the elimination of the gas

Nitrogen gas is physiologically inert because its tension is the same in venous blood and lung alveoli (570 mm Hg)

4 After this exchange of gases, the blood becomes arterial

Oxygen tension of arterial blood 100 mm Hg

Co_2 tension of arterial blood 40 mm Hg

Nitrogen tension of arterial blood 570 mm Hg

These gases are dissolved in the blood

5 The quantities of each of these gases which can be dissolved (calculated content in blood) is compared with the actual quantities found in the blood by the following data

	ml /dl		
	O_2	Co_2	N_2
Calculated content in blood	0 393	2 96	1 04
Actual quantities found in arterial blood	20 0	50 0	1 70
Actual quantities found in venous blood	14 0	56 0	1 70

THE TRANSPORT OF OXYGEN BY THE BLOOD

Function of hemoglobin

1 The transport of oxygen from the lungs to the tissues by the blood is due to the ability of hemoglobin to combine reversibly with oxygen

$$Hb + O_2 = Hbo_2$$

(Hb=Reduced (deoxygenated) hemoglobin , Hbo_2=Oxyhemoglobin)

2 At a tension of 100 mm Hg or more, hemoglobin is completely saturated The oxygen carrying power of the blood is absolutely a function of the hemoglobin (red cell) concentration

Dissociation of oxyhemoglobin

1 The vital relationship between the saturation of hemoglobin and the oxygen tension is shown below by the dissociation curve of oxyhemoglobin in which the per cent saturation is plotted against the oxygen tension

2 The curve drawn with Co_2 at a tension of 40 mm Hg. is considered as representative of the normal physiologic condition

3 The hemoglobin is 95-98% saturated when the oxygen tension is 100 mm Hg. in arterial blood There is a slight effect on the saturation of hemoglobin with the further increase in oxygen tension

4. The saturation of hemoglobin declines slowly with the fall in oxygen tension and a rapid evolution of oxygen takes place at the oxygen tension of 50 mm. Hg. This is the "unloading tension" of hemoglobin.

5. In the tissues, the oxygen tension is about 40 mm. Hg and the oxyhemoglobin dissociates and oxygen is readily available to the cells.

6. During the passage of blood through the tissues the oxygen content of the blood falls from 20 to 15 vol%. This gives a considerable reserve of oxygenated blood in the event of inadequate oxygenation at the lung.

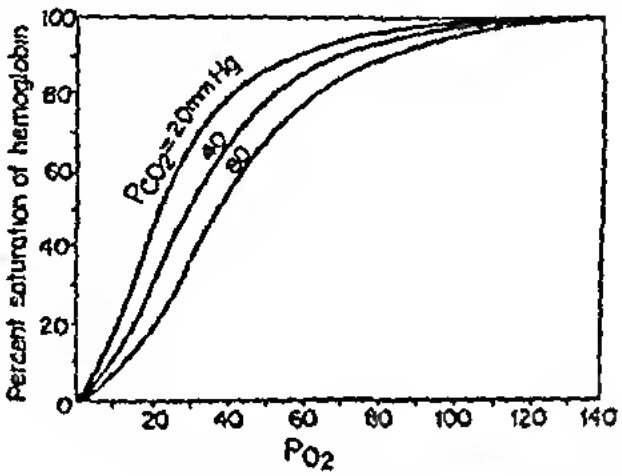


Fig. 12.1: The dissociation of hemoglobin.

Factors affecting the dissociation of oxyhemoglobin :

1. **Temperature :**

(a) A rise in temperature decreases hemoglobin saturation.

(b) At 25°C, hemoglobin is 88% saturated but at 37°C, it is only 56% saturated. Therefore, hemoglobin gives up oxygen more readily while passing from high to low oxygen tension (as from lungs to tissues) in warm-blooded animals than in cold-blooded animals.

2. **Electrolytes :**

At low oxygen tensions, oxyhemoglobin gives up oxygen more readily in the presence of electrolytes.

3. **Effect of CO_2 :**

(a) The influence of CO_2 on the shape of the dissociation curve is actually the effect of carbonic acid formation with the lowering of the P^H of the environment.

(b) The increase in acidity facilitates the dissociation of oxyhemoglobin.

(c) The ability of CO_2 to shift the slope of the oxyhemoglobin dissociation curve to the right is known as the *Bohr effect*. This effect is often described as causing a shift of the P-50 to the right. P-50 is the partial pressure (mm. Hg.) at which hemoglobin is 50% saturated. 2, 3-biphosphoglycerate, a compound formed during glycolysis in the red cells, also causes a significant shift of the P-50 to the right.

Carboxyhemoglobin :

1. Hemoglobin combines with carbon monoxide more readily than with oxygen (210 times as fast) to form cherry-red carboxyhemoglobin.

2 This reduces the amount of hemoglobin to carry oxygen

3 When the carbon monoxide in the inspired air is 0 02% headache and nausea occur

4 In case, the carbon monoxide concentration is only $\frac{1}{210}$ that of oxygen in the air (about 0 1% carbon monoxide), unconsciousness occurs in 1 hour and death in 4 hours

Clinical signs of variation in hemoglobin saturation

1 A decrease in normal oxygenation of blood gives a characteristic bluish appearance to the skin This is said to be cyanosis It is characteristic of cyanide poisoning where respiration is also impaired.

2 In severe anemia, the concentration of hemoglobin is too low and cyanosis does not take place although the oxygen content of the blood is reduced

3 In Co poisoning the formation of cherry red carboxyhemoglobin often produces a ruddy appearance in the lips

THE TRANSPORT OF CO_2 IN THE BLOOD

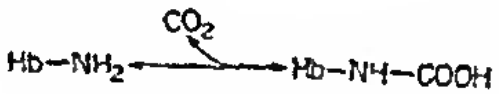
CO_2 is carried in the cells and in the plasma by the blood It exists in three forms The three main fractions are

1 A small amount of carbonic acid

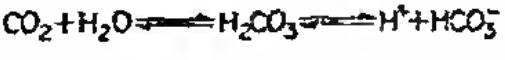
2 The "carbamino bound CO_2 which is transported in combination with proteins (mainly hemoglobin)

3 That carried as bicarbonate in combination with cations sodium or potassium

The carbamino-bound CO_2 is important in the exchange of this gas because of the high rate of reaction



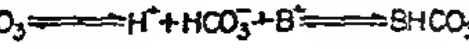
The amount of CO_2 dissolved in the blood is not large, but it is important because any change in its concentration causes the following equilibrium to shift



The above reaction is catalyzed by the enzyme *carbonic anhydrase*

Effect of CO_2 on blood P^H

1 CO_2 evolved from the tissues forms carbonic acid Most of the carbonic acid formed is promptly converted to bicarbonate as shown in the equation below (B^+ represents principally Na^+ or K^+)



2 At the P^H of blood (7 4), a ratio of 20 1 must exist between the bicarbonate and carbonic acid This ratio is calculated from the Henderson-Hasselbalch equation Any change in H^+ activity is met by an adjustment in the reaction Any alteration in the ratio disturbs the acid base balance of the blood in the direction of acidemia or alkalemia

THE BUFFER SYSTEMS OF THE BLOOD

1 Venous blood carries more CO_2 than arterial blood Hence, the P^H of venous blood is more acid than that of arterial blood by 0 01 0 03 units i e P^H 7 40 and 7 43 respectively

2 The blood buffers consist of the plasma proteins, hemoglobin, oxyhemo globin, bicarbonates and inorganic phosphates

3 When CO_2 enters the venous blood, the small decrease in P^H shifts the ratio of acid to salt in all the buffer pairs When the ratio is shifted to form more of the acid cations become available to form additional bicarbonate In this respect, the plasma phosphates and bicarbonates play a minor role

4 The buffering action of the plasma proteins is important because they release sufficient cations for the carriage of about 10% of the total CO_2

5 The phosphates in the red cells carry 25% of the total CO_2

6 The most important buffering role of hemoglobin and oxyhemoglobin which carries 60% of the CO_2 of the whole blood

The hemoglobin buffers

At the *lungs* the formation of oxyhemoglobin from reduced hemoglobin releases hydrogen ions which react with bicarbonate to form carbonic acid The low CO_2 tension in the lung shifts the equilibrium towards the production of CO_2 which is continually eliminated in the expired air

$$H^+ + HCO_3^- \longleftrightarrow H_2CO_3 \longleftrightarrow H_2O + CO_2$$

In the *tissues* the oxygen tension is reduced and hence oxyhemoglobin dissociates delivering O_2 to the cells and reduced hemoglobin is formed CO_2 produced by metabolism enters the blood where it is hydrated to form H_2CO_3 which ionizes to form H^+ and HCO_3^- Reduced hemoglobin acting as an anion accepts the H^+ ions forming acid reduced hemoglobin (HHb) Very little change in P^H occurs because the newly arrived H^+ ions are buffered by formation of very weak acid

When the blood returns to the lungs, these H^+ ions are released as a result of the formation of a stronger acid (oxyhemoglobin) and the newly released H^+ ion

is promptly neutralized by HCO_3^- This reaction is necessary for the liberation of CO_2 in the lungs

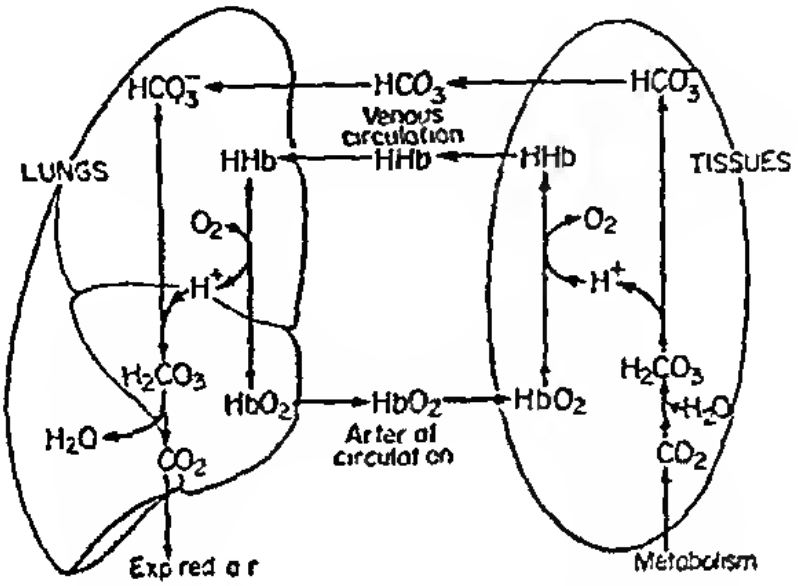


Fig 12.7 The buffering action of hemoglobin

The chloride shift

1 CO_2 reacts with water to form carbonic acid (H_2CO_3) mainly inside the red cell by the enzyme carbonic anhydrase present in the red cells

2 The carbonic acid is then buffered by the intracellular buffers (phosphate and hemoglobin) combining with potassium

3 Bicarbonate ion also returns to the plasma and exchanges with chloride which shifts into the cell when the tension of CO_2 increases in the blood

4 When the CO_2 tension is reduced, chloride leaves the cell and enters the plasma

5 Under normal conditions the red cell is impermeable to sodium or potassium But it is permeable to hydrogen, bicarbonate and chloride ions and intracellular sources of cation (potassium) are indirectly available to the plasma by chloride (anion) exchange This permits the carriage of additional CO_2 (as sodium bicarbonate) by plasma

6 The CO_2 entering the blood from the tissues passes into the red cells where it forms carbonic acid by carbonic anhydrase Some of the carbonic acid returns to the plasma The remainder reacts with hemoglobin buffers to form bicarbonate which then returns to the plasma in exchange of chloride The chloride is neutralized by potassium in the red cells

7 All of these reactions are reversible At the lung when the blood becomes arterial, chloride shifts back into the plasma, thus liberating intracellular

potassium to buffer the oxyhemoglobin and in the plasma, neutralizing the sodium which is liberated by the removal of CO_2 during respiration

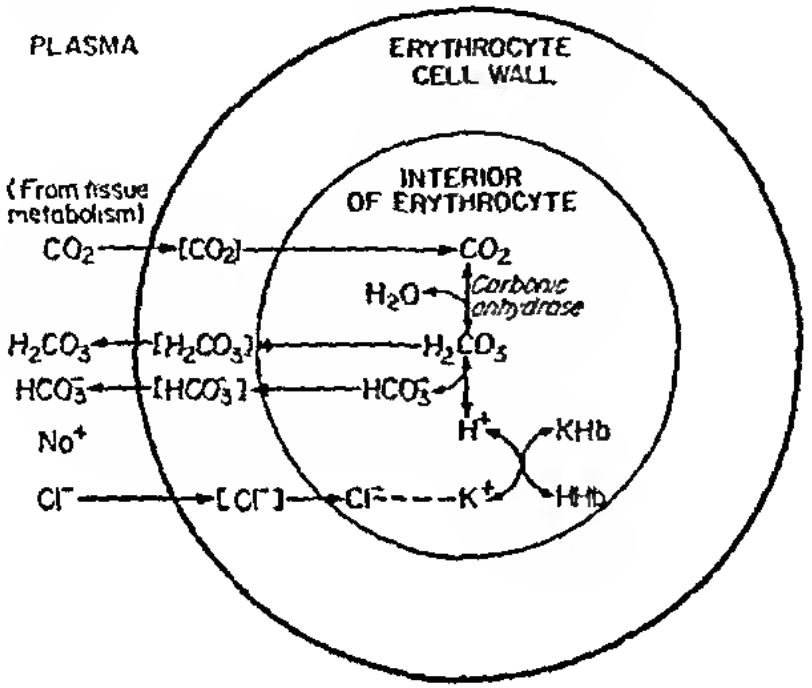


Fig 12 3 Chloride shift

ACID BASE BALANCE

Respiratory acidosis—This condition occurs when the accumulation of H_2CO_3 is caused in the blood under certain circumstances

Respiratory alkalosis—This condition occurs when the rate of elimination of CO_2 is excessive following the reduction of H_2CO_3 in the blood

Metabolic acidosis—This occurs in the deficit of bicarbonate without any change in H_2CO_3

Metabolic alkalosis—This happens in the excess of bicarbonate

Causes of disturbances in acid base balance

A Metabolic acidosis

1 It occurs in uncontrolled diabetes with ketosis

2 It occurs in some cases of vomiting when the fluids lost are not acid

3 It also occurs in renal disease, poisoning by an acid salt excessive loss of intestinal fluids (as in diarrhea or colitis) and excessive losses of electrolytes

4 Increased respiration is an important sign of uncompensated acidosis

B Respiratory acidosis

1 This occurs in any disease which impaires respiration such as pneumonia, congestive failure, asthma

2 It is also found to occur in the depression of the respiratory center (as by morphine poisoning)

3 A poorly functioning respirator also causes this condition

C. Metabolic alkalosis

1 Causes

(a) By the ingestion of large quantities of alkali in the treatment of peptic ulcer

(b) In the high intestinal obstruction (as in pyloric stenosis)

(c) After prolonged vomiting or after the excessive removal of gastric secretions containing hydrochloric acid.

(d) In Cushing's disease and during corticotropin or cortisone administration

2. Symptoms

(a) Tetany is caused due to the decrease in ionized serum calcium

(b) The respirations are slow and shallow

(c) The urine is alkaline

(d) Elevated blood bicarbonate

(e) Deficiency of potassium

D Respiratory alkalosis

1 This is brought about by hyperventilation

2. It may occur in patients in hepatic coma

The role of the kidney in acid base balance

1 The non volatile acids including lactic and pyruvic acids, hydrochloric acid phosphoric acid and sulphuric acid are produced by metabolic processes. About 50-150 mEq of the inorganic acids are eliminated by the kidneys in 24 hours These acids are partially buffered with cation, largely sodium In the distal tubules of the kidney some of the cations are reabsorbed and the PH of the urine falls

2 Kidney can buffer acids and conserve fixed base in the production of ammonia from amino acids The ammonia is utilized to neutralize acids when formed in excess

3 In kidney disease tubular reabsorption of sodium in exchange for hydrogen is poor and excessive retention of phosphates and sulphates. As a result acidosis occurs

HYPOXIA

Hypoxia means oxygen deficiency in any condition of insufficiency of tissue oxidation processes There are four types of hypoxia

1 Hypoxic hypoxia 2 Anemic hypoxia 3 Stagnant hypoxia. 4 Histotoxic hypoxia

1 Hypoxic hypoxia

This is characterized by normal oxygen capacity but diminished oxygen tension in the arterial blood with the result of varying degree of hemoglobin unsaturation

(a) **High altitude**—Hypoxia is caused by the diminished oxygen tension in the atmospheric air and consequently in the alveolar air and blood stream This leads to respiratory alkalosis

(b) **Rapid, shallow respiration**—Shallow breathing is due to insufficient oxygenation of blood This happens in diseases

(c) **Congenital heart disease**—In the congenital cardiac defect a portion of the blood may flow directly from the right to the left side of the heart without passing through the lungs As a result, the mixture of aerated and non aerated blood in the systemic circulation causes hypoxic hypoxia.

2 **Anemic hypoxia**

This is characterized by a diminution in the oxygen capacity of arterial blood due to the decrease in the amount of functioning hemoglobin

3 **Stagnant hypoxia**

This is due to circulatory insufficiency, the rate of blood flow through the tissues being retarded, with resulting increase in the percentage volume of oxygen removed from the blood in its passage through the capillaries

4 **Histotoxic hypoxia**

In this condition the oxygen supply is normal in every respect but the degree of oxygen utilization by the tissues is diminished because the tissue cells are poisoned in such a way that they cannot use oxygen properly

Exercise

1 Discuss the transport of O_2 and CO_2 in the blood (Lucknow Uni 71A)

2 Explain the buffer systems of the blood (Bombay Uni 72A)

3 Write short notes on

(a) Acid base balance (R U 73A)

(b) Diffusion of gases in the lungs (M U 74S)

CHAPTER 13

MUSCLE TISSUE

Chemistry

There are three types of muscle tissue in the body

1 Striated (voluntary) or skeletal muscle
2 Non striated (Involuntary) or smooth muscle
3 Cardiac muscle

Chemical composition of skeletal muscle

Water	75%
Protein	20%
Inorganic material certain organic extractives and carbohydrate (glycogen and its derivatives)	5%

MUSCLE STRUCTURE

1 Skeletal muscle is composed of fibrils surrounded by electrically excitable membrane, the *sarcolemma*

2 The individual muscle fibre consists of a bundle of many myofibrils arranged in parallel These are embedded by intramuscular fluid termed the *sarcoplasm*

3 The fluid contains glycogen the high energy compounds ATP and phosphocreatine and the enzymes of glycolysis

4 The functional unit of muscle is *sarcomere* It exists along the axis of a fibril at distances of 2.5 μ m

5 Alternating dark and light bands (A bands and I bands) are present in the myofibrils The central region of the A band (the H zone) is less dense than the rest of the band The I band is bisected by a very dense and narrow Z line

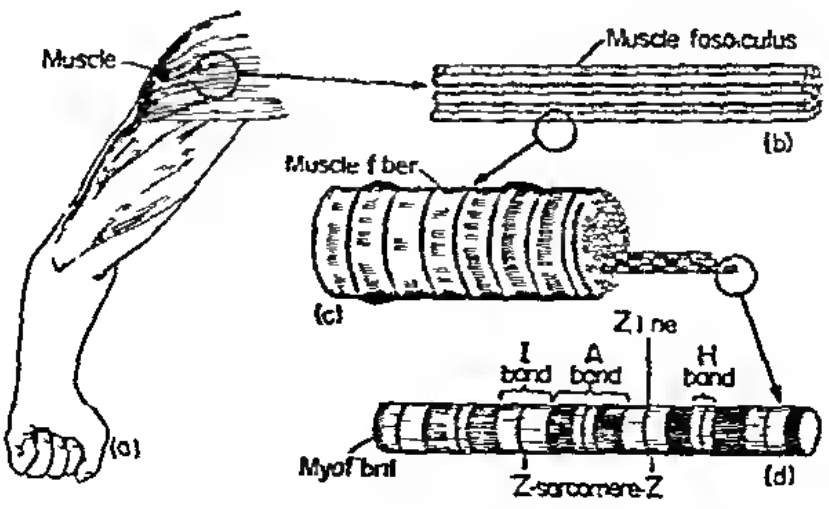


Fig 13 1 The structure of voluntary muscle

6. The cross section of myofibril under electron microscope show that myofibrils is constructed of two types of longitudinal filaments. One type confined to the A band (the thick filament) contains mainly the protein *myosin*. These filaments are about 16 nm in diameter. The other filament (the thin filament) lies in the I band but extends also into the A band. The thin filaments are smaller than those of myosin (about 6 nm in diameter).

7. The thin filaments contain the proteins actin, tropomyosin and troponin. Each thin filament lies between 3 thick filaments.

8. When the muscle contracts, the length of the A bands remains the same but the I bands disappear.

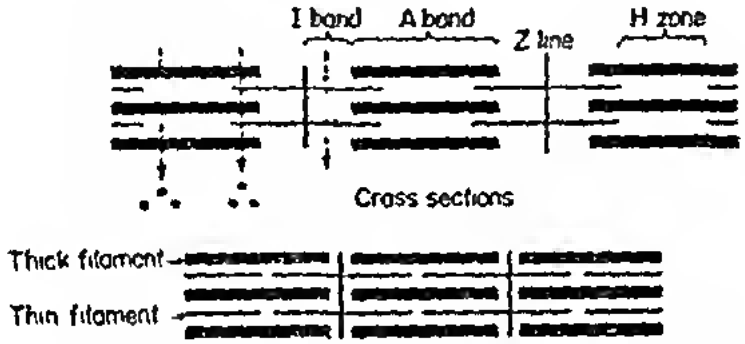


Fig. 13.2: Arrangement of filaments in striated muscle.

THE PROTEINS IN MUSCLE

The muscle fibrils are composed of:

(1) Proteins—20%.

(2) Water—75%.

(3) Inorganic materials, certain organic extractives and carbohydrates (glycogen and its derivatives)—5%.

Muscle proteins are characterized by their elasticity.

Myosin:

1. Myosin is the richly abundant muscle protein and it is a globulin.

2. It is soluble in dilute salt solutions and insoluble in water.

3. Its molecular weight is 5,00,000 containing 2 major chains and 4 light chains.

4. The enzyme *trypsin* cleaves myosin into two components—*meromyosins*—of unequal size. Therefore, they are termed as *light* and *heavy* meromyosins.

5. It has the adenosine tripbosphatase (ATPase) activity.

6. Myosin binds actin forming *actomyosin*. Light meromyosin does not combine *with actin*. But heavy *meromyosin* combines *with actin*.

7. Heavy meromyosin is a rod-shaped protein attached to the two globular components of myosin. The rod portion can be split off of the globular region by the action of papain and the resulting portions are termed as HMM (heavy meromyosin) S-2 (the rod) or HMM S-1 (the globular portion). Each

HMM S-1 possesses an active site for ATPase activity and a binding site for actin

8 The 4 light chains of myosin molecule are bound to the HMM S-1 fragments The light chains function as modulators of ATPase activity

9 Actomyosin is formed by 3 myosin molecules with 1 actin molecule

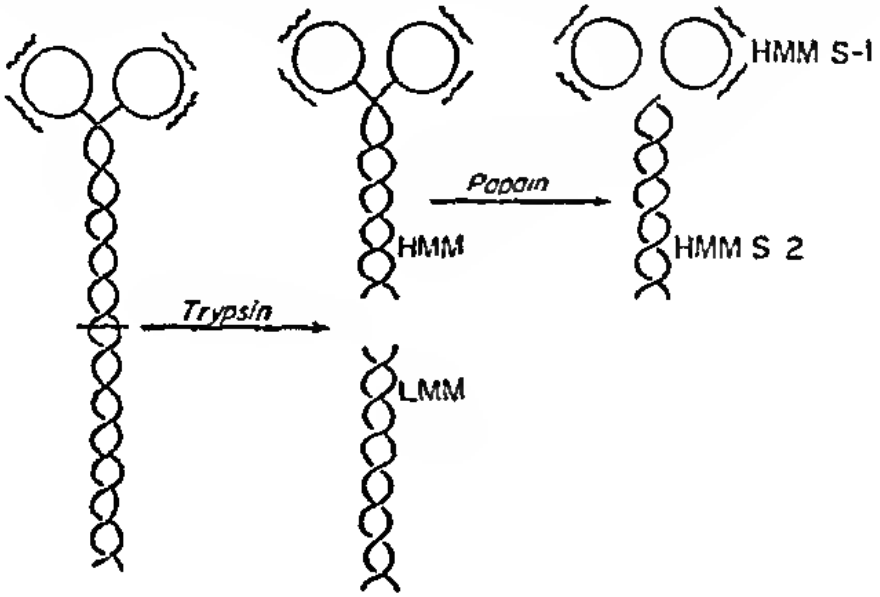


Fig 13 3 Enzymatic cleavage of myosin
HMM—Heavy meromyosin
LMM—Light meromyosin

Actin

1 Actin is a globulin of molecular weight 60,000

2 It is the major constituent of thin filaments in striated muscle

3 During the preparation of actin by extraction with solution of low ionic strength it is obtained as a molecular weight of 42,000 in a globular configuration called *G actin.*

4 G actin polymerizes to the fibrous form F actin in the presence of Mg^{++} and ATP is hydrolyzed to ADP and P_i is released Hence, ATP must be added to get depolymerization of F-actin to G actin

5 Actomyosin is formed by actin and myosin But ATP dissociates it into actin and myosin

$$\text{Actin} + \text{Myosin} \underset{\text{ATP}}{\rightleftharpoons} \text{Actomyosin}$$

Tropomyosin and Troponin .

1 Tropomyosin and troponin complex are proteins present in the thin filaments of muscle

2 Calcium ion has the effect on the interaction of actin and myosin which in turn is mediated by tropomyosin and troponin

3 Tropomyosin is a double stranded α helical rod of molecular weight 70,000 and present between the 2 strands of F-actin

4 Troponin is a complex of 3 polypeptide chains—TPC, TPI, TPT

5 The troponin complex is present in the thin actin filaments at intervals of 38 5 nm

6 A troponin complex with tropomyosin molecule regulates the activity of 7 actin monomers

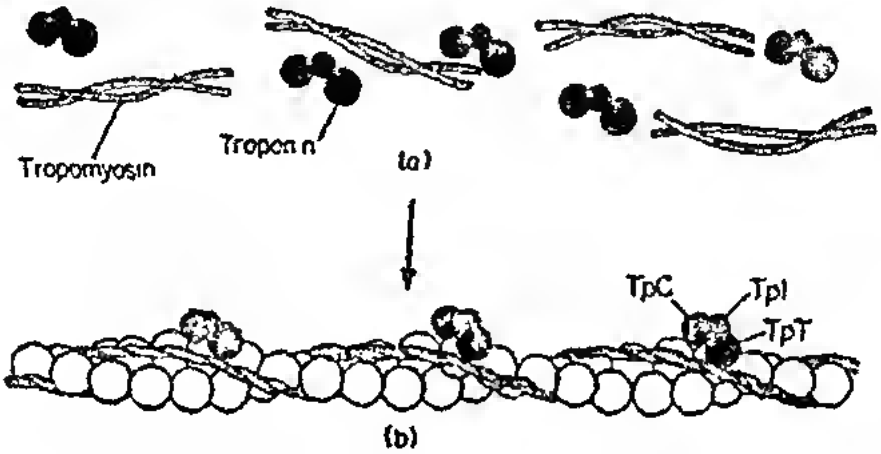


Fig 13 4 The representation of the thin filament

MOLLECULAR EVENTS IN

MUSCLE CONTRACTION

1 In absence of calcium ions, the interaction of actin and myosin is inhibited by troponin and tropomyosin

2 Calcium ions are released from the sarcolemma by the excitation of a motor nerve to the muscle.

3 The released calcium binds to the TPC portion of the troponin complex and are transmitted to tropomyosin and then to actin This allows actin to interact with myosin giving rise to muscular contraction with the hydrolysis of ATP which acts as an energy source This process continues until calcium ion is removed

Ca^{++} ⟶ Troponin ⟶ Tropomyosin ⟶ Actin ⟶ Myosin

Fig 13 5 Muscle contraction

4 When the nerve impulse arrives at the junction between the nerve ending and the muscle, the outer membrane of a muscle fibre is depolarized This depolarization is transmitted to the interior of the muscle fibre by the *sarcoplasmic reticulum* Calcium ions are remained in the sarcoplasmic reticulum in the resting state of the muscle by the help of ATP This system lowers the concentration of calcium ions in the sarcoplasm (cytoplasm) of resting muscle cells but increases calcium ions within the sarcoplasmic reticulum forming calcium

—binding—protein called calsequestrin Muscle contraction starts with the release of calcium from the sarcoplasmic reticulum via the troponin tropomyosin system mentioned above

MUSCLE PHOSPHAGENS

1 ATP is the immediate source of energy for muscular contraction But the amount of it in muscle is very small It is just enough to cause contraction for a fraction of a second

2. In vertebrate muscle, the source of high-energy phosphate is phosphocreatine which can transfer higher phosphate groups than ATP, and can donate a high-energy phosphate group to ADP to reform ATP

Phosphocreatine is the vertebrate phosphagen , whereas *phosphoarginine* is the invertebrate phosphagen

3 In the *resting state*, mammalian muscle contains 4-6 times phosphocreatine than ATP The enzyme *creatine kinase* (creatine phosphokinase, CPK) catalyzes the hydrolysis of creatine phosphate transferring high-energy phosphate from creatine phosphate to ADP (the Lohmann reaction) The reaction is reversible and the resynthesis of creatine phosphate takes place when ATP becomes available during the recovery period following a period of muscular contraction The transfer of phosphate from ATP to creatine to form creatine phosphate is catalyzed by the enzyme ATP-creatine transphosphorylase (shown in the figure 12 6)

4 ATP is also available in the muscle from ADP by the enzyme myokinase (adenylate kinase) which catalyzes the transfer of a high-energy phosphate from ADP and AMP is also formed

$$2\ ADP \xleftrightarrow{\text{Myokinase}} AMP + ATP$$

5 Iodoacetate can block resynthesis of ATP and phosphocreatine by preventing glycolysis

6 ATP is also formed in the muscle from glycogen under anaerobic conditions in which glycogen is converted to pyruvate or lactate by glycolysis. The initial breakdown of glycogen takes place by the enzyme phosphorylase which is activated by the calcium ions Lactate thus formed is reconverted to glucose in liver by *Cori cycle* and re-enters muscle

ATP is also formed from the oxidation of other fuels such as free fatty acids, ketone bodies and glucose Muscle contraction generally takes place under aerobic conditions

7 Fat is the ultimate source of energy for long periods of muscular exertion as in the case of migratory birds, whose total carbohydrate stores are quite inadequate to act as the sole source of energy and whose fat stores become depleted during migration The flight muscles of birds (red meat) are particularly well developed for aerobic oxidation of pyruvate, free fatty acids and ketone bodies and these muscles are also having a well developed vasculature to increase oxygenation and a high content of enzymes of the respiratory chain as well as of cytochromes and myoglobin

8 Heart muscle is also similar to the flight muscles of birds It can utilize free fatty acids, ketone bodies and even lactate which are oxidized under aerobic conditions

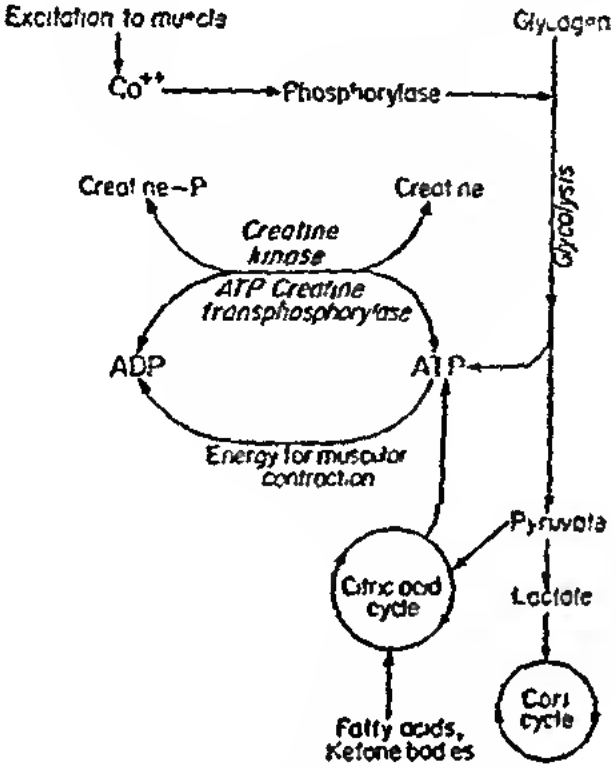


Fig 13 6 Muscle contraction

INORGANIC CONSTITUENTS OF MUSCLE

1 The cations of muscle (potassium, sodium, magnesium and calcium) are the same as in extracellular fluids excepting potassium which predominates in muscle

2 The anions are phosphate, chloride and small amounts of sulphates

3 A good amount of potassium is incorporated into the tissue during deposition of glycogen in the muscle and synthesis of protein Muscle weakness is a sign of potassium deficiency

4 Muscle calcium and magnesium act as activators or inhibitors of intra muscular enzyme systems

CELL MOTILITY AND THE CYTOSKELETON

Nonmuscle cells perform mechanical work, morphogenesis, cleavage, endocytosis, exocytosis and intracellular transport These cellular functions are carried out by an extensive intracellular network of filamentous structures Constituting the cytoskeleton All eukaryotic cells contain three types of filamentous structure

1 Actin filaments (7 9 5 nm in diameter)

2 Microtubules (25 nm in diameter)

3 Intermediate filaments (10 12 nm in diameter)

Actin filaments

1 The G-actin protein of nonmuscle cells has a molecular weight of about 43 000 and contains N methylhistidyl residues

2 This actin spontaneously polymerizes in the presence of magnesium and potassium chloride to form the double helical *F actin filaments* like those in muscle

3 There are two types of actin in nonmuscle cells β actin and γ actin Both types can coexist in the same cell and even copolymerize in the same filament

4 Actin forms *microfilaments* of 7 9 5 nm in the cellular cytoplasm The bundles of filaments are prominent just underlying the plasma membrane of resting cells and are there referred to as *stress fibres* These stress fibers disappear as cell motility increases

5 Actin microfilaments are associated with other muscle like proteins in nonmuscle cells α Actinin is present at the plasma membrane sites to which microfilaments are attached

6 Myosin is found in association with actin microfilaments at the bases of microvilli

7 Tropomyosin promotes the formation of bundles of actin stress fibres α Actinin promotes the attachment of actin microfilaments to membranes substratum and other cell organelles

Microtubules

1 Microtubules are the integral component of the cellular cytoskeleton They consist of cytoplasmic tubes 25 nm in diameter and of indefinite length They are also necessary for the formation and function of the *mitotic spindle*

2 They perform a number of other cellular functions and are responsible for the intracellular movement of endocytotic and exocytotic vesicles

3 They form the major structural component of *cilia and flagella*

4 They are major protein component of axons and *dendrites*, where they maintain the structure and participate in the axoplasmic flow of material along this neuronal processes

5 They are the cylinders of 13 longitudinally arranged protofilaments, each consisting of dimers of α *tubulin and* β *tubulin* which are closely related protein molecules

6 Many important alkaloids can present microtubule assembly

7 The movement of chromosomes during anaphase of mitosis is dependent upon microtubules

Intermediate filaments

1 They are distinct from microfilaments and microtubules There are five major classes of these filaments

2 Each filament consists of distinct subunits biochemically and immunologically

COLLAGEN

1 Collagen is the major macromolecule of connective tissues and it is the most common protein in the animal kingdom

2 In mammalian tissues, there are *five distinct types* of collagen and they exist as a family of molecules sharing many properties

3 The most important property of collagen molecules is their *triple helix*, a coiled coil of three polypeptide subunits Each polypeptide subunit is twisted into a left handed helix of 3 residues per turn Three of these left handed helices are then wound to a right handed superhelix to form a stiff rodlike molecule 1 4 nm in diameter and about 300 nm long These triple helical molecules are associated into fibrils There is a gap between the end of one triple helix and the beginning of the next where there is the deposition of hydroxyapatite crystals in bone formation

4 The collagen fibers range from 10 to 100 nm in diameter and are visible by microscopy as banded structures in the extracellular matrix of connective tissues

5 Glycine is the only amino acid to exist in the limited space available down the central core of the triple helical molecule

6 In mammalian collagen, about 100 of the X positions are proline and 100 of the Y positions are 4 hydroxyproline They also contain 3 hydroxyproline in some X positions and 5 *hydroxylysine in Y positions*

The synthesis of collagen

1 Collagen is an extracellular protein but it is synthesized as an intracellular precursor molecule before becoming a mature collagen fibril

2 **Preprocollagen,** a precursor of collagen, contains a leader or signal sequence of about 100 amino acids at its amino terminus This preprocollagen is generated by ribosomes attached to the endoplasmic reticulum

3 When the signal sequence penetrates into the vesicular space of the endoplasmic reticulum, the leader sequence is cleaved off and the amino-terminal end of procollagen continues to protrude into the endoplasmic reticular space At this site, *prolyl 4 hydroxylase* and *lysyl hydroxylase* acts on prolin or lysine residues respectively in the Y position of the (Gly—X—Y)n Peptide A prolyl 3 hydroxylase in the X position immediately preceding a 4 hydroxyproline in the Y position

4 The procollagen molecule contains at its aminoterminus a 20 000—MW peptide and at its carboxy terminus a 30 35 thousand—MW peptide Neither of these is present in mature collagen

5 After formation of triple helix, further hydroxylation of prolyl and lysyl residues cannot take place

6 After this intercellular processing, the glycosylated procollagen molecule reaches the outside of the cell by way of the Golgi complex *Extracellular procollagen aminoprotease and procollagen carboxyprotease* remove the amino-terminal and carboxy terminal propeptides respectively The newly formed collagen molecules have about 1000 amino acids and spontaneously assemble into *collagen fibrils* that are indistinguishable from the mature fibrils found in tissues These fibrils do not have the tensile strength of mature collagen fibrils until they are *cross linked by a series of covalent bonds*

Inherited defects of collagen

Type VI Ehlers-Danlos Syndrome:

1 This is an inherited deficiency of lysyl hydroxylase

2 This disease is characterized by frequent abnormalities of the eye, severe scoliosis (abnormal vertebral column curvature) and hyperextensibility of the skin and joints

Type V Ehlers-Danlos Syndrome:

1 This is due to the deficiency of lysyl oxidase activity The absence of this enzyme prevents normal cross linking of collagen Lysyl oxidase is a copper requiring enzyme

2 There is severe arteriovascular and skeletal change

Type VII Ehlers-Danlos Syndrome:

1 This is caused due to the non-serving of procollagen as a substrate for the procollagen aminoprotease

2 The patients have hip dislocations, increased skin elasticity, and short stature

Exercise

1	Discuss the chemistry of muscle	(M U 74S)
2	Discuss the muscle contraction	(P U 72A)
3	Describe the structure of muscle	(R U 71A)
4	Write short notes on	
	(a) Actomyosin	(M U 73S)
	(b) Tropomyosin	(Bh U 72S)

CHAPTER 14

VITAMINS

History :

1. Lunin (1881) and Pakelharing (1905) showed in an experiment that the cessation of growth and death of animals had been prevented on the addition of small amounts of milk to their diet.

2. Eijkmann (1906) concluded that the dietary deficiency of a substance which was present in rice polishings caused the disease beri-beri.

3. Hopkins (1906—1912) by experiments gave more importance of the "growth-promoting" substance in milk. These experiments had been confirmed by Osborne and Mendel and by McCollum and Davies (1913).

4. Hippocrates (Ca. 400 B.C.) recommended ox liver and honey for nigbt-blindness.

5. Indians in Quebec (1935) cured the outbreak of scurvey in cartier's men by the use of green leaves.

6. Cod liver oil was used in Manchester (Ca. 1770) for rickets.

7. In the Japanese navy (Ca. 1860) barley was used to prevent beri-beri.

8. The names accessory food factors, food hormones, vitamines (vital amines) were derived from a group of substance responsible for these deficiencies. They are now called vitamin (without the "e").

Definition :

The diet coatainiag proteins, fats, carbohydrates and minerals are not sufficient to sustain life. Additional factors present in natural foods are also required although in small quantities. Tnese "accessory food factors" which are organic in nature and necessary for the normal functioning of the tissues are called vitamins.

Classification :

1. Fat-soluble vitamins—Vitamins A, D, E and K.
2. Water-soluble vitamins—Vitamin C and B-complex. (B_1, B_2, B_6, niacin, pantothenic acid, biotin, lipoic acid, folic acid, B_{12}, inositol)

Commoa properties of vitamins :

A. *Fat-soluble vitamins* :
   1. They are soluble in fat.
   2. Bile salts and fats are essential for their absorption.
   3. They are generally stored in liver.
   4. Normally they are not excreted in urine.

B. *Water-soluble vitamins* :
   1. They are soluble in water.
   2. They are easily absorbed.
   3. They are not stored in the body (except B_{12}).
   4. They have a threshold for urinary excretion.

FAT-SOLUBLE VITAMINS

VITAMIN A

Chemistry :

1. Vitamin A_1 aldehyde (retinal) is derived from β carotene by cleavage at the midpoint of polyene chain connecting the β-ionone rings

2 The *biosynthesis* of vitamin A is likely a dioxygenase reaction in which molecular oxygen reacts with the two central carbon atoms of β-carotene followed by the cleavage of the central double bond of β-carotene to produce 2 moles of vitamin A_1 aldehyde (retinal) This aldehyde is then reduced by *retinene reductase* depending on NADH to yield vitamin A alcohol (retinol) The reaction is shown below

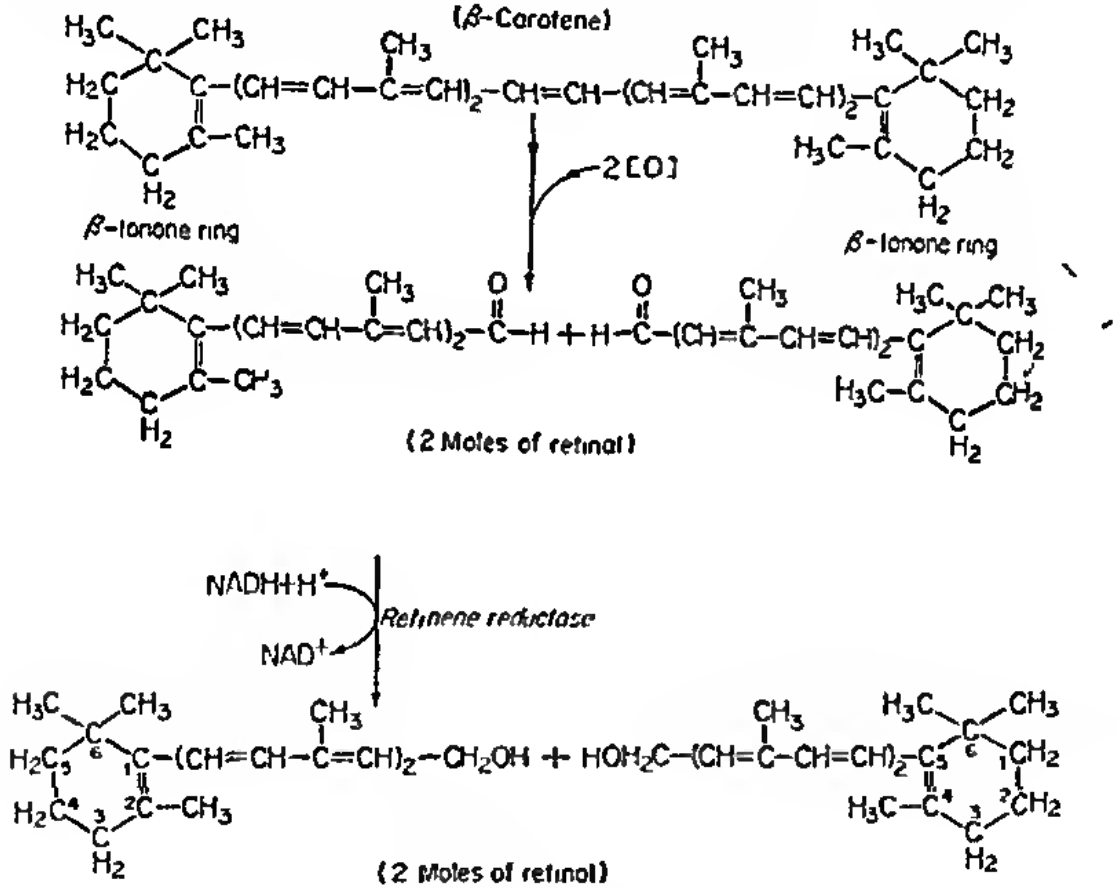


Fig 14 1 Vitamin A biosynthesis

3 The potency of vitamin A_2 (3-dehydroretinol) is 40% that of vitamin A_1 and it differs from A_1 structurally by the presence of an additional double bond between carbon 3 and 4 of the β-ionone ring

4 Retinol exists, as an ester with higher fatty acids, in the liver, kidney, lung and fat depots

5 The plant pigments, *carotenes or carotenoids*, synthesized by all plants except parasites and saprophytes are said to be provitamins A In the human diet, vitamin A is derived from the preformed vitamin (retinol) and the provitamin

carotenoids *β carotene* has the highest vitamin A activity and is the most abundant in human diets

6 Carotenes are only active as a vitamin precursor when it is converted to retinol This conversion takes place in the intestinal wall in rats, pigs, goats, rabbits, sheep and chickens although liver also participates but in man, it takes place in the liver only

Retinol is transported to the blood as *retinol binding protein* (retinol attached to α_1-globulin) Whereas, the carotenes are transported with the lipoproteins

Absorption

1 The dietary intake of vitamin A in the form of vitamin A esters are hydrolyzed in the lumen of the intestine by the enzyme *lipase* in presence of bile salts and fats tocopherol prevents the oxidative destruction of the vitamin

2 The vitamin and the carotene are taken up by the intestinal mucosa where the vitamin is esterified and carotene is first converted to retinal and then to retinol by retinal dehydrogenase which is then esterified mostly with palmitic acid

3 These esters together with some unchanged retinal and carotenoids which are not precursors of vitamin A are absorbed and enter the intestinal lymphatics and eventually the circulation, in chylomicrons

4 In the blood, the vitamin esters are attached to β lipoproteins and are then taken up by the liver (Kupffer cells) which contains almost all the body store

5 The vitamin is then released as retinol binding protein for use elsewhere, e g in the retinal rods

6 Since retinol dehydrogenase is present in the liver serum retinal can be converted to retinol

7 The impaired absorption of dietary fat (e g obstructive jaundice, chronic pancreatitis) leads to the impaired absorption of vitamin A in the absence of pancreatic enzymes (impaired hydrolysis of vitamin A esters) and in celiac disease or other conditions

8 In subjects with liver damage, the capacity for storage and formation of vitamin A is impaired and its concentration in the blood is also decreased

Storage

1 95 per cent of vitamin A in the form of esters is stored in the liver The hepatic storage increases with age The quantity stored in the liver varies in different species but the storage capacity in man is relatively large

2 A small amount of it is also present in other tissues, e.g lactating breast, adrenals, lung, intestine

Sources

1 Plant sources—All pigmented (particularly yellow) vegetables and fruits (e g sweet potatoes, carrots, pumpkins, papayas, tomatoes, apricots and peaches) and the leafy green vegetables which supply provitamin A (carotene) in the diet Cereals also contain carotene

2 Animal sources—Preformed vitamin A (retinol) is supplied by foods of animal origin , they are liver, milk, butter, eggs, kidney, the fat of muscle meats and fish liver oil which is very rich in the vitamin

Daily requirements

Adults	5000 I U.
During pregnancy and lactation	6000—8000 I U
Children	2000—3000 I U
Infants	1500 I U

1 I U = 0 3 μg of retinol
= 0 6 μg of β-carotene

Normal concentration in blood .

24—60 I U /dl
or
0 84—2 10 μmol/l

Physiological functions

1 Vitamin A helps in maintaining the integrity of epithelial tissue such epithelial layer of skin, respiratory mucosa, oesophagus and genitourinary trac

2 It functions in the preservation of the structural integrity and the norm permeability of the cell membrane as well as that of the membranes of subcellul particles such as lysosomes and mitochondria

3 It accelerates the normal formation of bones and teeth.

4 Wald and Morton gave more emphasis on the specific role of vitamin in the physiologic mechanism of vision The retinal pigment, *rhodopsin* or visu purple present in the rod cells of the retina is a conjugated protein with a molecul weight of about 40 000

When light strikes the retina, rhodopsin splits into its protein compone *opsin* and the non protein carotenoid *retinene* (retinal) The retinal is th converted to retinol by reduction catalyzed by the enzyme *retinene reductas* accompanied by the coenzyme NAD Retinene reductase is very similar alcohol dehydrogenase of liver

During the liberation of retinal from rhodopsin 2 coloured intermedia compounds are formed One is a red or orange-red compound, *lumirhodopsi* which is stable only at temperatures below —50°C. Beginning at a temperatu of about —20°C lumirhodopsin is converted to *metarhodopsin*, which is al orange-red in colour This compound hydrolyzes to retinal and opsin at tem peratures below —15°C and in the presence of water

Regeneration of rhodopsin takes place in the dark which is shown the figure below

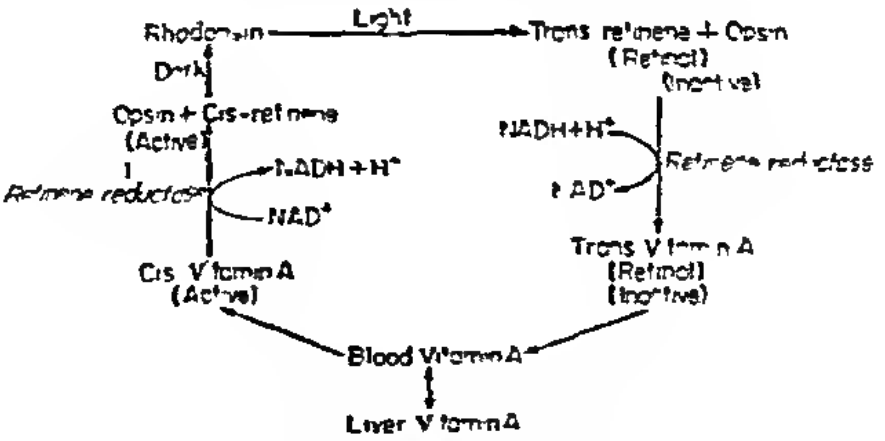


Fig. 14 2 Rhodopsin—vitamin A cycle

Deficiency symptoms

1 Xerophthalmia—Thickening and loss of transparency of the bulbar conjunctiva with yellowish pigmentation The keratinization of ocular tissue progresses to blindness in the late result of vitamin A deficiency It is a major cause of blindness in childhood which is more prevalent in Hong Kong, Manila and Dacca etc

2 Follicular conjunctivitis

3 Keratomalacia—Softening of the cornea, in advanced cases, with ulceration and necrosis Defective vision due to keratinization of epithelium of cornea

4 Impairment of dark adaptation progressing to *night blindness* (*nyctalopia*)

5 Follicular hyperkeratosis of the skin

6 Keratinizing metaplasia of epithelium of nose, respiratory mucosa oesophagus and genitourinary tract

7 Urolithiasis—Formation of urinary calculi

Hypervitaminosis

1 Acute symptoms are developed on the ingestion of very large amounts of vitamin A These include drowsiness, sluggishness, severe headache vomiting and peeling of the skin about the mouth and elsewhere In infants and young children, there may be a sudden rise of intracranial pressure

2 Continued intake of excessive amounts, especially in children, produces roughening of the skin, irritability, coarsening and falling of the hair, anorexia, loss of weight, headache, hyperesthesia, occasionally anemia, leukopenia

3 Serum level of vitamin A, lipid and acid phosphatase are elevated

All these symptoms are vanished on withdrawal of vitamin A

Coenzyme activities

Vitamin A has no coenzyme activities

Determination of vitamin A

Colorimetric determination utilizes the *Carr Price reaction*, in which a blue colour is obtained when a solution of antimony trichloride in chloroform is added to the vitamin-containing mixture This is used to determine the vitamin A content of blood plasma

THE VITAMINS D

The vitamins D are a group of compounds All are sterols occurring chiefly in animal organisms. Provitamins D possess the property of curing or preventing rickets when subjected to long wave ultraviolet light (about 265 nm)

Chemistry

1 The most important D vitamins are D_2 (activated ergosterol) or ergocalciferol or viosterol and D_3 (activated 7-dehydrocholesterol, cholecalciferol) on the basis of nutrition.

2 Provitamin D_2 (ergosterol) occurs in the plant kingdom ; whereas vitamin D_3 occurs in fish liver oil in nature

3 The structure of vitamin D_2 is the same as that of D_3 except that the side-chain on position 17 is that of cholesterol

4 Provitamin D_3 are synthesized in the body of man and other mammals. This is then activated in the skin by sunlight or ultraviolet rays and carried to various organs in the body for utilization or storage in the liver

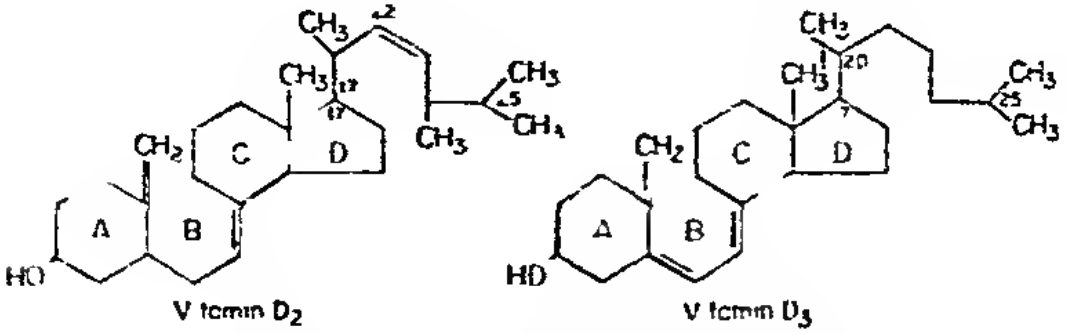


Fig 14.3 Structure of vitamin D_2 and vitamin D_3

5 The D vitamins are more stable than vitamin A and are not lost by ordinary cooking and preserving processes

Absorption and Storage

1 The D vitamins are readily absorbed in the small intestines.

2. Since they are fat soluble their absorption is enhanced by factors which favour fat absorption such as sufficient quantity of bile salts

3 After absorption, the vitamin is stored largely in the liver, kidneys intestines, adrenals and bones

4 A small amount is excreted in the bile but is partly reabsorbed in the intestine Not a single amount is eliminated in the urine

Sources

In the active form vitamin D is not well distributed in nature

Rich sources—The liver and viscera of fish and the liver of animals which feed on fish

Good sources—Eggs and butter

Poor sources—Milk The amount of the vitamin can be increased by providing additional vitamin D in the cow's diet.

Cheapest source—The cheapest source is sunlight which forms D_3 from 7-dehydrocholesterol in the skin

Daily requirements

Infants and children	400 I U
Adults	200 I U
Women during pregnancy and lactation	400 I U
1 I U = 0 025 μgm of cholecalciferol	

Normal concentration in blood

In adults	700—3100 I U /l
In children	860—2100 I U /l

Physiological functions :

1. Vitamin D directly stimulates the intestinal absorption of calcium and indirectly that of phosphate.

2 The major function of vitamin D is to stimulate transcription of the mRNA for a calcium transport protein Thus, it is concerned with intestinal absorption, renal excretion and bone metabolism of calcium Its ultimate effect is very much related to the concurrent activity of parathormone and calcitonin

3 It increases the excretion of phosphate by kidney and decreases the concentration of serum phosphate

4 It decreases the PH in the lower intestinal tract which aids in increasing the absorption of calcium and phosphorus

5 It is necessary for the development of bones and normal growth of the body

6 It stimulates the calcification of bones in both adults and growing children

7 It increases the citrate level of blood, bone, kidney and heart tissues and also the excretion of citric acid

8 It stimulates the activity of phytase which catalyzes the hydrolysis of phytic acid in the intestine

Deficiency symptoms .

1 During the period of skeletal growth vitamin D deficiency results in *rickets* (rachitis), with a characteristic defect in endochondral bone growth and mineralization of the zone of provisional calcification of long bones and corresponding areas of flat bones

2 In fully grown bones in *adults*, there is a type of defective mineralization of osteoid tissue termed "osteomalacia"

Causes of deficiency of vitamin D

1 Dietary insufficiency or insufficient exposure to sunlight
2 Gastrointestinal disorder
3 Chronic obstructive jaundice
4 Prolonged treatment with anticonvulsant drugs

Hypervitaminosis D

Extremely large amounts (500 to 1000 times the normal requirements) cause hypervitaminosis D

1 The early symptoms are anorexia thirst, constipation and polyuria, followed later by nausea, vomiting and diarrhoea Hyperphosphatemia also occurs

2 Increased urinary excretion of calcium and phosphate may lead to urinary lithiasis, and the hypercalcemia and hyperphosphatemia may lead to metastatic calcification The kidney, arteries, muscles and gastric mucosa are mainly involved The development of renal failure leads to death

Coenzyme activities—No coenzyme activities

VITAMIN E (TOCOPHEROLS)

Chemistry

1 Vitamin E refers to a group of compounds koown as tocopherols

2 Four tocopherols oamely α, β-, γ- and δ- have been isolated β- and γ tocopherols have two methyl groups in the aromatic nucleus whereas α tocopherol has three methyl groups in the aromatic nucleus and δ- has only one methyl group

3 Tocopherols are soluble in fat solvents and destroyed to an alkaline medium The vitamin activity is destroyed by oxidation,

4 The tocopherols are largely methyl derivatives of the parent compound tocol and they are yellow oily substances

5 Some tocopherols are derivatives of tocotrienol which contains three double bonds in the aliphatic side chain and is therefore terpenoid in structure

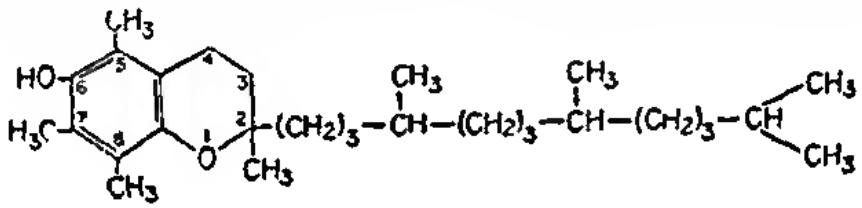


Fig 14 4 α Tocopherol

Absorption and Storage

1 It is absorbed in the intestine in the presence of bile salts

2 It is stored in the liver (mitochondria , microsomes) and fatty tissues

3 It is present in high conceotration in the adrenals, the pituitary, the uterus and the testes

Sources

Good sources—Eggs, meats, liver, fisb chicken, oatmeal, corn oil and cotton seed oil

Daily requiremeots

Adults —25 30 mg

Normal concentratioo In blood

10 mg /I

Physiological functions

1 Vitamin E protects the polyuosaturated fatty acid from oxidatioo by molecular oxygen in the formation of peroxides

2 Vitamin E and other antioxidant such as vitamio C are importaot io inhibiting damage to luog tissue from oxidaots in the air

3 The tocopherols play a part in the stabilization of cellular membraoes and play some part in electron transport together with appropriate proteio

4 Tbe tocopherols preveot tbe oxidation of vitamio A.

5 Vitamin E protects enzymes in muscle, nerves or gonads from estruction

6 It prevents the hepatic necrosis produced by the lack of sulphur ontaining amino acids in dietary proteins

7 It prevents the development of cerebral disorder

8 It seems to be involved in heme synthesis

Deficiency manifestations

1 Increased susceptibility of erythrocytes to hemolysis by hydrogen peroxide and decrease in the erythrocyte life span in human adult males

2 Anemia, edema and skin changes are observed in infants wheo fed un saturated oils

3 Decreased erythrocyte life span, hemolysis creatinuria and ceroid deposition in malahsorption syndromes in children

4 Smaller testis and permanent sterility in male rats

5 Muscular dystrophy in some animals exhibiting iocreased respiration

6 Resorption of foetus in female rats

7 Increased oxygen consumption by skeletal muscle

8 Hepatic necrosis

Coenzyme activities—No coenzyme activities

THE VITAMINS K

Dam (1935) named it vitamin K since it is a *koagulation vitamin* Dam Karrer and co workers (1939) isolated pure vitamin K_1 In the same year, Doisy and co-workers isolated pure vitamin K_2

Chemistry

1 The several substances (natural and synthetic) with vitamio K (antihemorrhagic) activity are naphthoquinones

2 Two naturally occurring vitamins K have been identified Vitamin K_1 was isolated originally from alfalfa and vitamio K_2 was origioally isolated from putrid fish meal Menadione is the most important of the synthetic vitamins K

3 The natural vitamios K are "fat soluble" vitamins The synthetic vitamins K are lacking the loog hydrocarbon chain aod hence soluble in water to some extent.

4 Vitamin K_1 possesses a phytyl radical on position 3 which occurs in plaots and vitamin K_2 (menaquinooe, farnoquinone) possesses a difarnesyl radical which occurs in hacteria

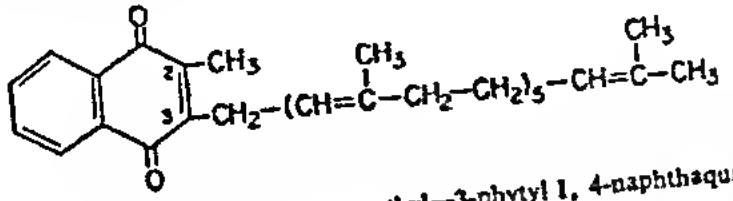


Fig 14 5 Vitamin K_1 (2-methyl—3-phytyl 1, 4-naphthaquinone)

VITAMIN E (TOCOPHEROLS)

Chemistry

1 Vitamin E refers to a group of compounds known as tocopherols.

2 Four tocopherols namely α, β, γ and δ- have been isolated β- and γ tocopherols have two methyl groups in the aromatic nucleus whereas α tocopherol has three methyl groups in the aromatic nucleus and δ- has only one methyl group

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4 The tocopherols are largely methyl derivatives of the parent compound tocol and they are yellow oily substances

5 Some tocopherols are derivatives of tocotrienol which contains three double bonds in the aliphatic side chain and is therefore terpenoid in structure

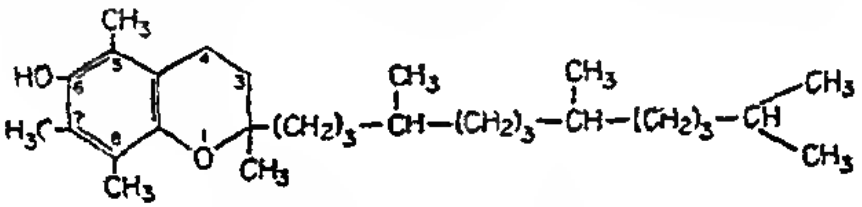

Fig 14 4 α Tocopherol

Absorption and Storage

1 It is absorbed in the intestine in the presence of bile salts

2. It is stored in the liver (mitochondria , microsomes) and fatty tissues

3 It is present in high concentration in the adrenals, the pituitary, the uterus and the testes

Sources

Good sources—Eggs, meats, liver, fish chicken, oatmeal, corn oil and cotton seed oil

Daily requirements

Adults —25-30 mg

Normal concentration in blood

10 mg/L

Physiological functions

1 Vitamin E protects the polyunsaturated fatty acid from oxidation by molecular oxygen in the formation of peroxides

2 Vitamin E and other antioxidant such as vitamin C are important in inhibiting damage to lung tissue from oxidants in the air

3 The tocopherols play a part in the stabilization of cellular membranes and play some part in electron transport together with appropriate protein

4 The tocopherols prevent the oxidation of vitamin A

5. Vitamin E protects enzymes in musele, nerves or gonads frnm estruction.

6. It prevents the hepatic necrnsis produced hy the lack nf sulphur ontaining aminn acids in dietary proteins.

7. It prevents the development of cerebral disorder.

8. It seems to he invnlved in heme synthesis.

Deficiency manifestations :

1. Increased susceptibility of erytbrocytes to hemolysis hy hydrogen pernxide and decrease in the erythrocyte life span in human adult males.

2. Anemia, edema and skin changes are ohserved in infants when fed unsaturated oils.

3. Decreased erythrocyte life span, hemolysis, creatinuria and cernid depnsitinn in malahsorptinn syndrnmes in children.

4. Smaller testis and permanent sterility in male rats.

5. Muscular dystrophy in some animals exhibiting increased respiratinn.

6. Resnrption of fnetus in female rats.

7. Increased nxygen ennsumption by skeletal muscle.

8. Hepatic neerosis.

Cnenzyme activities—Nn coenzyme activities.

THE VITAMINS K

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Chemistry :

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3. The natural vitamins K are "fat-soluble" vitamins. The synthetic vitamins K are lacking the lnng hydrocarbon chain nnd hence soluble in water tn snme extent.

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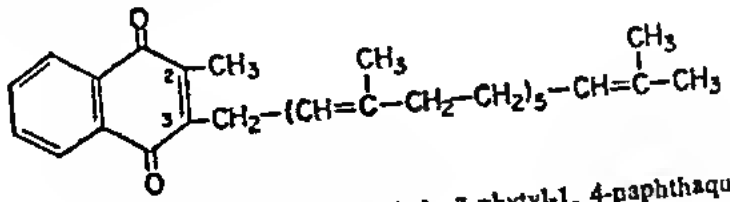


Fig. 14.5. Vitamin K_1 (2-methyl—3-phytyl-1, 4-naphthaquinone).

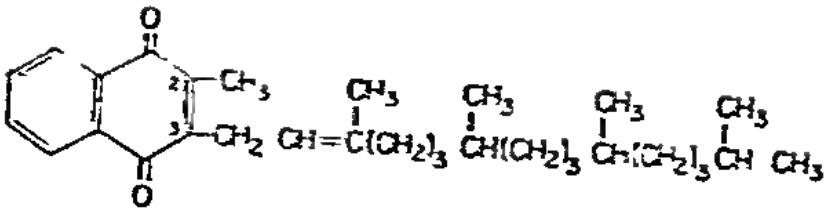


Fig. 14.6 Vitamin K_2 (2-methyl-3-difarnesyl-1, 4-naphthaquinone).

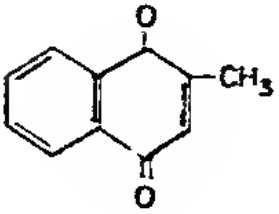


Fig. 14.7 Menadione (2-methyl-1, 4-naphthaquinone)

Absorption and Storage

1. Being fat soluble, its absorption is enhanced by sufficient amount of bile salts mainly to the jejunum by way of lymphatics.
2. Absorption is diminished by large amounts of liquid petrolatum.
3. Its absorption is interrupted leading to hemorrhage in jaundice and other liver diseases when the bile secretion is very less.
4. It is present in blood stream in significant amounts.
5. Liver stores appreciable amounts.
6. All tissues contain small amounts of vitamin K.
7. The feces contain large amounts of it which is produced by the bacterial flora, e.g. E. Coli.

Sources

Vitamin K_1

Best sources—Green leafy vegetables, e.g. alfalfa, spinach, cabbage, kale etc.

Good sources—Cauliflower, soyabean, wheat germ etc.

Fair sources—Carrots and potatoes.

Poor sources—Milk, meat and fish.

Vitamin K_2 is produced by most bacteria present in the human intestine if it is not supplied in the diet

Daily requirements

The average diet contains adequate amounts of vitamin K_1 and K_2 being synthesized by bacteria in the intestine. So the vitamin K deficiency has not been reported in healthy individuals except in new born infants fed on mother's milk when the mother's diet has a low vitamin K content.

Physiological functions

1. Vitamin K catalyzes the synthesis of prothrombin by the liver after transcription from information carried on messenger RNA

2 It reduces the prothrombin time

3 It regulates the synthesis of plasma clotting factors (factors VII, IX and X)

4 Vitamin K_1 is an essential component of the phosphorylation processes involved in photosynthesis in green plants It is also involved in oxidative phosphorylation in animal tissues

Deficiency manifestations

The deficiency of Vit K leads to a lowering of prothrombin level and increased clotting time of blood This may lead to hemorrhagic conditions

Hypervitaminosis K

The parenteral administration of too large doses of vitamin K (e g 30 mg / day for 3 days) to infants has been shown to produce hyperbilirubinemia in some cases

Coenzyme activities—No coenzyme activities

WATER-SOLUBLE VITAMINS

ASCORBIC ACID (VITAMIN C)

Introduction

1 The isolation of vitamin C was carried out by Zilva during 1917 1927 He obtained the highly potent substance and noted its reducing properties

2 In 1928, Szent Gyorgy isolated an acid with strong reducing properties from cabbage, adrenal glands and oranges He called it Hexuronic acid

3 In 1932, Waugh and King isolated vitamin C in a crystalline form from lemon juice

4 In 1933, vitamin C was named ascorbic acid owing to its antiscrorbutic properties

Chemistry

1 Haworth and co workers in 1933 established the chemical structure of ascorbic acid

2 The synthesis of vitamin C was reported in 1933 by Haworth and co workers in England and Reichstein and co workers in Switzerland

3 The chemical structures of L-ascorbic acid and L-dehydro ascorbic acid are given below

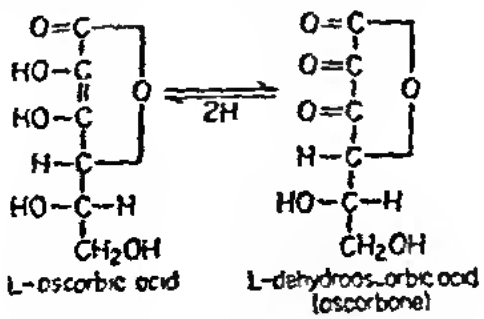


Fig 14 8 Structure of ascorbic acid

4 The structure of L-ascorbic acid shows that it is a derivative of hexose called L-gulose

Properties

1 Ascorbic acid is a white crystalline water soluble substance with sour taste

2 It is chemically an enediol lactone which is oxidized to dehydroascorbic acid (ascorbone) Both these forms are biologically active

3 D ascorbic acid does not possess any antiscorbutic activities

4 The oxidation of ascorbic acid is catalyzed by copper and silver ions and the oxidation is faster at higher temperatures, e g during cooking of foods

5 It is a powerful reducing agent which can reduce Fehling's solution in the cold

6 It can reduce 2, 6 dichlorophenolindophenol to the colourless leuco base

7 It is easily destroyed by cooking

8 It is stable below P^H 6 8 at room temperature but readily oxidized in an alkaline medium

Absorption and Storage

1 Ascorbic acid is readily absorbed from the small intestine, peritoneum and subcutaneous tissues

2 It passes through the portal vein to the general circulation and to all tissues

3 It is supplied to the fetus from the maternal circulation passing the placental barrier readily The placenta is also able to concentrate this vitamin

4 It is not stored in any particular organ and is distributed throughout the body

5 Each organ or tissue has an optimal saturation level of ascorbic acid Excessive intake of ascorbic acid do not increase the saturation level but the excess is excreted in the urine

Sources

Richest source—Amla

Good sources—Citrus fruits, tomatoes, green peppers, raw cabbage, guava, cauliflower adrenal cortex

Fair sources—Grapes, apple, banana, jackfruit, milk, liver, green leafy vegetables particularly salad greens, fresh potatoes, freshly killed meat, fresh raw fish, papiya

Ascorbic acid is not synthesized in the body of the human beings Dietary sources can only provide this vitamin The artificial food or sterilised milk is devoid of vitamin C

Daily requirement

Infants	35 mg
Children	40 mg
Adults	45 mg
Pregnant women	60 mg
Lactating women	80 mg

Normal concentration in blood plasma

0 6—1 5 mg /100 ml of blood plasma

In erythrocytes 1½ times of that of plasma and in white blood cells and platelets 20 40 times of that of plasma

Physiological functions

1 Since it is very sensitive to reversible oxidation (ascorbic acid⇌ dehydroascorbic acid) it is involved in the oxidation reduction reactions of the cell

2 It is involved in the conversion of folic acid to folinic acid (citrovorum factor)

3 It is involved in the hydroxylation of steroids in the adrenal cortex

4 It is required in the metabolism of tyrosine and phenylalanine and also in tryptophan

5 It is required for the absorption of iron and incorporation of plasma iron in ferritin

6 It is involved in the formation of norepinephrine

7 It is essential for the normal regulation of the colloidal condition of intercellular substances including the fibrils and collagen of connective tissue, osteoid tissue, dentine, the intercellular 'cement substance' of the capillaries

8 It is concerned in the hydroxylation of proline and hydroxyproline is an important constituent of collagen

9 It has an inhibitory effect on the hyaluronidase-hyaluronic acid system

Deficiency manifestations

Severe ascorbic acid deficiency produces scurvy The signs of this deficiency are entirely confined to supporting tissues of mesenchymal origin (bone dentine cartilage and connective tissue)

Scurvy is characterized by failure in the formation and maintenance of inter cellular materials which causes typical symptoms such as

1 Internal haemorrhages
2 Loosening of the teeth
3 Poor healing of wounds
4 Swelling of long bones
5 Easy fracturability of bones
6 Swelling sponginess tenderness and bleeding of gums
7 Anemia
8 Susceptibility to infections
9 General weakness

Toxic effects

Ascorbic acid in daily doses of 100 to 200 mg over long periods does not produce any harmful effects in human beings

THE VITAMINS OF THE B COMPLEX

1 Thiamine (vitamin B_1, antiberiberi substance, antineuritic vitamin, aneurine)

2 Riboflavin (vitamin B_2 lactoflavin)
3 Niacin (pellagra preventive factor, nicotinic acid)
4 Pyridoxine (vitamin B_6, rat antidermatitis factor)
5 Pantothenic acid (filtrate factor, chick antidermatitis factor)
6 Lipoic acid (thioctic acid protogen, acetate replacement factor)
7 Biotin (vitamin H, anti-egg white injury factor)
8 Folic acid group (vitamin M, streptococcus lactis R (SLR) factor, Vitamin B_c, fermentation residue factor, pteroyl glutamic acid, liver lactobacillus casei factor)
9 Inositol (mouse anti alopecia factor)
10 Vitamin B_{12} (cyanocobalamin, cobamide, antipernicious anemia factor, extrinsic factor of castle)

THIAMINE (VITAMIN B_1)

Introduction

1 In 1885, Takaki prevented the occurrence of beriberi in the Japanese navy by altering the diet

2 In 1897, Eijkmann produced polyneuritis in fowls by feeding them a diet consisting of washed polished rice and showed that the birds recovered after the administration of extracts of rice polishings

3 In 1926, Jansen and Donath isolated vitamin B_1 in crystalline form from rice polishings

4 In 1931, Windaus and co workers isolated vitamin B_1 in crystalline form from yeast and established its emperical formula

5 In 1934, Williams and co workers and in 1933 and 1935 Peters and co workers made improvements in the methods of isolation of vitamin B_1

Chemistry

1 Williams and co workers in 1936 established the chemical structure of thiamine The chemical structure is given below

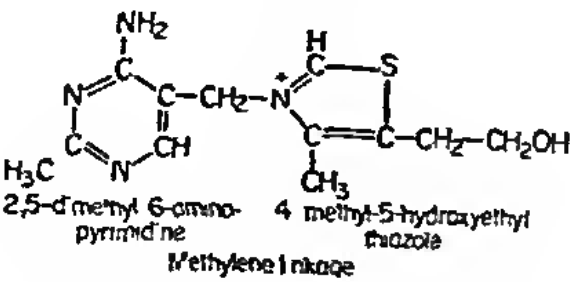


Fig 14 9 Structure of thiamine

2 Thiamine contains a pyrimidine ring and thiazole ring

3 Lohmann and Schuster in 1937 isolated thiamine pyrophosphate (co-carboxylase) from yeast

Properties

1 Thiamine is readily soluble in water

2 It is stable in acid medium

3 It is destroyed when autoclaved at 120°C for 30 minutes

4 It is destroyed even at room temperature in an alkaline medium

5 When it is dissolved in sodium bisulphite solution at PH 4 8 to 5 0 it is cleaved into pyrimidine half and thiazole half

6 It is when oxidized with potassium ferricyanide in alkaline solution, it is converted into thiochrome which has a strong fluorescence in ultraviolet light

Absorption and Storage

Free thiamine is readily absorbed from the small intestine but not TPP Excess thiamine administered is not stored in the tissues A part of the excess thiamine is excreted in urine and some of it is destroyed

Sources

Rich sources—Rice polishings wheat germ and yeast

Good sources—Cereals pulses nuts oilseeds

Fair sources—Meat fish eggs milk vegetables and fruits

Thiamine is practically present in all plants and animal tissues commonly used as food The thiamine contained in these foods is destroyed with improper cooking

Daily requirement

It is difficult to fix a single requirement of vitamin B_1 The requirement is increased when metabolism is elevated as in fever, hyperthyroidism, increased muscular activity, pregnancy and lactation Fat and protein reduce while carbohydrate increases the daily requirement of the vitamin Some of the thiamine is synthesized by the bacteria in the intestine Deficiencies of the vitamin occur not only by poor dietary intake but also in persons suffering from organic diseases

Infants	0 3—0 5 mg
Children	0 7—1 2 mg
Adult (males)	1 2—1 5 mg
,, (females)	1 0—1 1 mg
Pregnant women	1 3—1 5 mg
Lactating women	1 3—1 5 mg

[Recommended daily dietary allowance (Revised 1974)]

Normal concentration of vitamin B_1 in the blood plasma

1 About 1 μg /100 ml of blood plasma in the free form

Physiological role

1 Thiamine is essential for growth

2 It is essential for maintaining the nerves in normal condition

Coenzymic activities

(a) Thiamine in the form of pyrophasphate (TPP), the active form of the vitamin, is involved in oxidative decarboxylation of certain important intermediates in carbohydrate metabolism, e g pyruvic acid and α-ketoglutaric acid It is therefore referred to as "cocarboxylase".

(b) Succinyl CoA formed from α-ketoglutaric acid plays an important role in the biosynthesis of the porphyrin ring of hemoglobin and certain oxidases

(c) TPP also acts as a coenzyme for transketolase activity in the hexose monophosphate (HMP) shunt or the pentose phosphate cycle This cycle supplies NADPH required for fatty acid synthesis

Deficiency manifestation

1 Its deficiency causes beriberi—Wet beriberi and dry beriberi and neurological manifestations

2 The early clinical features in both forms of beriberi are common The first symptoms are

(a) Anorexia and dyspepsia associated with heaviness and weakness of the legs

(b) There is tenderness in the calf muscles with complaints of 'pins and needles' on pressure and pain and numbness in the legs

(c) The knee jerks are usually sluggish

(d) The subjects feel weak and get easily exhausted while working

(e) If not treated, the subjects develop wet or dry beriberi

Wet beriberi

(a) Oedema is the important feature

(b) Palpitation and breathlessness are present

(c) The pulse is fast

(d) The heart becomes weak.

Dry beriberi

(a) The muscles become wasted and weak and difficult to walk.

(b) The subject needs stick to stand and walk and finally becomes bedridden

(c) The concentrations of pyruvic acids and lactic acids are increased

(d) The transketolase activity of red cells is decreased

Toxic effects

If the vitamin is taken in excessive amounts the excess vitamin is promptly excreted in the urine As a result, there is no evidence of toxicity

RIBOFLAVIN (VITAMIN B_2)

Introduction

1 As early as 1879 the water soluble, yellow green, fluorescent pigment in milk whey was noted This substance was not isolated in pure form until 1932

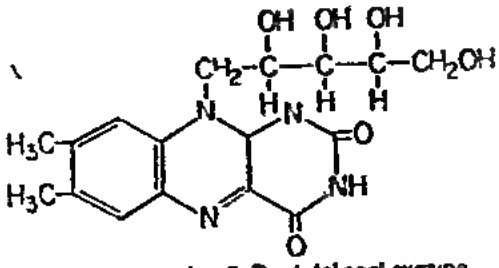
2 It was first isolated from milk, hence the earlier name lactoflavin

3 It was shown to be a constituent of oxidative tissue-enzyme system and an essential growth factor for laboratory animals

Chemistry

1 Kuhn Karrer and co-workers (1934, 1935) accomplished the synthesis of riboflavin

2. It contains an isoalloxaziae nucleus The chemical structure of it is given below.



6,7 dime hy 9-D rib tolsod oxazine

Fig 14 10 Structure of riboflavin

3 It is water soluble, heat stable and sensitive to light

4 The solution of vitamin B_2 when exposed to ultra violet light emits a strong greenish yellow fluorescence

5 On oxidation with ultraviolet rays or visible light it undergoes irreversible decomposition

6 It is rapidly destroyed when the solution is exposed to bright light

7 Reducing agents such as stannous chloride and hydro sulphite convert it into a colourless compound without any fluorescence

8 Riboflavin in alkaline solution when exposed to ultra violet light is converted into lumiflavin which has a greenish yellow fluorescence in ultra violet light

Absorption and storage

The vitamin is phosphorylated in the intestinal mucosa during absorption It is absorbed from the small intestine through the portal vein and is passed to all tissues being stored in the body The major part is excreted in urine and a small part is metabolized in the body

Normal concentration in blood plasma

2 5 to 4 0 μg/100 ml of blood plasma

Daily requirements

Recommended daily dietary allowances (Revised 1976)

Infants	0 4—0 6 mg
Children	0 8—1 2 mg
Adults (males)	1 5—1 8 mg
„ (females)	1 1—1 4 mg
Pregnant women	1 4—1 7 mg
Lactating women	1 6—1 9 mg

Sources

Riboflavin is widely distributed throughout the plant and animal kingdoms

Rich sources—Anaerobic fermenting bacteria
Good sources—Milk, liver, kidney, heart fish etc
Fair sources—Cereals, roots, germinating wheat and barley

Physiological functions :

1. Riboflavin is involved in the regulatory functions of some hormones connected with carbohydrate metabolism.

2. The free riboflavin present in retina is converted by light to a compound involved in stimulation of the optic nerve.

Coenzymic activities :

(a) It is a constituent of some enzymes called flavoproteins which are involved in intermediary metabolism. They play a role in the electron transport system in mitochondria.

(b) Riboflavin mononucleotide (FMN) is a constituent of the warburg yellow enzyme, cytochrome C reductase, and the L-amino acid dehydrogenase.

(c) Flavin adenine dinucleotide (FAD) is the prosthetic group of diaphorase, glycine oxidase, xanthine oxidase, and the D-amino acid dehydrogenase.

(d) Riboflavin, in the form of FMN and FAD, is a coenzyme in oxidation-reduction reactions which are intimately concerned with a number of vital metabolic processes.

(e) It is also the prosthetic group of acyl-CoA dehydrogenase which is involved in the oxidation of fatty acids

Deficiency manifestations :

1. Characteristic lesions of the lips.
2. Fissures at the angles of the mouth (cheilosis)
3. Dermatitis of the face.
4. Magenta tongue.
5. Certain functional and organic disorders of the eyes.

Toxic effects : No toxic effect.

NIACIN (NICOTINIC ACID)

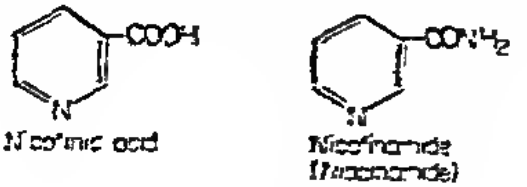
Introduction :

1. During 1922—1928 Goldberger and co-workers treated the disease pellagra in human beings and in 1928, they showed that boiled yeast extract can cure pellagra. Hence Goldberger named niacin as the Pellagra-Preventive (P-P) factor

2. In 1937, three groups of workers independently reported that nicotinic acid was effective in curing Pellagra in human beings.

Chemistry :

1. Niacin is pyridine 3-carboxylic acid. It occurs in tissues as niacinamide (nicotinamide). The chemical structures of both are given below.



Nicotinic acid Nicotinamide (Niacinamide)

Fig. 14.11 Structure of niacin.

2 It is soluble in water

3 It is stable to heat and not destroyed by autoclaving at 120°C for 20 minutes in acid or alkaline medium

4 Nicotinamide when heated in a strong alkaline or acid solution it is converted into nicotinic acid

5 Nicotinamide exists in human and animal tissues as coenzyme I (DPN now called NAD) and coenzyme II (TPN now called NADP)

Absorption and Storage

1 Nicotinic acid and nicotinamide are absorbed from the intestine through the portal vein into the general circulation

2 Excess nicotinic acid is not stored in the body

3 The majority of the excess nicotinic acid is excreted in urine in the form of N methylnicotinamide 6-pyridone of N methylnicotinamide N methylnicotinic acid and the glycine conjugates of these methyl derivatives Methylation takes place in the liver Methionine is the principal source of these methyl groups

4 Niacin in the form of *niacytin* present in maize is not absorbed unless the food is prepared with alkali (tortilla)

Sources

Richest sources—Yeast rice polishings
Good sources—Meat liver and poultry
Fair sources—Milk eggs tomatoes leafy green vegetables
Poor sources—Most fruits and vegetables

The amino acid *tryptophan* present in the dietary proteins is converted into niacin in the body 60 mg of tryptophan produce 1 mg of niacin So tryptophan present in the foodstuff also provides additional niacin

Daily requirements

Recommended daily dietary allowances (Revised 1974)

Infants	5—8 mg
Children	9—16 mg
Adult (males)	16—20 mg
Adult (females)	12—16 mg
Pregnant women	14—18 mg
Lactating women	16—20 mg

Normal concentration of niacin in the whole blood

0 5—0 8 mg /100 ml of whole blood

Physiological functions

1 Nicotinic acid is essential for the normal functioning of the skin intestinal tract and the nervous system

2 Coenzymic activities

(a) Nicotinic acid principally occurs as nicotinamide or niacinamide This niacinamide is a component of two coenzymes *NAD* and *NADP* This reduced form of NAD is dihydronicotinamide adenine dinucleotide (NADH) and that of NADP is dihydronicotinamide adenine dinucleotide phosphate (NADPH)

(b) These coenzymes play an important role in metabolism by acting as hydrogen and electron transfer agents by means of reversible oxidation and reduction Hence the great importance of niacin in human nutrition as well as in the requirements of many other organisms including bacteria and yeast is stated

The mechanism of transfer of hydrogen from a metabolite to oxidized NAD causing the oxidation of the metabolite and the formation of reduced NAD is shown below

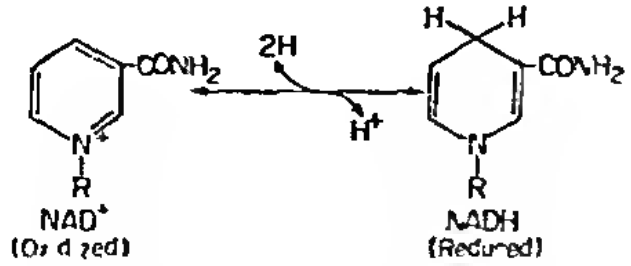


Fig 14 12 Formation of NADH

(c) NAD and NADP take part in many enzyme reactions involving dehydrogenases Some of these include oxidation of alcohol to aldehyde, of glucose to gluconic acid, of malic acid to oxaloacetic acid of lactic acid to pyruvic acid, of glycerophosphate to phosphoglyceraldehyde, of glucose 6-phosphate to 6-phosphogluconate, of pyruvic acid to acetyl CoA and of α ketoglutarate to succinyl CoA etc

(d) The reduced NADP (NADPH) is also involved in enzyme reaction in fatty acid synthesis synthesis of cholesterol and of steroid hormones and also in the formation of tetrahydrofolate (H_4 folate)

Deficiency manifestations

The deficiency of niacin causes the disease *pellagra* The clinical features of the disease include three *D S—dermatitis* (lesion of skin of face, neck, knees, breasts thick and scaly skin) *diarrhoea dementia* (headache, depression, anxiety, insomnia and forgetfulness)

Toxic Effects In large doses nicotinic acid causes burning sensation that may ultimately alarm the patient

PYRIDOXINE (VITAMIN B_6)

Introduction

1 In 1938, pyridoxine was isolated in a pure form by five different groups of workers

2 In 1939, the vitamin was synthesized independently by two groups of workers in Germany and U S A respectively

Chemistry

1 Since pyridoxine occurs in nature it is a mixture of three compounds (pyridoxine, pyridoxal and pyridoxamine) The structure of these three are given below The more active derivatives are pyridoxal and pyridoxamine phosphates Pyridoxine is 3 hydroxy 4, 5-dihydroxymethyl 2 methyl pyridine. Pyridoxal

contains an aldehyde instead of hydroxyl methyl in No 4 and pyridoxamine contains a primary amine side chain in No 4 of pyridine nucleus These three compounds are interchangeable

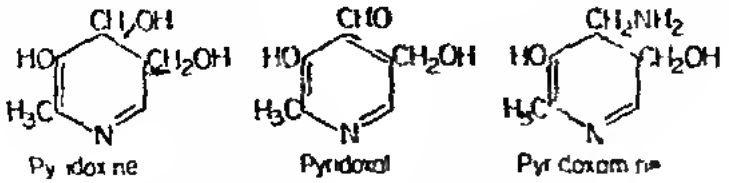


Fig 14 13 Structure of pyridoxine

2 Pyridoxine is readily soluble in water

3 When it is in alkaline solution, it is slowly destroyed by exposure to light

4 It reacts with phenol reagent or 2 6 dichloroquinone chlorimide producing coloured compounds

Absorption and Storage

It is readily absorbed from the small intestine The excess amount if ingested is not stored in the body but is excreted in urine

Sources

Rich sources—Yeast, rice polishings certain seeds such as wheat and corn
Good sources—Milk, meat, eggs, leafy vegetables, liver
Fair sources—Fish, fruits, vegetables

Intestinal bacteria can also synthesize this vitamin

Daily requirements

Recommended daily dietary allowances (Revised 1974)

Infants	0 3 mg
Childreo	0 6—1 2 mg
Adult (males)	1 6—2 0 mg
Adult (females)	1 6—2 0 mg
Pregnant women	2 5 mg
Lactating women	2 5 mg

Physiological functions

1 Pyridoxine is essential for the growth of infants

Coenzyme activities

(a) Pyridoxal phosphate, the active derivative of pyridoxine, functions as *codecarboxylase* in the decarboxylation of tyrosine, arginine glutamic acid and certain other amino acids

(b) The *deaminases* (dehydrases) for serine and threonine are also catalyzed by pyridoxal phosphate acting as coenzyme

(c) Pyridoxal phosphate acts as *cotransaminase* in the transamination reactions

(d) Pyridoxal phosphate acts as a coenzyme for *kynurerinase* in the synthesis of niacin from tryptophan

(e) Muscle phosphorylase also contains pyridoxal phosphate as coenzyme

(f) Pyridoxal phosphate acts as coenzyme in the *transulfuration* reaction in the transfer of sulphur from methionine to serine to form cysteine

(g) Pyridoxal phosphate is also involved in the process of absorption of amino acids from the intestine

(h) Pyridoxal phosphate is also involved in the desulphuration of cysteine and homocysteine

(i) Pyridoxal phosphate is required for the synthesis of α aminolevulinic acid which is an important intermediate in the synthesis of porphyrin and heme nuclei

(j) This pyridoxal phosphate especially applies to brain metabolism because it is necessary for the formation of serotonin, γ aminobutyric acid and the catecholamines

(k) Pyridoxal phosphate has also important relationship to oxalate metabolism Hyperoxaluria occurs in deficiency states

(l) Pyridoxal phosphate is involved in the synthesis of coenzyme A from pantothenic acid

(m) Pyridoxal phosphate is concerned with immune response

Deficiency symptoms

Fortunately deficiency of vitamin B_6 is rare because of its easy availability in most foodstuffs

1 Deficiency gives rise to irritability and depression. In some subjects there are lymphopenia and peripheral neuropathy

2 Deficiency of this vitamin occurs in infants on inadequate milk formulas The major symptoms are convulsions due to depletion of brain γ amino butyric acid content

3 The drugs isonicotinic acid hydrazide and hydrazaline act as B_6 antagonist causing deficiency symptoms including hypochromic anemia and peripheral neuropathy

4 In the deficiency states there are inborn errors of metabolism including cystathioninuria, familial xanthurenic aciduria and some pyridoxol responsive anemias

Toxic effects No toxic effects are yet reported

PANTOTHENIC ACID

Introduction

1 In 1938, Williams and co workers isolated this vitamin in a pure form as its calcium salt

2 In 1940, its synthesis was accomplished by several groups of workers

Chemistry

1 Pantothenic acid consists of β alanine and pantoic acid joined through a peptide bond The chemical structure is given below

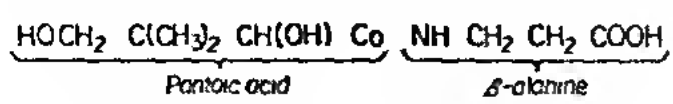


Fig 14 14 Structure of pantothenic acid

2 It is highly soluble in water

3 It is stable to autoclaving at 120°C for 30 minutes in neutral solution but is destroyed in acid or alkaline medium

4 It is not obtained in crystalline form but its sodium, potassium or calcium salts crystallise readily

5 Pantothenic acid exists in the tissues in the active form as coenzyme A which contains pantothenic acid, β mercaptoethylamine adenine, ribose and phosphoric acid

Absorption and Storage

Pantothenic acid and its salts are readily absorbed from the small intestine through the portal vein into the general circulation

If ingested in excess of the requirements it is not stored in the body, but is excreted in urine or metabolised by the tissues

Sources

Richest source—Jelly
Rich sources—Yeast, liver, rice polishings and wheat germ
Good sources—Milk, meat eggs and leafy vegetables
Fair sources—Fruits and other vegetables

Daily requirements

Infants	1—2 mg
Children	4—5 mg
Adults (males & females)	5—10 mg
Pregnant & Lactating women	10—15 mg

Physiological functions

1 Pantothenic acid is essential for the growth of infants and children

2 Coenzymic activities

(a) Pantothenic acid as a constituent of coenzyme A is required for several fundamental reactions in metabolism

(b) Coenzyme A combines with acetate to form 'active acetate' (acetyl coenzyme A) which is directly utilized by combination with oxaloacetic acid to form citric acid which initiates the citric acid cycle

(c) Acetic acid derived from carbohydrates, fats or many of the amino acids undergoes further metabolism through the 'common metabolic pathway

(d) In the form of active acetate, acetic acid also combines with choline to form acetylcholine or with the sulfonamide drugs which are acetylated prior to excretion

(e) The decarboxylated produet of α ketoglutarate in the citric acid cycle is a coenzyme A derivative called "active" succinate (succinyl CoA) Succiny-CoA and glycine are involved in the first step leading to the biosynthesis of heme So anemia occurs in the deficiency of this vitamin

(f) In lipid metabolism, coenzyme A has got significant role In the first step of oxidation of fatty acids the fatty acids are to be activated by coenzyme A catalyzed by the enzyme thiokinase In each turn of the β-oxidation cycle, one molecule of acetyl CoA is released This acetyl CoA directly enters the citric acid cycle for degradation to carbon dioxide and water or two molecules of acetyl-CoA condense to form ketone bodies

(g) Coenzyme A in the form of acetyl CoA is also required for the synthesis of cholesterol and thus of the steroid hormones

(h) A significant amount of the cellular pantothenic acid is protein bound This form is contained in a compound known as acyl carrier protein, a coenzyme required in the biosynthesis of fatty acids

(i) Coenzyme A is also involved in the metabolism of propionate and of branch chain fatty acids

Deficiency symptoms

Deficiency of this vitamin in man results in nausea, vomiting certain gastro-intestinal disorders irritability, inadequate growth, anemia, fatty liver, failure in gaining weights

Toxic effects No ill effects are still reported

THE FOLIC ACID GROUP

Introduction

1 In 1934, Wills showed that tropical macrocytic anemia in human beings was cured by a vitamin present in autolysed yeast extract

2 In 1947, Pifiner et al isolated folic acid in a crystalline form from liver

Chemistry

1 Folic acid (folacin, pteroylglutamic acid) is a compound made up of the Pteridine nucleus, P aminobenzoic acid and glutamic acid There are at least three nutritionally important and chemically related compounds which occur in natural products belong to the folic acid group These three compounds only differ in the number of glutamic acid residues attached to the Pteridine amino-benzoic acid complex The chemical structure of folic acid is given below Folic acid is synonymous with vitamin Bc

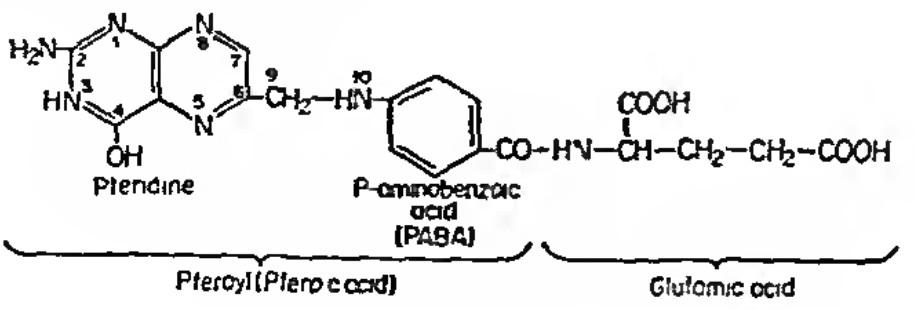


Fig 14 15 Structure of folic acid

2 Folinic acid (leucovorin folinic acid—SF [synthetic factor]) is the reduced form of folic acid with a formyl group on position 5 (N^5 formyl tetrahydrofolic acid)

3 Folic acid is soluble in water

4 It is stable to heat at neutral PH

5 *Its activity is not lost if it is heated at 120°C for 30 minutes at neutral PH*

6 Riboflavin accelerates the photo oxidation of folic acid

Absorption and transport

1 Absorption of folic acid takes place along the whole length of the mucosa of the small intestine

2 Monoglutamates are produced from polyglutamates which is ingested within the intestinal mucosa and dihydrofolates are further reduced to tetrahydrofolates by *folic acid reductases*

3 The tetrahydrofolates are then converted to methyltetrahydrofolate which enter the portal blood to be transported to the liver

4 The vitamin then appears in the systemic circulation to supply the tissue

5 The vitamin is transported to the plasma as methyltetrahydrofolate bound to protein The folate level of plasma obtained from umbilical cord blood is about 3 times that of the maternal plasma

Excretion

1, 20% of ingested folate that remains unabsorbed is excreted in the faces

2 2—5 μg of folic acid is excreted in the urine daily This may be increased after an oral dose of folate if the tissues are saturated

3 Some folate are also excreted in saliva, sweat and bile

Folic acid in tissues

1 Tissue folate is about 70 mg in the whole body

2 About one-third (5—15 μg/g) is in the liver

3 Folate is incorporated into the erythrocytes during erythropoiesis

Normal level of folic acid in serum

3—25 μg/l of serum in healthy subjects

Sources .

Richest sources—Yeast, liver, kidney
Good sources—Meat, fish, green leafy vegetables
Fair sources—Milk, fruits
Intestinal bacteria also synthesize folic acid

Daily requirements :

Recommended dietary daily allowances (Revised 1974)

Infants	50 μg
Children	100—300 μg.
Adult (males)	400 μg
Adult (females)	400 μg
Pregnant women	800 μg
Lactating women	600 μg

Physiological functions :

Coenzymic activities :

1 Folic acid as a coenzyme is involved in the transfer and utilization of the single carbon (C-1) moiety

Before functioning as a C-1 carrier, folic acid is first reduced to 7, 8-dihydrofolic acid (H_2 folate) and then to the tetrahydro compound (H_4 folate) catalyzed by *folic acid reductase* which use NADPH as hydrogen donor. The reactions are given below

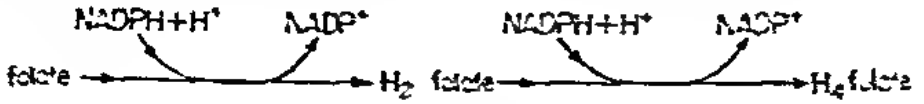


Fig. 14 16

The "one carbon" moiety may be formyl (—CHO), formate (H^+COO^-), methyl (CH_3^-) or hydroxymethyl ($-CH_2OH$) These are metabolically interchangeable

Folinic acid (formyl tetrahydrofolate, f^5-H_4 folate) is involved in the formylation of glutamic acid during the metabolic degradation of histidine ; otherwise it is metabolically inert. But f^{10}—H_4 folate is metabolically active. However, f^5 can be converted to f^{5-10} by formyl tetrahydrofolic acid isomerase as follows :

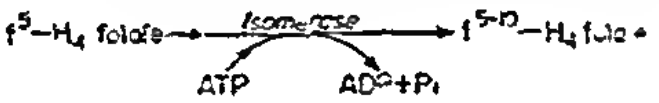


Fig 14 17

f^{5-10}—H_4 folate is also converted to N^5-methyltetrahydrofolic acid by an NAD-dependent reductase and this methyl group is then transferred to deoxyadenosyl -B_{12} (cobamide coenzyme) to form methyl -B_{12} which is an important donor of methyl group in the formation of methionine from homocysteine.

The other sources of the one-carbon moiety are the methyl groups of methionine, choline (by way of betaine) and thymine. These methyl groups are oxidized to $-CH_2OH$ groups and carried as such on f^{5-10}—H_4 folate The beta carbon of serine contributes hydroxymethyl group to the formation of a single carbon moiety

The single (formyl) carbon present on the tetrahydrofolic acids is utilized in the following ways

(i) As a source of carbons 2 and 8 in the purine nucleus

(ii) As a source of the formyl group on N-formyl methionine -tRNA which initiates synthesis of peptide chains on ribosomes in microorganisms

(iii) As a source of the formyl carbon in the formation of the beta carbon of serine in conversion of glycine to serine

(iv) In the synthesis of methyl groups for methylation of homocysteine to form methionine or methylation of uracil to form thymine and for the synthesis of choline by the way of methyl groups from methionine.

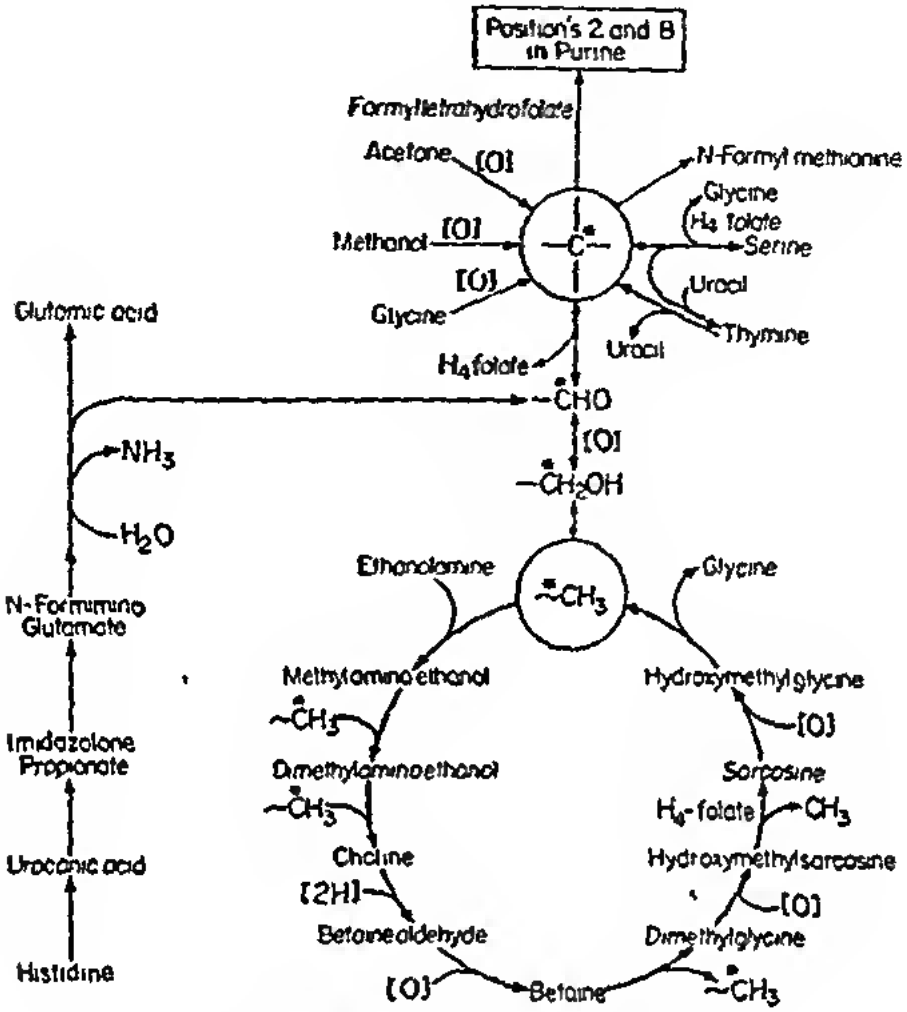


Fig. 14.18 Sources and utilization of the one carbon moiety.

2. Since the folic acid coenzymes take part in the synthesis of purines and thymine (the methylated pyrimidine of DNA) they are fundamentally involved in growth and reproduction of cells.

3. Folic acid coenzymes not only confined to the hematopoietic system but also generalized throughout the body.

4. In the metabolism of histidine, there is a folic acid—dependent step at the point where formiminoglutamic acid (Figlu) is converted to glutamic acid.

Folic Acid Antagonists :

1. The competitive inhibitor *aminopterin* (4-amino folic acid) is the most potent folic acid inhibitor.

2. Another antagonist is *amethopterine* (4-amino -10-methyl folic acid).

3 In animals the inhibitory effect of aminopterin cannot be reversed by folic acid but only by folinic acid.

Importance

1 They have got clinical application in the treatment of malignant disease and confirmation of the action of folic acid in cell growth.

2. Aminopterin has been used in the treatment of leukemia, particularly in children. A remission is temporarily observed in some patients but after a time the leukemic cells acquire the power to overcome the effects of the antagonist.

Deficiency symptoms

Folic acid deficiency can result from low dietary intake, in intestinal malabsorption syndromes and during pregnancy A similar deficiency can occur as a result of prolonged administration of anticonvulsant drugs (phenytoin sodium and *primidone) Folic acid antagonists also cause deficiency of folic acid.*

The deficiency gives rise to a megaloblastic anemia. The nuclei of the neutrophil polymorphonuclear leukocytes contain more than the normal number of lobes Other deficiency manifestations include retardation of growth, weakness, infertility, inadequate lactation in females and increased output of formiminoglutamic acid (FIGLU) in the urine after histidine loading.

Toxic effects

Renal injury has been observed in animals receiving large doses (50 mg./Kg. body wt.) of folic acid intravenously

VITAMIN B_{12}

Introduction

1 In 1926 Minot and Murphy discovered that liver can cure pernicious anemia

2. In 1948, Smith and Parker in Great Britain and Rickes et al in U.S.A. independently isolated Vitamin B_{12}

3 It is identical with extrinsic factor present in liver which cures pernicious anemia. It was found effective in curing pernicious anemia when administered intramuscularly in small quantities (5 to 10 μg.)

Chemistry

1 The structure of vitamin B_{12} is given below The central portion of the molecule consists of 4 reduced and substituted pyrrole rings surrounding a single cobalt atom. The central structure is referred to as a "Corrin" ring system. Two of the Pyrrole rings (rings I and IV) are joined directly rather than through a single methylidyne carbon. Below the corrin ring system there is a 5, 6-dimethylbenzimidazole riboside that is connected at one end to the central cobalt atom and at the other end from the ribose moiety through phosphate and amino-propanol to a side chain on ring IV of the tetrapyrrole nucleus. Addition of cyanide forms "*Cyanocobalamin*" which is identical with the originally isolated vitamin B_{12} and the removal of cyanide group results in the formation of the compound "*Cobalamin*". Cyanide group if substituted by hydroxy group

forms "*hydroxocobalamin*", by a nitro group forms "*nitro cobalamin*" by a methyl group forms "*methylcobalamin*". It contains cobalt 4 to 5 per cent

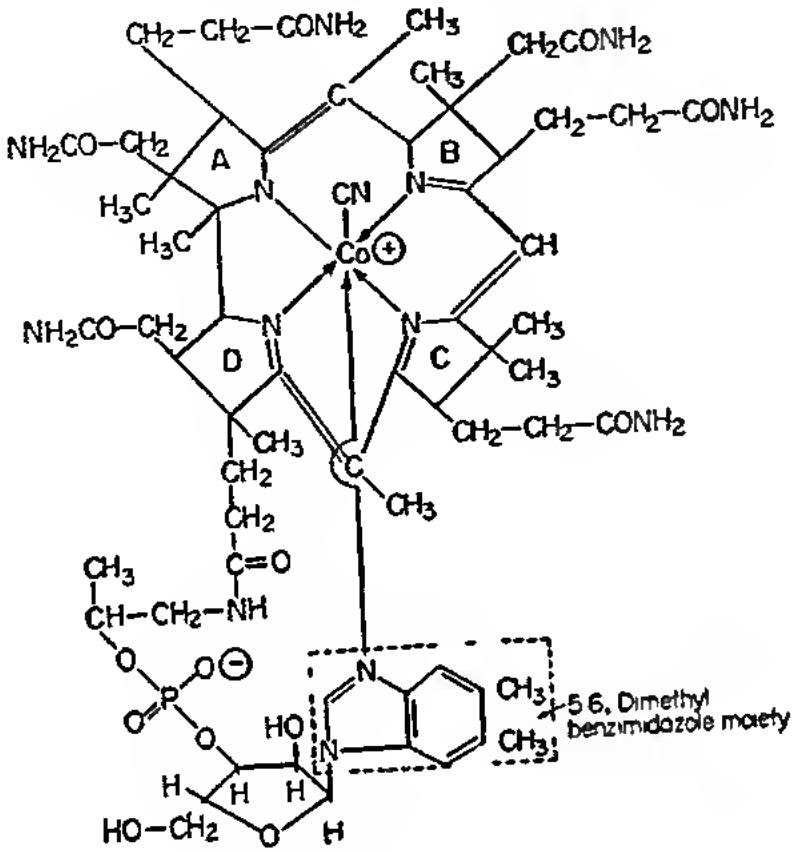


Fig 14 19 Structure of vitamin B_{12}

2 It has molecular weight of 1355 and an emperical formula C_{63} H_{88} N_{14} O_{14} P CO.

3 Aqueous solution of vitamin B_{12} when exposured to sunlight results in the destruction of the vitamin

4 If ascorbic acid is added to a solution of vitamin B_{12} the B_{12} activity is slowly destroyed due to the reducing action of ascorbic acid on vitamin B_{12}

5 Crystalline vitamin B_{12} is stable to heating at 100°C for long periods and aqueous solutions at PH 4 0—7 0 can be autoclaved with very little loss Destruction is rapid when the vitamin is heated at PH 9 0 or above

Absorption :

1 Vitamin B_{12} is absorbed from the ileum Its absorption depends on the presence of hydrochloric acid and a constituent of normal gastric juice called the *intrinsic factor* (IF), a mucopolysaccharide secreted by the parietal cells of the gastric mucosa

2 Free vitamin B_{12} (cobalamin) is bound to intrinsic factor in the proportion of 2 mol of cobalamin to 1 mol of IF Intrinsic factor is found in the cardia and fundus of the stomach The combination of B_{12} with intrinsic factor

results in the formation of a complex which is resistant to intestinal digestion Vitamin B_{12} also binds to the proteins of gastric juice, bile and saliva

3 It is currently believed that intrinsic factor possesses 2 receptor sites, one for B_{12} and the other for ideal intestinal microvilli The former receptor-site requires a neutral Pn and the presence of calcium ions (Ca^{++}) The latter receptor site is readily saturated which limits the absorption of B_{12} to 1 5 μg after a single dose The intrinsic factor is released within the intestine liberating B_{12} to pass into the intestinal mucosal cell Intrinsic factor protects vitamin B_{12} peptide against bacterial attack and in the peptide form the vitamin is absorbed

4 About 1% of the large concentra'ion of the vitamin is also absorbed by passive mechanism

Transport

1 Vitamin B_{12} is present in plasma as methylcobalamin, 5'-deoxyadenosyl cobalamin or hydroxocobalamin bound to proteins, *transcorrin I* and *transcorrin II* Transcorrin I is a strong binder of cobalamin and transcorrin II is a weaker binder of cobalamin 1—10% of B_{12} carried on transcorrin II is attached immediately after absorption and is readily released to be excreted in the urine

2 Cobalamin bound to plasma protein fraction is carried to the tissues where it is bound to a variety of protein receptors Any excess is stored in the liver as 5' deoxyadenosylcobalamin The total amount of cobalamin in the bodies of adults is 2 5 mg of which about 1 5 mg is in the liver

Excretion

1 B_{12} is excreted mainly by the way of the bile and by this pathway about 40 μg pass into the jejunum daily By an interohepatic circulation most of this is reabsorbed in the ileum using the intrinsic factor mechanism

2 Small amounts of the vitamin also enter the intestine from the gastric, pancreatic and intestinal secretions

3 The unabsorbed vitamin leaves the body in the feces This amount together with that produced in the colon by bacterial synthesis is about 3 6 μg daily

4 Cobalamin unbound to protein is excreted in the urine This amounts to 0 25 μg /day

Normal concentration in serum

160—1000 Pg /ml

Sources

Vitamin B_{12} is present only in the foods of animal origin It is not present in foods of vegetable origin Bacterial synthesis of cobalamin occurs in the human colon but it is not absorbed The only source of cobalamin in nature is via synthesis by micro organisms in soil, water and the animal intestine

Richest sources—Liver and Kidney
Good sources—Meat, fish, eggs
Fair sources—Milk, cheese

Daily requirements

Recommended daily dietary allowances (Revised 1974)

Infants	0 3 μg
Children	1—2 μg
Adult (males)	3 0 μg
Adult (females)	3 0 μg
Pregnant women	4 0 μg
Lactating women	4 0 μg

Functions

1 Vitamin B_{12} along with folic acid is required for the development of red blood cells beyond megaloblast stage

2 It stimulates the appetite and general health of the subject

3 It cures the neurological symptoms of pernicious anemia

4 Coenzymic activities

(a) Vitamin B_{12}, as the coenzyme, is involved with tetrahydrofolate in the synthesis of labile methyl groups which can be transferred to homocysteine to form methionine This is not sufficient to supply the body with its total methionine requirement and the amino acid is therefore a member of the essential amino acid group

(b) The vitamin is also a coenzyme for the mutase which converts methylmalonyl COA into succinyl COA Hence methylmalonate accumulates in the urine in deficiency states

(c) The vitamin is also involved in the maintenance of sulfhydryl groups in the reduced state The deficiency of the vitamin produces the reduction of glutathione in the blood

(d) Vitamin B_{12} is also involved in the conversion of ribonucleotides to deoxyribonucleotide

(e) It is also required in the dehydrase reactions in the conversion of ethylene glycol to acetaldehyde and glycerol to β hydroxypropionaldehyde

(f) It is as coenzyme required for the isomerisation of glutamic acid to threo-β methyl aspartate

(g) It is involved in the biosynthesis of proteins

Deficiency Symptoms

In man deficiency of vitamin B_{12} may result from poor dietary intake which occurs in the tropics, in strict vegetarians (vegans) and occasionally in the elderly It may result from a deficiency of intrinsic factor or from interference with the function of intrinsic factor and from intestinal malabsorption and also from the prolonged use of anticonvulsant drugs and of paraminosalicylic acid which is used in the treatment of tuberculosis

In deficiency states, the symptoms are

1 Megaloblastic macrocytic anemia (Pernicious anemia)
2 Mucosal atrophy and inflammation of tongue (glossitis) and mouth
3 Severe disease of the nervous system both central and peripheral
4 Psychiatric symptoms are not uncommon
5 There may also be amblyopia
6 A severe form of acidosis in children due to the excessive production of methylmalonate due to congenital defect in the biosynthesis of 5 -deoxyadenosyl cobalamin

Toxic Effects

No ill effects on excessive ingestion of the vitamin

BIOTIN

Introduction

1 Boas (1927) observed that when raw egg white was given as the main source of protein in the diets of rats, they developed symptoms of dermatitis retarded growth, loss of hair and loss of muscular control All these symptoms were prevented by egg yolk The factor was called 'anti-egg white injury factor'

2 In 1931, Gyorgy named this factor 'vitamin H'

3 In 1939, vitamin H was isolated by Gyorgy, Kuhn and Lederer

4 In 1942, Melville et al isolated vitamin H from milk and named this vitamin 'Biotin'

Chemistry

1 The chemical structure of Biotin in the free state is given below

2 The carboxyl group of biotin combines with the terminal nitrogen of lysine residue of enzyme protein forming *biocytin* (Lysine-biotin conjugate)

3 It is highly soluble in hot water

4 It forms salts with alkali hydroxides

5 It is stable to autoclaving at 120°C for 30 minutes at neutral PH

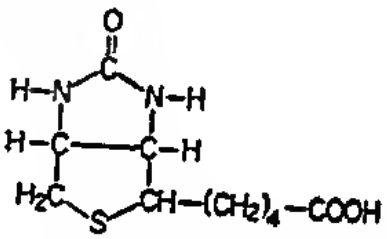


Fig 14.20 Structure of Biotin.

Absorption and Storage

Biotin is readily absorbed from the small intestine through the portal vein into the general circulation

Excess of the requirements is not stored in the body but is mostly excreted in the urine

Sources

Biotin is widely distributed in natural foods Intestinal bacteria also supply biotin in large proportion

Rich sources—Egg yolk liver, kidney

Good sources—Yeast milk, tomatoes

Fair sources—Fruits vegetables human milk.

Daily requirements

Since intestinal bacteria and diets supply biotin in adequate amounts the deficiency of this vitamin in human beings is rare

Infants	10—15 μg
Children	20—40 μg.
Adults	50—60 μg

Normal concentration in blood

12—24 μg /dl in adults

14—55 μg /dl. in infants

Functions

Coenzymic activities

Biotin is connected with the carboxylation reactions (CO_2 "fixation') The biotin coenzyme--apoenzyme complex attaches CO_2 which is afterwards transferred to other substances The following reactions are accomplished by this mechanism

1 Biotin acts as coenzyme along with acetyl CoA carboxylase to convert acetyl CoA to malonyl CoA which is an important step for fatty acid synthesis in extramitochondrial pathway

2 The conversion of pyruvate to oxaloacetate is also a biotin—dependent carboxylation reaction catalyzed by pyruvate carboxylase

3 Biotin acts as a coenzyme in the conversion of propionic acid to succinic acid

4 Biotin is involved in the fixation of CO_2 for the formation of carbon 6 in purine synthesis

5 Succinic acid dehydrogenase succinic acid decarboxylase, aspartic acid deaminase, serine deaminase and threonine deaminase are influenced by biotin

6 β methylcrotonyl CoA-carboxylase converts β methylcrotonyl CoA to β methylglutaconyl CoA in presence of biotin

Deficiency symptoms

The deficiency of Biotin may result from the destruction of intestinal bacteria by sulfonamide drugs or from the adequate intake of raw egg white which contains the protein *avidin* (interfere absorption of biotin) or from breast milk (containing low biotin) in association with diarrhoea in case of infants

The deficiency develops nausea, aneroxia, anemia, muscular pain dermatitis of extremities

LIPOIC ACID

Introduction

Lipoic acid was first detected in laetic acid bacteria It had replaced growth stimulating effect of acetate Hence, it was designated as "*acetate replacement factor*" This vitamin wasalso required for the nutrition of the protozoon Tetra bymena gelei for which it was termed as "*protogen*

Chemistry

1 It is a sulfur containing fatty acid [6 8-dithiooctanoic acid (thioctic acid)] The chemical structure is given below

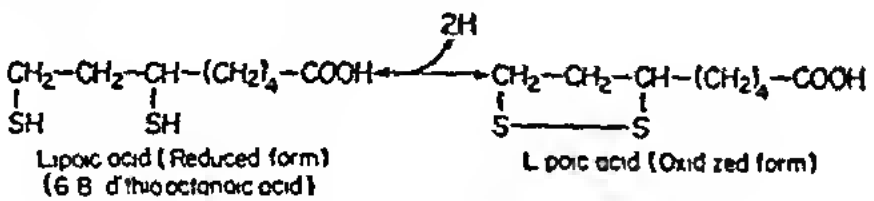


Fig 14 21 *Structure of lipoic acid*

2 It is soluble in water

Sources

It is widely distributed in natural foods.

Daily requirements : Not yet established

Functions :

Lipoic acid acts as a coenzyme in the oxidative decarboxylation of pyruvic acid and α-ketoglutaric acid

Deficiency symptoms .

No deficiency symptoms have so far been established for this vitamin

INOSITOL

Introduction :

1 Both plant and animal tissues contain inositol

2. For a good length of period the hexaphosphoric ester of inositol (phytic acid) present in cereals has been known

3 Seven optically inactive and two optically active forms of inositol are present in nature of which only one inactive form has got nutritional value

4 In 1940, Woolley et al reported that inositol can prevent the development of *alopecia* in mice.

Chemistry :

1 Inositol (meso-inositol or myo-inositol) is hexahydroxycyclohexane The structure is given below

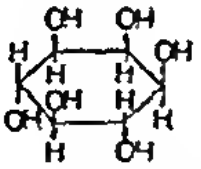


Fig 14.22 Structure of inositol

2. Meso-inositol is biologically most effective
3 It is highly soluble in water
4 It is a crystalline compound and has a sweet taste
5 It is stable to heat in neutral acid and alkaline medium

Absorption and Storage :

Inositol is absorbed readily from the small intestine through the portal vein and passes to the tissues through the general circulation

Excess of the requirement of this vitamin is not stored in the body but is excreted in the urine and feces or metabolised.

Existance of inositol :

1 The greater part of inositol exists in bound form , although a small amount exists in the free state in muscle and other tissues

2 It exists in the form of phospholipids in animal tissues, whereas it exists in the form of phospholipids, phytic acid and phytin in plant tissues

3 Brain and liver contain phospholipids containing inositol Mitochondria and microsomes also contain large amount of inositol containing phosphatides

4 Cereals oilseeds and nuts contain large amounts of phytic acid and phytin (calcium or magnesium salt of phytic acid) Phytic acid (inositol hexaphospboric acid) is hydrolyzed by an enzyme *phytase* present in plants into inositol and phosphoric acid Unless phytic acid is hydrolyzed it is not absorbed from the intestines Only a small percentage of the phytic acid present in the diet is hydrolyzed and absorbed due to the low concentration of phytase in the intestinal juice

Sources

Yeast, meat fruits, milk, nut, vegetables and grains contain inositol

Daily requirements Not yet reported

Functions

1 Along with choline, inositol exerts a lipotropic effect
2 Large concentration of inositol in heart muscle increases the rate of contraction
3 Labelled inositol gives rise to labelled glucose in the body
4 It is oxidized to glucuronic acid in the liver by *oxygenase*
5 It stimulates the growth of yeast and fungi
6 It occurs in brain tissues as phosphatidyl inositol (lipositol)
7 It causes an increase in nerve chronaxia in the rat
8 In animals it increases the peristalsis of the small intestines

Deficiency symptoms

Deficiency symptoms in mice include retarded growth, failure of lactation, alopecia (loss of hair over the body) spectacled eye (a condition due to loss of hair around the eye) and those in chicks include encephalomalacia

CHOLINE

Introduction

1 In 1934, Best and Huntsman discovered that choline deficiency produces fatty liver in rats

2 Choline is very essential for life processes It is an important metabolite although it cannot be classified as a vitamin Furthermore, the choline requirement is more than most substances and the deficiency symptoms are suggestive of vitamin deficiency diseases Hence, it is considered as vitamin

Chemistry

1 Choline is hydroxyethyl trimethylammonium hydroxide The chemical structure is given below

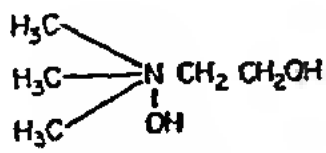


Fig 14.23 Structure of choline

2. It is a strong base and a colourless crystalline compound.
3 It is soluble in water
4 Dilute solutions of choline (less than 4 per cent) are stable to heat.

Absorption and Storage

Choline in the free state present in food is easily absorbed from the small intestine through the portal vein into the general circulation.

Excess ingestion of choline than the requirements is not stored in the tissues Part of it is converted into phospholipids but a greater part is metabolised in the liver into trimethylamine which is excreted in the urine

Sources

Rich sources—Liver, egg yolk
Good sources—Rice polishing, meat, cereals
Fair sources—Milk, fruits, vegetables

Requirements

Choline requirements of human beings are not yet established However, the diets commonly consumed contain large amounts of choline.

Normal level of free choline in plasma in male adults

About 4 4 μg/ml

Functions

1 Acetylcholine is a chemical mediator of parasympathetic and other types of activity in the nervous system It is formed as follows

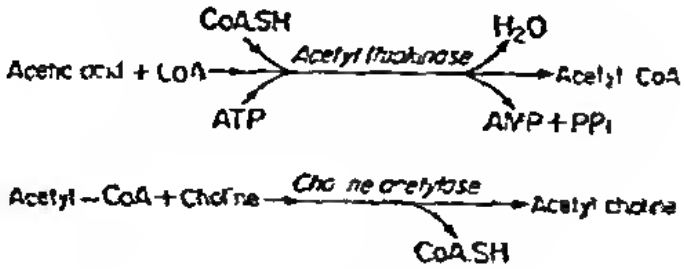


Fig 14.24

Acetylcholinesterase is an enzyme present in many tissues which can hydrolyze acetylcholine to choline and acetic acid Red blood cells can synthesize acetylcholine.

Both choline acetylase and acetylcholinesterase are present in red cells. Choline acetylase is also present in brain, skeletal muscle, spleen and placental tissue When choline acetylase becomes inactive due to drug inhibition or lack of substrate, the red cells lose their selective permeability and undergo hemolysis

2. Choline is a constituent of phospholipids which are essential constituents of all cells in the body

3 Choline takes part in transmethylation reactions in the formation of methionine from homocystine

$$\text{Choline} \xrightarrow{\text{Oxidation}} \text{Betaine}$$

$$\text{Betaine} + \text{homocystine} \longrightarrow \text{Methionine} + \text{dimethylglycine}$$

4 It prevents accumulation of fat in the liver of many animals

5. It is essential for growth of many animals

6. Its deficiency reduces egg formation in the hen

Deficiency symptoms .

1 Deficiency of choline causes fatty liver in rats

2. In the young growing rat there is hemorrhagic degeneration of the kidneys and hemorrhage into the eye balls and other organs and ultimately leads to cirrhosis

3 In chicks, its deficiency causes slipped tendon disease in which there is a defect at the tibiotarsal joint of the bird

Metabolism of choline

A Biosynthesis

1 Serine is decarboxylated to ethanolamine in presence of pyridoxal phosphate

2 Ethanolamine is progressively methylated to choline by the incorporation of one-carbon fragment into a methyl group of methionine

$$\text{Serine} \longrightarrow \text{Ethanolamine} \longrightarrow \text{choline}$$

B Catabolism

1 Choline is first oxidized to betaine aldehyde and then to betaine Choline acts as a methyl donor only after oxidation to betaine

2 After loss of methyl group in the use of homocystine to methionine, betaine is converted to dimethylglycine Dimethylglycine produces N hydroxymethyl sarcosine after oxidation

3 Sarcosine is then formed by transferring hydroxymethyl to tetrahydrofolic acid

4 Sarcosine is then converted to glycine by oxidation

5 Glycine is converted to serine by the addition of a hydroxymethyl group derived from formylated tetrahydrofolic acid derivatives

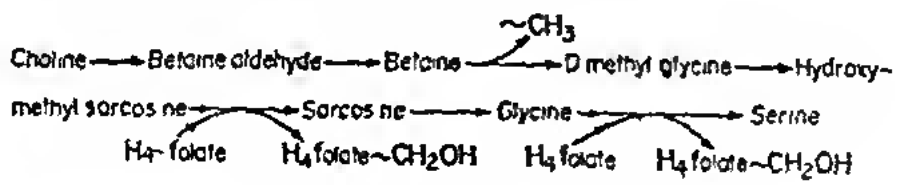


Fig 14 25

Short notes on .

RICKETS

Causes

Vitamin D deficiency causes the disease rickets in children.

Symptoms

1 It is characterised by bone deformities.

2. The earliest bony les on is often craniotabes—small round unossified areas in the membranous bones of the skull

3 Another early sign is the beading' at the costochondral junctions of the ribs known as 'Rickety rosary'

4 Later features are 'Bossing of the frontal and parietal bones and delayed closure of the anterior fontanelle and deformities of the chest such as undue prominence of the sternum known as Pigeon chest

5 The bones are soft due to the non-deposition of calcium salts and hence they are easily bent by the weight of the body

6 In case, the rickets continue for two to three years there is serious bone deformities such as bow legs deformities of the spine and pelvis. There is extension and widening of the epiphysis at the growing points

7 Renal rickets is caused by defective transport of phosphate by the renal tubules

Prevention

It can be prevented by the administration of vitamin D and calcium. Vitamin D in ordinary cannot cure the renal rickets and hence renal rickets are sometimes referred to as vitamin D resistant rickets

OSTEOMALACIA

Causes

Vitamin D deficiency causes the disease osteomalacia in adults It generally occurs in pregnant women of low income groups who consume a diet devoid of vitamin D and calcium and those who observe 'purdha', also It is prevalant in India, Pakistan China and middle-east countries.

Symptoms

1 The changes in the blood and bone are similar to those in rickets.

2. Progressive decalcification of bones and bones become soft.

3 Bone deformities occur in pelvis legs, ribs, sacrum and lower lumbar vertebrae

4 Owing to the deformity of the pelvis normal delivery of the baby becomes difficult

Prevention

This disease can be prevented by the administration of vitamin D and calcium

SCURVY

Causes

Severe deficiency of ascorbic acid leads to the disease scurvy.

Symptoms

A Scurvy in adults

1 The first symptoms are weakness, easy fatigue and listlessness These are followed quickly by pain in bones, joints and muscles of the extremities, shortness in breath

2 Hemorrhages in muscle occur particularly in calf, thigh and forearm Hemorrhages may occur in joints with swelling and pain

3 As the deficiency advances, the gums become swollen, blue red and spongy The gums may be infected by bacteria There is loss of teeth

B Infantile scurvy

1 The prescorbutic infants become anorexic and listless for a few days

2 With the beginning of the disease, the infant lies with legs drawn up on the abdomen

3 The infant cries when touched especially when its legs or arms are moved or lifted

4 Extreme tender swellings may be felt at the end of the long bones

5 The sternum may sink slightly inward

6 Purpura occurs in the skin

7 If treatment is delayed, dyspnea, cyanosis, convulsions and death may occur

Prevention

1 The disease can be prevented by the administration of high dose of ascorbic acid

2 Citrous fruits are to be taken regularly

BERIBERI

Causes

The disease beriberi is caused by the deficiency of thiamine (vitamin B_1)

Types

There are three types of beriberi—wet beriberi dry beriberi and infantile beriberi

Symptoms

A Wet beriberi

1 The important feature of wet beriberi is edema

2 It is rapidly developed and not only the legs but also the face, trunk, serous cavities are involved

3 The calf muscles are slightly swollen and tender on pressure

4 Palpitation and breathlessness appear.

5 The diastolic blood pressure is low while the systolic is high

6 The pulse is fast

7 The heart becomes weak and death occurs as a result of heart failure

B Dry beriberi

1. The muscles are progressively wasted and weak and difficult to walk

2 The affected individual takes the help of sticks to stand and walk and ultimately he becomes bedridden

3 The patient dies if untreated

4 There is cerebral disorder

C Infantile beriberi

1 Those infants will suffer from the deficiency of the vitamin whose mother's breast milk contains low thiamine

2 The infants face restlessness and sleeplessness

3 Anorexia, vomiting and breathlessness develop

4 Most of the symptoms are due to cardiac dilatation and failure

5 Sudden death occurs if not treated with thiamine immediately

Prevention

The disease can be prevented by the administration of thiamine by intramuscular injection

PELLAGRA

Causes

1 The deficiency of niacin causes the disease pellagra

2 It occurs whenever corn (maize) is the main diet and the corn protein is deficient in some of the essential amino acids, notably tryptophan and lysine Hence niacin is not formed in the body

3 It is also caused by acute or chronic infection

Characteristics

Pellagra is characterised by three D's—dermatitis, diarrhoea and dementia

Symptoms

A Dermatitis

1 The bright red erythema resembling sunburn occurs over the exposed parts of the body

2 The commonest sites are the back of the fingers and hands, the forearms, dorsum of the feet and ankles and the neck

3 At the onset, the skin becomes red and slightly swollen

4 Secondary infection is always present

5. The dermatitis is precipitated by exposure to sunlight

B Diarrhoea

1 In most cases, nausea and vomiting are found

2 The diarrhoea ranges from a few to several loose stools a day with blood and mucosa.

C Dementia

1 Dementia more frequently occurs in chronic cases

2 Irritability, changes in disposition depression inability to concentrate are found in milder mental disturbances.

3 In mild cases, poor memory is common

4 In chronic cases, spasticity, ataxia the involvement of the bladder and rectal sphincters are seen

Prevention

1 Niacinamide in doses of 15—25 mg three times a day are to be prescribed

2 Adequate quantities of meat eggs milk and vitamin B-complex are given

3 Any accompanying infection should be treated with the proper antibiotic

PERNICIOUS ANEMIA

Causes

The lack of 'intrinsic factor in the stomach resulting the failure of absorption of vitamin B_{12} causes the disease pernicious anemia

Symptoms

1 The RBC count is low 1 5 to 2 5 million per cubic millimetre

2 The average diameter of the cell is above normal

3 Excessive destruction of the abnormal circulating red cells causes the increase in the serum bilirubin

4 The hemoglobin content is as low as 8 to 9 per cent

5 The nucleated red cells of the bone marrow is highly increased

6 The cells of stage I are peculiar and are called megaloblasts

7 The cells of the stomach responsible for acid and enzyme secretions are atrophied So the gastric secretions are devoid of acid pepsin and intrinsic factor (IF)

8 The tongue is sored and inflammated

9 Numbness and tingling occur in fingers and toes

10 Signs of involvement of the spinal cord (vitamin B_{12} neuropathy) are observed

11 In advanced cases demyelation of the white fibres of the spinal cord occurs.

Prevention

It can be prevented by the administration of vitamin B_{12}

Daily requirements of vitamins

	Fat soluble vitamins				Water soluble vitamins	
	A	D	E	K	B_1	B_2
Adults	5000 I U	200 I U	25—30 mg	1—2 mg	1.2—1.5 mg	1.5—1.8 mg
Women during pregnancy & lactation	6000—8000 I U	400 I U			1.3—1.5 mg	1.6—1.9 mg
Infants	1500 I U	400 I U			0.3—0.5 mg	0.4—0.6 mg
Children	2000—3000 I U	400 I U			0.7—1.2 mg	0.8—1.2 mg
	1 I U = 0.3 μg of retinol = 0.6 μg of β carotene.	1 I U = 0.025 μg of cholecalciferol				

	Water soluble vitamins						
	Niacin	Pyridoxine	Pantothenic acid	Folic acid	B_{12}	C	Biotin
Adults	16—20mg	1.6—2.0 mg	5—10 mg	400 μg	3.0 μg	45 mg	30—60 μg
Women during Pregnancy & lactation	16—20mg	2.5 mg	10—15mg	600—800 μg	4.0 μg	60—80 mg	30—60 μg
Infants	5—8mg	0.3 mg	1—2 mg	50 μg	0.3 μg	30 mg	10—15 μg
Children	9—16mg	0.6—1.2 mg	4—5 mg	100—300 μg	1—2 μg	40 mg	20—40 μg

Exercise

1 Give the chemistry, functions and deficiency manifestations of vitamin A (M U 75S)

2 Mention the sources of vitamin A Describe the function and effects of deficiency of vitamin A What is the daily requirement ? (M U 73A)

3 Describe the role of vitamin A in our food requirements What are the manifestations of deficient intake of this vitamin ? (P U 64A)

4 Describe briefly the chemical nature, action in the body, dietary sources and nutritional importance of vitamin A (P U 65S)

5 What do you know about vitamin D ? (Luck 69S)

6 Name the fat soluble vitamins their occurrence daily adult requirements and their importance to the body (Punjab 60A)

7 Give a brief account of the chemistry, sources daily requirement and deficiency states of thiamine Describe the mechanism of its action (P U 71A)

8 Describe briefly the chemical nature mode of action dietary sources and nutritional importance of thiamine (R U 67A)

9 Describe the chemistry, sources daily requirement and metabolic functions of (a) thiamine, (b) niacin Mention the disease caused by their deficiency in diet (R U 76A)

10 Name the vitamins with coenzyme activities Give an account of the sources properties, effects of deficiency and functions of pyridoxine (M U 72A)

11 Discuss the role of Riboflavin in the body (PUN 69A)

12 What is riboflavin ? Discuss its metabolic role in the body and the deficiency symptoms (P U 72S)

13 What are vitamins ? Describe the functions and properties of any two vitamins of B-complex group (Muz 74A)

14 Mention the sources of vitamin B_{12} and the effect of its deficiency Describe the metabolic functions of vitamin B_{12} (M U 74S)

15 Describe the sources, requirements functions and deficiency manifestations of vitamin C (M U 73S P U 68A)

16 Name the vitamins with coenzyme activities Discuss the biological importance of flavoproteins (P U 73A)

17 *Write short notes on*

(i) Vitamin A (Muz 75A)
(ii) Deficiency symptoms of vitamin D (M U 74A)
(iii) Vitamin D (R U 64S)
(iv) Rickets (Muz 74A , Mith 67A)
(v) Vitamin K (Pun 66A)
(vi) Tocopherol (M U 75A , Bh U 75A)
(vii) Thiamine (R U 74A , M U 73A , Mith 71A)
(viii) Thiamine deficiency (M U 72A)
(ix) Coenzymic activity of thiamine (Bh U 76A)
(x) Functions of riboflavin (Mith 65A)
(xi) Nicotinic acid (Bh U 75S P U 68S R U 69A)

(xii) Pyridoxine (R. U 75A)
(xiii) Folic acid (M U 76A , Math 67S , T U 70A)
(xiv) Vitamin C (P U 68S , R U 64A , Math 74A)
(xv) Cyanocobalamin (P U 72A)
(xvi) Scurvy (Bh U 74A ; R U 70S)
(xvii) Choline (R U 74A)
(xviii) Pellagra (P U 74A)
(xix) Beriberi (P U 72S)
(xx) Pernicious anemia (M U 76A)
(xxi) Osteomalacia (R U 74A)
(xxii) Ascorbic acid (Bh. U 72S)
(xxiii) Manifestation of Niacin deficiency (C.U 1982)

18 Discuss the importance of ascorbic acid as an essential food factor (C.U 1981)

CHAPTER 15

DIGESTION AND ABSORPTION FROM THE GASTROINTESTINAL TRACT

Unless tbe foodstuffs taken are broken down into smaller molecules, tbey are not absorbed from tbe digestive tract The disintegration nf foodstuffs into assimilable form constitutes the process nf digestion

The digestion in tbe alimentary canal takes place by tbe hydrolytie enzymes witb tbe very small expenditure of energy. During digestion the vitamins and the minerals are made more assimilable This is true to the fat-soluble vitamins wbicb are absorbed nnly wben fat digestion proceeds normally.

Advantages nf digestion ·

1 The composition of tbe body pnteins, fats and polysaccbarides is not the same as that nf proteins, fats and polysaccbarides nf food Only tbe units of tbese complex substances are tbe same in the body and tbe blood Tbe food protein must be bnken down into amino acids fnm wbicb tbe tissue protein ean be formed Similarly, tbe polysaccbarides and fats are formed in the body.

2 Dissaccbarides whicb are absorbed easily are not used in the body as such and are exereted as fnreign substances But after digestion to bexoses, tbey are utilized in tbe body

3 Digestion prevents the undesirable effects of the introduction of the foreign proteins into tbe blood converting tbem into amino acids whicb have no harmful effect

4 It prevents the too rapid absorptnn nf food and thus enables tbe blood to distribute the absorbed substances without uudesirable effects.

5 The power nf digestion increases the variation in our diet and our enjoyment

DIGESTION IN THE MOUTH

Constituents nf saliva :

1 Saliva is colourless, slightly acid (PH 6 4—7 1) rather tban alkaline, viscous fluid secreted mainly by the parntid, submaxillary and sublingual salivary glands The submaxillary secretion contains most nf the glycoproteins (mucin) but tbe parotid contains none

2 The amount nf secretion from each gland varies with tbe stimulus and nature of the food. The amount is stated tn be 1000—1500 ml each day

3 A saliva nf 0 6% solids will contain 0 4% organic and 0.2% inorganic material

4 The chief organic substances are glycoprotein (mucin) salivary amylase (ptyalin), small amounts nf albumin nnd globulin, urea and uric acid, traces of thiocyanic acid.

5 The inorganic substances are Cl^-, Na^+ K^+, Ca^{++}, Mg^{++}, HCO_3^- and HPO_4^{--} Chloride ion is an important activator of amylase Calcium ion helps to stabilise the amylase

Salivary digestion

1 The salivary amylase or ptyalin brings about the hydrolysis of starch and glycogen to maltose at the optimum PH 6 8 Chloride ion activates the enzyme

2 The enzyme can act on the food for a short time (5 to 6 minutes) which is of little significance

3 Salivary amylase is readily inactivated at PH 4 0 or less So the digestive action on food in the mouth is soon ceased in the acid environment of the stomach

4 In many animals, salivary amylase is entirely absent

Functions of saliva

1 Saliva moistens dry food and facilitates swallowing by the lubricating action of the glycoprotein

2 The digestion of starch begins in the mouth

3 Saliva contains buffering substances e g bicarbonate, phosphate and mucin

4 It keeps the mouth at a neutral Ph and thus protects the teeth from decalcification and also keeps the mouth and teeth clean

DIGESTION IN THE STOMACH

Gastric secretion

1 Nervous or reflex mechanisms cause the initiation of gastric secretion

2 The continued gastric secretion is regulated by the hormone gastrin (gastric secretion) The chemical stimulant is produced by the gastric glands and absorbed into the blood, which carries back to the stomach where it stimulates gastric secretion

3 Histamine, the decarboxylated product of the amino acid histidine also stimulates the secretion of gastric juice

Constituents of gastric juice

1 Normal gastric juice is a thin light coloured fluid which is strongly acidic

2. Hydrochloric acid secreted by the oxyntic cells (parietal cells) is 0 55 per cent which is equivalent to PH 0 9 The high acidity is neutralised by the high water content of saliva, the glycoprotein of saliva, the proteins of the food functioning as weak acid and the mucus of the stomach

3 The gastric juice contains 97—99% water and about 0 55% solids of which about 0 4 per cent is organic

4 The organic substances are the enzyme pepsin, glycoprotein, traces of lipase, rennin in infants

5 The inorganic substances are mainly Cl^-, K^+, Na^+, traces of Ca^{++}, Mg^{++}, phosphate and sulfate

6 On ordinary diet, the amount of secretion is 2—3 litres daily by an adult

7 The intrinsic factor (HCl and mucoproteins), present in the gastric juice, helps in the absorption of vitamin B_{12} The mucopolysaccharide present in the intrinsic factor contains fucose, hexosamine, hexoses and neuraminic acid

Gastric digestion :

An ordinary meal is evacuated from the stomach in about four hours The time may be more if the food is not properly masticated or if there is an excess of fat or gastric secretion is disturbed by emotion

A. Hydrochloric acid :

Hydrochloric acid is formed by the parietal cells and the process is similar to that of the 'chloride shift'.

Carbonic anhydrase catalyzes the formation of H_2CO_3 from H_2O and CO_2 as follows :

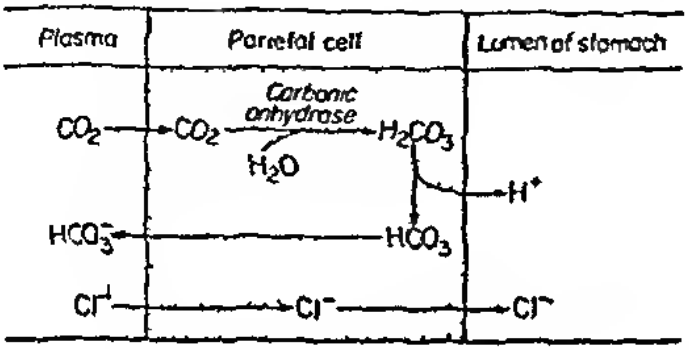


Fig 15 1 Production of gastric hydrochloric acid

Production of no hydrochloric acid leads to the condition achlorhydria

Functions of hydrochloric acid :

1 It maintains the optimal PH (1 2—1.5) for digestion of proteins by pepsin.

2. It converts inactive pepsinogen into active pepsin.

3. It stimulates duodenum to liberate secretin.

4 It helps the absorption of iron by converting ferric hydroxide of the food into ferric form which is changed to the ferrous form by reduction.

5. It denatures food proteins making them more readily digestible

6 It has a germicidal effect on micro-organisms and hence prevents the growth of micro-organisms in the stomach

B. Pepsin :

1. It is secreted in the inactive form pepsinogens which is activated to pepsin by the action of HCl and a small amount of pepsin can cause the activation of the remaining pepsinogen.

2. It converts native *protein into proteoses* and *peptones*

3 It has a molecular weight of 32,700.

4. It contains large amount of acidic amino acids

C. Rennin (Chymosin, Rennet) :

1. This enzyme occurs in the stomach of infants and is absent from the stomach of adults.

2. It causes the coagulation of milk and prevents the rapid passage of milk from the stomach.

3 It changes the casein of milk to paracasein in presence of calcium at P^H 5 Paracasein is then acted on by pepsin

$$\text{Casein} \xrightarrow[Co^{++}]{Rennin} \text{Calcium paracaseinate} + \text{Peptides}$$

Fig 15 7

D Lipase

1 The gastric lipase has mild lypolytic action which is of no significance

2 It can act only at the P^H 5 to 7 Hence it is not so active at P^H 1.3—1 5

PANCREATIC DIGESTION

Pancreatic secretion

1 The secretion of pancreatic juice is controlled by both nervous and hormonal means

2 Hydrochlorie acid, fats, proteins carbohydrates and partially digested foodstuffs entering the duodenum and the upper jejunum stimulate the secretion of hormone *secretin* and *pancreozymin*

Secretin stimulates the pancreas to produce thin, watery fluid, high in bicarbonate but low in enzyme content Pancreozymin stimulates the pancreas to produce a viscous fluid low in bicarbonate but high in enzyme content

Constituents of pancreatic juice

1 It is a clear alkaline fluid with a P^H about 8 0

2 It contains 1 8% of solids (including HCO_3^-) of which 0 6% is organic substances

3 The organic substances include proteins, the enzymes trypsinogen, chymotrypsinogen, carboxypeptidase, lipase, amylase, maltase, phospholipase, ribonuclease, deoxyribonuclease, cholesteryl ester hydrolase and collagenase

4 The inorganic substances are Na^+, Cl^-, HCO_3^- with small amounts of K^+, Ca^{++} and HPO_4^{--}

5 The amount of juice secreted each day is 600—800 ml.

Pancreatic digestion ·

A Trypsin

1 It is secreted in the inactive form trypsinogen which is converted into trypsin by the enzyme enterokinase secreted by the duodenal mucosa.

2 It attacks the native protein, proteoses and peptones to produce polypeptides

3 It attacks peptide linkages containing arginine or lysine residue

B Chymotrypsin .

1 It is also secreted in the inactive form chymotrypsinogen which is converted into chymotrypsin by the action of trypsin

2. It also attacks the native protein, proteoses and peptones to produce polypeptides

3 It attacks peptide linkages containing tyrosine and phenylalanine residues

C. Carboxypeptidase :

1. It is a zinc containing enzyme.

2. Two carboxypeptidases (A and B) occur in the pancreatic juice.

3. They are exopeptidase and hydrolyze only the terminal peptide linkage.

4. Carboxypeptidase A hydrolyses terminal peptide linkage containing tyrosine, phenylalanine and tryptophan while carboxypeptidase B hydrolyses terminal peptide linkages containing lysine and arginine.

D. Amylase :

1. Pancreatic amylase is similar in action to salivary amylase.

2. It is an α-amylase and an endoamylase.

3. It hydrolyses starch and glycogen into maltose and a mixture of branched of (1 : 6) oligosaccharides into glucose.

4. In pancreatitis, serum and urine amylase level is increased showing diagnostic importance.

E. Lipase :

1. Pancreatic lipase is the most important in the digestion of fats. It hydrolyses fats into diglyceride, monoglyceride glycerol and fatty acids.

2. It is specific for the hydrolysis of primary ester linkages which occurs in position 1 and 3 of triglycerol.

3. The hydrolysis of fat by lipase is increased when the fat is emulsified by bile salts. This is due to the larger surface exposed owing to the reduction of surface tension to the action of lipase.

F. Aminopeptidase and Dipeptidase :

The aminopeptidase attacks the terminal peptide bond at the free amino end of the chain.

G. Phospholipases :

These enzymes hydrolyse phospholipids (lecithin and cephalin).

H. Cholesteryl ester hydrolase (Cholesterol esterase) :

This enzyme hydrolyses the esterification of free cholesterol with fatty acids.

J. Collagenase :

This enzyme hydrolyses collagen present in meat and fish.

J. Ribonuclease (RNAase) and Deoxyribonuclease (DNAase) :

1. These enzymes are specific for the hydrolysis of RNA and DNA respectively.

2. These are endonucleases. Both the enzymes are capable of cleaving internal phosphodiester bonds to produce a 3′-hydroxyl and a 5′-phosphoryl or a 5′-hydroxyl and a 3′-phosphoryl terminus.

3. Some are capable of hydrolyzing both strands of a double stranded molecule whereas others can only cleave single strands of nucleic acids.

4. Some nucleases are exonucleases. These are capable of hydrolyzing a nucleotide when it is present at a terminus of a molecule.

DIGESTION IN THE INTESTINE

Intestinal secretion :

Hydrochloric acid, fats, proteins carbohydrates and partially digested foodstuffs entering the duodenum and the upper jejunum stimulates the secretion of *enterocrinin* which induces the flow of intestinal juice

Constituents of intestinal juice

1 It is an alkaline fluid of P^H about 7 8

2 The amount of the juice secreted each day by an adult is 2 to 3 litres

3 It contains about 1 5 per cent solids of which nearly two-thirds is inorganic and the remainder is organic substances

4 Half the inorganic part is $NaHCO_3$ and half NaCl

5 The organic substances are mainly enzymes and protein The enzymes include aminopeptidase and dipeptidase, lactase, maltase, sucrase, lipase, phosphatases, nucleases, nucleotidases, nucleosidases, phospholipases, enterokinase

Intestinal digestion .

1 **Disaccharidases (lactase, maltase, sucrase)**

They hydrolyse the corresponding disaccharides into monosaccharides for absorption

$$\text{Lactose} \xrightarrow{\text{Lactase}} \text{Glucose} + \text{Galactose}$$

$$\text{Maltose} \xrightarrow{\text{Maltase}} \text{Glucose} + \text{Glucose}$$

$$\text{Sucrose} \xrightarrow{\text{Sucrase}} \text{Glucose} + \text{Fructose}$$

2 **Phosphatases**

They remove phosphate from certain organic phosphates such as hexophosphate, glycerophosphate and the nucleotides derived from the food

3 **Nucleases**

They cause the hydrolysis of nucleic acids to mononucleotides

4 **Nucleotidases**

They hydrolyse nucleotides to nucleosides removing phosphate.

5 **Nucleosidases :**

They hydrolyse the nucleosides into purine or pyrimidine base and pentose sugars (ribose or deoxyribose)

6 **Phospholipases**

They hydrolyse phospholipids into glycerol, fatty acids, phosphoric acid and bases such as choline

7 **Enterokinase**

This is secreted by the duodenum It converts inactive trypsinogen to active trypsin

8 *Lipase*

This converts triglycerides and diglycerides into monoglycerides and fatty acids

THE BILE

1 Bile is absolutely necessary for the digestion of fat

2 Liver plays an important role in digestion by producing bile The gall bladder, attached to the hepatic duct stores a certain amount of bile produced by the liver between meals The composition of the bile in the gall bladder is modified by addition of mucin and other substances and by removal of water, bicarbonate and chloride by reabsorption by the bladder *mucosa During digestion* the gall bladder contracts by the stimulation of the hormone *cholecystokinin* which is produced by the small intestine and release bile rapidly to the small intestine by the way of common bile duct The pancreatic secretions mix with the bile

Properties of gall bladder bile

1 Gall bladder bile may be golden yellow brownish yellow or olive green in colour depending on the proportions of the bile pigments

2 It is a viscid fluid

3 It has a bitter taste and characteristic smell

4 The inorganic material is mainly Na^+, K^+ Ca^{++}, Cl^-, HCO_3^-

Daily formation

About 300 to 1200 ml of bile formed daily in adult human beings

Composition of bile

The composition of hepatic bile differs from that of gall bladder bile which is shown in the following table

Composition of human hepatic and gall bladder bile

Constituents	*Hepatic bile in per cent*	*Gall bladder bile in per cent*
Water	97 00	85 92
Solids	2 52	14 08
Bile acids	*1 93*	*9 14*
Mucin and pigments	0 53	2 98
Cholesterol	0 06	0 26
Fatty acids and fat	0 14	0 32
Inorganic salts	0 84	0 65
Specific gravity	1 01	1 04
pH	7 1—7 3	6 9—7 7

Bile acids :

1 Bile acids are synthesized in the liver from cholesterol. The synthesis of cholic acid from cholesterol is given below :

Cholesterol→7-hydroxy cholesterol→3, 7-dihydroxy cholestane→3, 7, 12-trihydroxycholestane→3, 7, 12-trihydroxy cholestanoyl CoA→cholyl-CoA→ cholic acid

2. The bile acids are derived from the parent acid called cholanic acid The structure of cholic acid and cholanic acid are given below

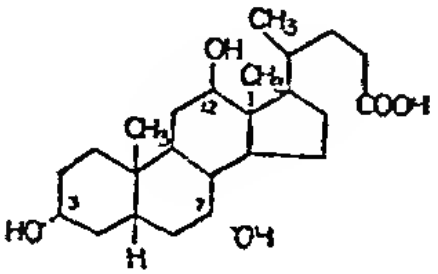


Fig 15 3 Structure of cholic acid (3 7, 12 trihydroxy cholanic acid)

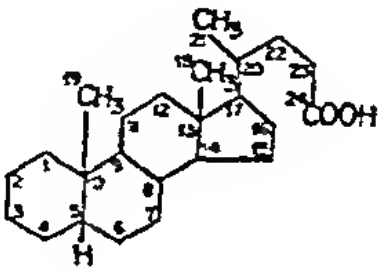


Fig 15 4 Structure of cholanic acid.

3 The different bile acids are hydroxy derivatives of cholanic acid which are given below :

Cholic acid	3, 7. 12-trihydroxycholanic acid.
Deoxycholic acid	3, 12-dihydroxycholanic acid
Chenodeoxycholic acid	3, 7-dihydroxycholanic acid.
Lithocholic acid	3-Hydroxycholanic acid.

4 Many substances such as fatty acids, phenols, higher alcohols, camphor, naphthalene, combine with deoxycholic acid in various molecular proportions. The resulting compounds are called *choleic acids* These choleic acids are water soluble. By such combinations insoluble fatty acids, cholesterol, fat-soluble vitamins, drugs are rendered soluble and diffusible and thus capable of being absorbed.

Bile salts

1 Cholic acid + Glycine→Glycocholic acid

2. Cholic acid + Taurine→Taurocholic acid.

Sodium or potassium glycocholate and sodium or potassium taurocholate are the two bile salts

3 In human bile, sodium or potassium glycocholate predominates It is three times as much as sodium or potassium taurocholate.

Functions

1 Bile salts act as emulsifying agents and emulsify fats increasing surface area and fats miscible with water. This helps to hydrolyse fats by pancreatic lipase

2 They activate pancreatic lipase and cholesterol esterase

3 They combine with free fatty acids and monoglycerides to form minute particles called miscelles and help in their absorption in the intestines

4 They stimulate intestinal peristalsis

5 They stimulate bile production in the liver They follow enterohepatic circulation and cause continuous secretion of bile by the liver

6 Cholesterol remains soluble in gall bladder bile by bile salts

7 They aid in absorption of fatty acids, chnlesterol, carotene, and the fat-soluble vitamins D and K by forming complexes more soluble in water (hydrotropic action)

Clinical significance

1 Bile salts in the blnod are increased greatly in clinical obstructive jaundice

2 After prnlonged obstruction, the concentratinn nf bile salts in the blood may diminish due to diminished synthesis of these substances as a result of progressive hepatocellular damage

3 In the absence of bile salts gall stones are formed

Bile pigments

1 Biliverdin and Bilirubin are the bile pigments formed from the breakdown of hemoglobin

2 They are excreted in the bile

3 They have no physiological role

The enterohepatic circnlntion

A portion of the bile ncids in the intestine undergoes changes by the netivity of the intestinal bacteria The deconjugation and 7α hydroxylation produce the secondary bile acids, deoxycholic acid from cholic ncid nnd lithocholic acid from chenodeoxycholic neid The conjugated nnd unconjugated bile salts are absorbed almost in the ileum As fecal bile ncids are present as the products of bacterial métabolism, therefore, it is assumed that metabolism within the intestinal lumen with reabsorption by passive diffusion is a component of the *enterohepatic circulation* This mechanism helps tn return 90% of the bile ncids secreted into the intestine to the liver each day But lithocholic acid is not reabsorbed to nny significant extent due to its insolubility

500 mg of bile salts per day are not absorbed and is eliminated in the feces The enterohepatic circulation of bile salts is sn efficient that a small amount of bile acids is cycled through the intestine 6-10 times a day with the loss of a small amount in the feces

Functions of bile

1 Bile salts help to lower the surface tension nf water nnd thus emulsify fats In the intestine nnd dissolve fatty acids nnd water insoluble soaps The presence of bile in the intestine helps the digestion and nbsorption of fats and the absorption of fat-soluble vitamins A, D, E and K

2 Bile salts are nctivators of lipase

3 Choleic acid formed by deoxycholic acid assists the nbsorption of many important insoluble compounds

4 Bile salts are reabsorbed from the intestine and pass back to the liver where they stimulate further secretion of the bile (cholagogue action)

5 Bile is an important source of alkali which helps to neutralize the acid chyme from the stomach

6. Bile is an important channel for the excretion of some substances like bile pigments, many drugs, toxins, and various inorganic substances such as copper, zinc and mercury

7 Fat digestion is impaired in the absence of bile. The fat then covers the other food particles and prevents enzymes from attacking them. These undigested food particles ultimately leads to putrefaction in the large intestine.

8 Bile salts keeps cholesterol in solution in gall bladder bile. In the absence of bile salts, cholesterol becomes precipitated This results in the formation of *gall-stones*

GALL-STONES

In the gall-bladder, cholesterol is solubilized by being held in micelles together with conjugated bile salts and phospholipids. The solubility depends on the ratio of cholesterol to bile salts plus phospholipids The secretion of phospholipids into the bile depends on the availability of bile salts. If bile salt content is decreased, the phospholipid content is also diminished and hence, the solubility of cholesterol is decreased causing crystallization These crystals grow to form stones

Gall stones are formed due to defects in the enterohepatic circulation and with the diseases of the terminal ileum as well as in patients with cirrhosis. In these cases, there is reduction in the bile salt pool

Infection of bile causes the deconjugation of bile acids with a decrease in their solubility This also results in the production of a phospholipase which converts lecithin to lysolecithin This decreases the stability of the micelles holding cholesterol in solution Infection can give rise to calcium-bilirubinate stones which were frequent in Japan

Chenodeoxycholic acid decreases the rate of secretion of cholesterol into the bile Bile then becomes unsaturated with respect to cholesterol and thus the cholesterol stone can be redissolved Unfortunately bacterial action in the intestine converts chenodeoxy acids to lithocholic acid which is very hepatotoxic in Rhesus monkeys producing proliferation of bile ducts.

Classification

Gall stones are mainly of three types

1 **Cholesterol stones**

(a) These stones may be single or multiple.
(b) They may be white or yellowish.
(c) They may be mulberry shaped
(d) They are not radio-opaque.

2 **Pigment stones**

(a) These stones are formed by bile pigments, organic material and calcium.
(b) They are small multiple stones.
(c) They are hard, dark green or black.
(d) They are rarely radio-opaque.

3 Mixed Gall stones

(a) These are composed of a mixture of cholesterol, bile pigments, Protein and Calcium
(b) They are faceted dark brown stones with a hard shell and soft centre.
(c) They may be radio-opaque
(d) They are the commest forms of gall stones

RENAL CALCULI

This incidence is more in North India due to the hot climates prevalent for more than six months in the year and the large amounts of tea (rich in oxalates) consumed by them In addition, the diet consumed by the people are rich in calcium, phosphates, and purines The urine volume of these people is small and they excrete more uric acid, calcium and phosphates

Conditions of Calculi formation

1 Low urine volume due to warm climates or low intake of water

2 Consumption of diets rich in calcium, phosphates, oxalates, and purines

3 Conditions accelerating increased excretion of uric acid, oxalic acid, calcium, and phosphates in urine

4 Urine infection and stagnation

Classification

Renal Calculi can be classified into the following four groups

1 Calculi due to calcium oxalate and phosphates High concentration of calcium oxalates and phosphates in urine forms stones which are hard, white, and radio-opaque

2 Urate stones This is due to hyperuricemia and gout These stones are usually small and yellowish brown in colour

3 Cystine stones These are rare but only happens in cystinuria

4 Xanthine stones These are also rare but only happens in xanthinuria.

INTESTINAL PUTREFACTION AND FERMENTATION

1 The residue of the food after absorption in the small intestine passes into the large intestine Water is also absorbed there and the residue becomes solid During this period, the activity of the bacteria takes place The bacteria produces various gases, such as CO_2 methane, hydrogen nitrogen and H_2S as well as acetic acid, lactic acid and butyric acid by fermentation and putrefaction Lecithin is decomposed to choline and neurine

2 Intestinal bacteria cause the decarboxylation of many amino acids into their amines such as

Histidine→Histamine.
Tyrosine→Tyramine
Arginine→Agmatine
Lysine →Cadaverine

Many of these amines are powerful vasopressor substances

3 After reduction, biliverdin and bilirubin produces urobilinogen and stercobilin and cholesterol to coprosterol, sulphur to H_2S

4 By a series of reactions, tryptophan forms indole and methylindole (skatole) which are responsible for the odour of the feces

5 Ethyl and methyl mercaptan and H_2S are formed from cysteine by a series of transformations

6 Intestinal bacteria putrefy nitrogenous substances to form ammonia which is absorbed into the portal circulation and is removed from the blood by the liver under normal conditions In the liver diseases, the concentration of ammonia in the peripheral blood rises to toxic levels Neomycin by its anti-bacterial action reduces the quantity of ammonia transported from the large intestine to the blood

7 The large intestine is the site for the reduction of biliverdin and bilirubin to urobilinogen and stercobilinogen, cholesterol to coprosterol, cystine to cysteine, sulphur to H_2S

Importance of intestinal bacteria

1 In man, intestinal bacteria synthesize certain vitamins, particularly vitamin K and possibly certain members of the B-complex

2 In herbivora, the intestinal bacteria are essential for the digestion of cellulose and make it available for absorption They also synthesize essential amino acids and vitamins

AUTOLYSIS

After death, the intracellular enzymes digest the tissues when kept under sterile conditions This self-digestion of tissues are called *autolysis*

The group of intracellular proteases present in all mammalian tissues is called *cathepsin* Cathepsins occur in the lysosomes There are four cathepsins which are analogous to pepsin, trypsin, aminopeptidase and carboxypeptidase.

Autolysis takes place in the living animal under pathological conditions, e.g. wasting in starvation and fevers Atrophy is a process of autolysis in the living animal

DIGESTION AND ABSORPTION FROM THE GASTROINTESTINAL TRACT

Digestion and absorption of carbohydrate

The carbohydrate diet mainly consists of polysaccharides (starch and *glycogen) and disaccharides (sucrose and milk lactose) It also contains* indigestible cellulose, hemicelluloses and pentosans etc

Digestion

Mouth Salivary amylase (ptyalin) starts the digestion of cooked starch in the mouth But very little digestion takes place in the mouth since the food remains in the mouth for a very short period of time

Stomach Since the food gets mixed with the gastric juice the action of amylase ceases due to high acidity Some of the sucrose present in the food get hydrolysed by the action of HCl in the stomach.

Small intestine The pancreatic amylase in the small intestine converts starch and glycogen into a mixture of maltose and isomaltose

$$\text{Starch and glycogen} \xrightarrow{\text{Pancreatic amylase}} \text{Maltose} + \text{isomaltose}$$

Then maltose and isomaltose along with sucrose lactose present in the diet are digested by the different disaccharidases present in the intestinal mucosa into their corresponding monosaccharides as stated below

$$\text{Maltose} \xrightarrow{\text{Maltase}} \text{Glucose} + \text{Glucose}$$

$$\text{Isomaltose} \xrightarrow{\text{Isomaltase}} \text{Glucose} + \text{Glucose}$$

$$\text{Sucrose} \xrightarrow{\text{Sucrase}} \text{Glucose} + \text{Fructose}$$

$$\text{Lactose} \xrightarrow{\text{Lactase}} \text{Glucose} + \text{Galactose}$$

Cellulose is not digested in human G I tract due to the absence of cellulase

Absorption

The comparative rates of absorption of monosaccharides taking glucose as 100 may be indicated as follows galactose (110) glucose (100), fructose (43), mannose (39), xylose (15) and arabinose (9) Galactose and glucose are absorbed at a faster rate than fructose Pentoses are slowly absorbed This is due to the fact that glucose and galactose are actively transported while fructose, mannose and pentoses are absorbed by simple diffusion

The monosaccharides are absorbed into the mucosal cells of small intestine and pass into circulation via portal vein A very small amount may be absorbed by the lymph The microvilli (brush border) lining the mucosa cells greatly help the absorption by increasing the surface area The rate of absorption of monosaccharides is independent of blood sugar concentration

Glucose and galactose for absorption follow the active transport against a concentration gradient Because they have the same chemical characteristics which are necessary for active transport mechanism The chemical characteristics are

(1) The OH on carbon 2 should have the same configuration as in glucose
(2) A pyranose ring should be present
(3) A methyl or a substituted methyl group should be present on carbon 5

Active transport of glucose

1 A mobile carrier which binds both glucose and Na^+ at separate sites and which transports them through the plasma membrane of the intestinal cell is required

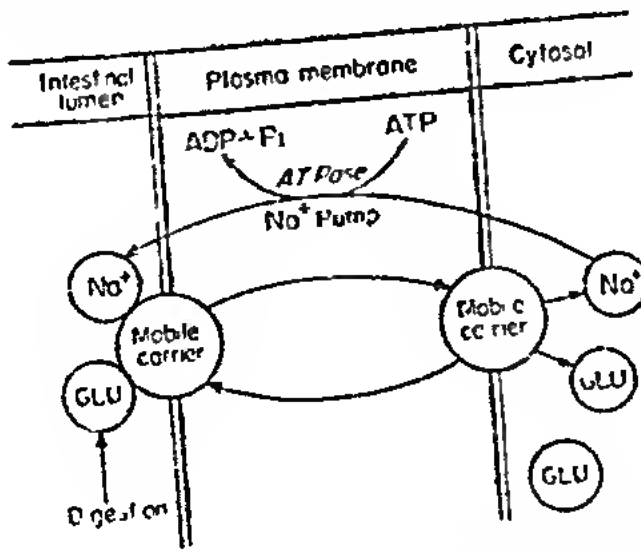


Fig 15 5 Active transport of glucose (GLU) across the intestinal mucosa

2 Both the glucose and Na^+ are released into the cytosol, allowing the carrier to return for more "cargo"

3 The Na^+ is transported down its concentration gradient and at the same time causes the carrier to transport glucose against its concentration gradient

4 The free energy required for this active transport is obtained from the hydrolysis of ATP linked to a sodium pump which expels Na^+ from the cell

Since polysaccharides and oligosaccharides are quickly hydrolysed the absorptive mechanism for glucose galactose and fructose are quickly saturated. But the hydrolysis of lactose proceeds at half the rate for sucrose This slower rate of hydrolysis of lactose shows that the digestion of lactose does not lead to saturation of the transport mechanism for glucose and galactose

Factors controlling active transport of glucose

1 Active transport of sugar is depressed by agents (cyanide, malonate, fluoroacetate) that inhibit respiration and by dinitrophenol which uncouples oxidative phosphorylation

2 Phlorhizin which interacts with the membrane site at which the sugar enters, inhibits intestinal absorption of glucose and galactose.

3 Ouabain (cardiac glycoside), an inhibitor of the sodium pump, inhibits active transport of glucose

4 The absorption of glucose is interferred by various intoxications, prolonged undernutrition and vitamin deficiencies especially of thiamine, pantothenic acid and pyridoxine

5 Absorption may be decreased in the presence of abnormalities (structural or functional) of the mucous membrane, as in inflammation (enteritis), edema and celiac disease

6 The absorption is retarded in hypothyroidism and accelerated in hyperthyroidism

7 Diminished absorption in adrenal cortical insufficiency is dependent upon the decreased concentration of sodium in the body fluid.

Disaccharidurea The intestine normally is virtually impermeable to disaccharides If it is absorbed, it is not metabolised. An increase in the excretion of intact disaccharides are found in some patients with *disaccharidase* deficiencies The patients with intestinal damage also excrete more disaccharides This condition is said to be disaccharidurea

Digestion and absorption of fats

Digestion

Stomach Lipase present in the stomach is unable to hydrolyze fats owing to the high acidity of the gastric contents Therefore, the major part of the ingested fat is digested in the small intestine

Small intestine The ingested fat reaching the duodenum is mixed with the bile and pancreatic juice which contains lipase The bile salts emulsify the fat before the action of lipase The emulsification is also brought about by monoglycerides, phospholipid and lysolecithin.

The secreted inactive pancreatic lipase is activated by bile and Ca^{++} The surface area of the emulsified fat becomes increased for which the rate of reaction of lipase is increased

Pancreatic lipase hydrolyzes 1- and 3-positions of the triglycerides leaving a mixture of 2 monoglycerides 1, 2 and 2, 3 diglycerides as well as the soaps of the free fatty acids

Triglyceride —(Pancreatic lipase)→ 1,2 diglyceride —(Pancreatic lipase)→ 2 monoglycerides

Fig 15 6

The pancreatic juice also contains phospholipase and cholesterol-esterase which hydrolyze phospholipids and esterified cholesterol

Intestinal juice also contains a lipase whose action is not of much importance as most of the fat is hydrolyzed by the pancreatic lipase

Absorption :

Several theories have been proposed for the mechanism of absorption of fats after digestion The important theories are A Lypolytic hypothesis B. Partition theory. C. More recent theory.

A **Lypolytic hypothesis :**

1 According to this theory, fat is completely hydrolyzed to fatty acids and glycerol which are absorbed.

2 The fatty acids combining with bile salts form a miscible complex which is absorbed into the intestinal mucosa

3. The fatty acids are then separated from bile acids and converted into triglycerides by combining with glycerol

4 The triglycerides are passed to the lacteals They then enter the lymphatics and reach the systemic circulation via thoracic duct

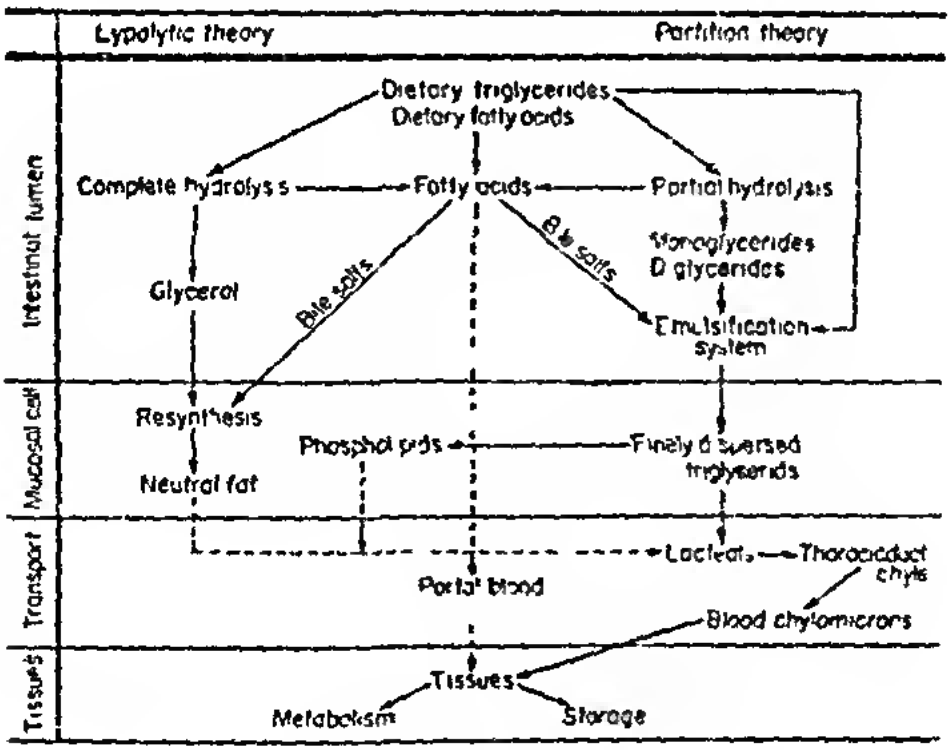


Fig 15 7. The old concept of fat absorption containing of lypolytic theory and partition theory.

B Partition theory

1 According to this theory, 30 per cent of the triglycerides are hydrolyzed to fatty acids and glycerol while 70 per cent remain unhydrolyzed.

2. The unhydrolyzed triglycerides are emulsified by monoglycerides and diglycerides in combination with bile salts to form a minute particle known as 'micelles' of size about 0 1 to 0 5μ

3 The resulting mixture is absorbed into the intestines, passed on to the lacteals and then to the lymphatics The mixture then reach to the systemic circulation viz thoracic duct.

4 The free fatty acids are absorbed as bile salt fatty acid complex into the intestinal mucosa The fatty acids are absorbed into the portal blood to reach the liver

C. Recent theory

1 The removal of the ester group of 2 monoacylglycerol requires isomerization to a primary ester linkage This is a slow process As a result, monoacylglycerols are the major end products of fat digestion and less than one-fourth of the ingested fat is completely broken down to glycerol and fatty acids

2. Within the intestinal wall, 2 monoacylglycerols are converted to triacylglycerols and 1 monoacylglycerols are further hydrolyzed to form free glycerol and fatty acids.

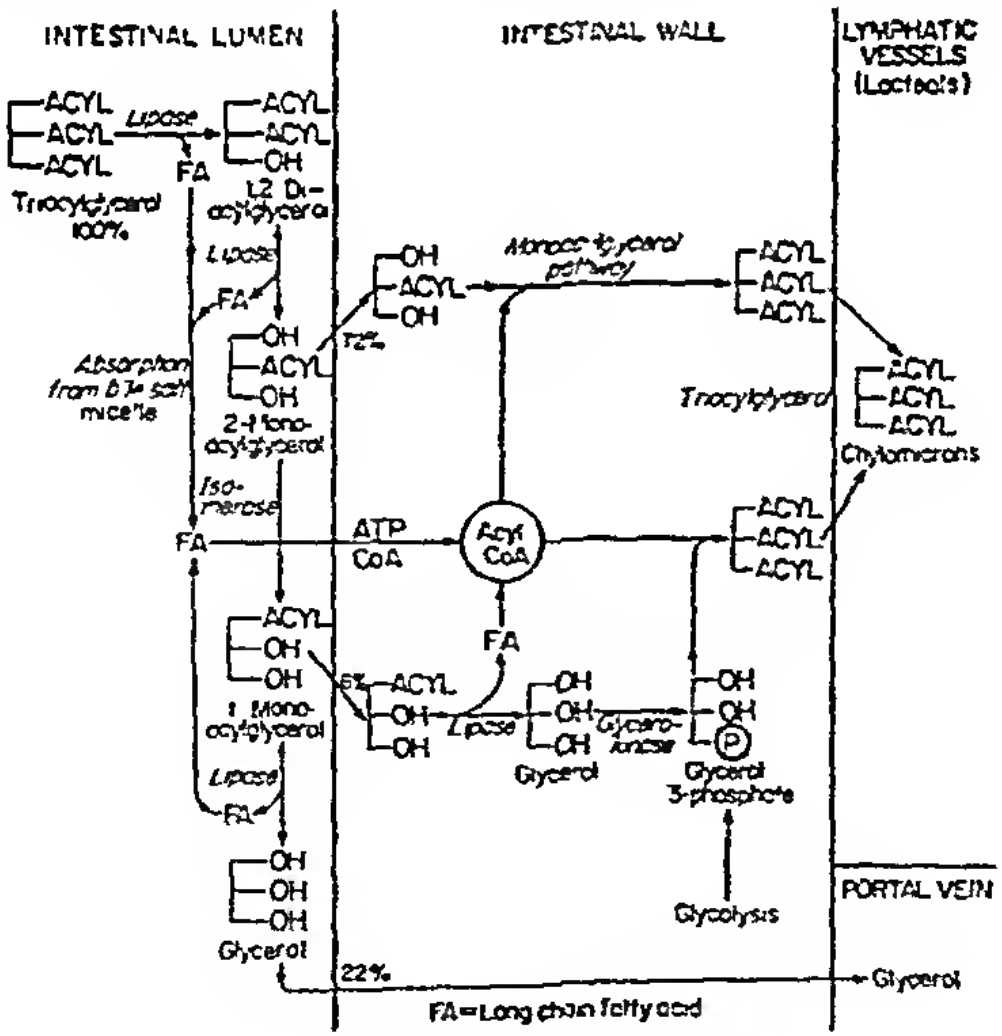


Fig 15.8 Digestion and absorption of fat

3 The fatty acids are then activated by *thiokinase* in presence of ATP and coenzyme A for the resynthesis of triacylglycerols

4 The free glycerol in the intestinal lumen is about 22 per cent of the total amount of triacylglycerol originally present This passes directly to the portal vein

5 The glycerol within the intestinal wall is activated by *glycerokinase* in presence of ATP to form glycerol 3 phosphate for the synthesis of triacylglycerol followed by the combination with acyl CoA present in the intestinal wall

6 All long chain fatty acids present in the intestinal wall are reincorporated into triacylglycerols which are transported to the lymphatic vessels of the abdominal region (the so-called lacteals) for distribution to the rest of the body

7 The great majority of absorbed fat appears in the form of chylomicrons which appear first at the lymphatic vessels of the abdominal region and later in the systemic blood The chylomicrons contains triacylglycerol, free and esterified cholesterol, phospholipid and 0 5 per cent protein

All of the factors relating to digestion and absorption of fat are mentioned in figure below .

Absorption of phospholipids : Phospholipids are split by phospholipases and their acyl chains are incorporated into chylomicrons Choline, the hydrophilic component, may be transported directly to the liver via the hepatic portal vein

Absorption of cholesterol It is absorbed into the lymphatics and recovered mainly as cholesteryl esters

Chyluria In this abnormality, the patient excretes milky urine because of the presence of an abnormal connection between the urinary tract and the lymphatic drainage system of the intestine, a so-called "chylous fistula' .

Digestion and absorption of protein

Digestion The proteolytic enzymes secreted in gastric juice, pancreatic juice and also present in the intestinal mucosa cause the hydrolysis of protein in the gastrointestinal tract

Stomach • Pepsin, the endopeptidase, is present in gastric juice and hydrolyzes the peptide bonds in the interior of the protein molecule Pepsin hydrolyzes the dietary protein into a mixture of polypeptides

$$\text{Dietary protein} \xrightarrow{\text{Pepsin}} \text{Polypeptides}$$

Rennin has a strong clotting action on milk This is very important in the digestion of milk proteins in infants The PH of the gastric juice becomes low in achlorhydria, achylia gastrica (both pepsin and HCl absent) and in pernicious anemia Then dietary protein will not be digested in the stomach

Small intestine • The polypeptides formed in the stomach are digested in the intestine by trypsin, chymotrypsin and carboxypeptidases secreted in pancreatic juice and aminopeptidases present in the intestinal mucosa Trypsin hydrolyzes peptide linkages containing arginine or lysine and chymotrypsin hydrolyzes peptide linkages containing tyrosine or phenylalanine

$$\text{Polypeptides} \xrightarrow{\text{Trypsin \& chymotrypsin}} \text{Peptides} + \text{Amino acids}$$

Fig 15 9

Carboxypeptidase A hydrolyzes the end group of peptides containing aromatic or aliphatic amino acid and releases free amino acids, Carboxypeptidase

B hydrolyzes peptides containing arginine and lysine residues. The intestinal mucosa also contains tripeptidase, dipeptidase etc., which hydrolyze tri- and dipeptides

$$\text{Peptides} \xrightarrow{\text{Carboxypeptidase}} \text{Amino acids.}$$

$$\text{Peptides} \xrightarrow{\text{Aminopeptidase}} \text{Amino acids.}$$

$$\text{Dipeptides} \xrightarrow{\text{Dipeptidase}} \text{Amino acids.}$$

$$\text{Tripeptides} \xrightarrow{\text{Tripeptidase}} \text{Amino acids.}$$

The final products of digestion of proteins are amino acids which are absorbed.

Absorption :

1. Three different active processes are involved in the transport of amino acids. One process involves cystine and the basic amino acids, another the amino acids proline and hydroxyproline and the third the neutral (L—) amino acids.

2. D-amino acids are absorbed by simple diffusion. But the neutral (L—) amino acids require a carrier system in the absorption. Na^+ is also required. This is similar to that of active transport of glucose. Vitamin B_6 (pyridoxal phosphate) is also involved in the process. The amino acid associates with the carrier and Na^+ in the microvilli and the complex travels to the inner side of the membrane where it dissociates, releasing the amino acid and Na^+ into the cytosol. The carrier returns back and functions repeatedly. Na^+ is then actively transported out of the cell.

3 If one amino acid is fed in excess, it retards the absorption of another This is similar to those made with respect to reabsorption of amino acids by the renal tubules.

4 Sometimes the whole protein is absorbed into the blood. A protein is antigenic and accounts for food allergies. In the young mammal, the permeability of the mucosa, in this respect, is greater than that in the adult.

5 Food proteins are generally readily digested (90 to 97 per cent) under normal conditions, very little escapes in the feces. The insoluble fibrous protein, keratin, is not hydrolyzed by enzymes of the human digestive tract. These are altered by heating to coagulation and hydrolyzed by superheated steam. The biological values of these proteins are not affected by such procedures. Cooked egg albumin is digested more readily than raw The nutritional value of cereal proteins is lowered by overheating or toasting

DIGESTION AND ABSORPTION OF NUCLEOPROTEINS AND NUCLEIC ACIDS

Digestion :

Stomach The nucleoproteins of many foods are cleaved by the acidity of the gastric juice into proteins (histones, protamines etc.) and nucleic acids.

$$\text{Nucleoproteins} \xrightarrow{HCl} \text{Nucleic acids} + \text{Proteins}$$

The proteins are hydrolyzed by proteases present in the digestive juices to amino acids in the same way as the dietary proteins

Small intestine Nucleic acids are hydrolyzed by three groups of enzymes

1 Nucleases 2 Nucleotidases 3 Nucleosidases

Nucleases are of two types 1 Ribonucleases 2 Deoxyribonucleases Ribonucleases hydrolyze RNA and deoxyribonucleases hydrolyze DNA The end products are nucleotides

Nucleotidases hydrolyze nucleotides into nucleosides and phosphoric acid

Nucleosidases hydrolyze nucleosides into purine or pyrimidine base and the pentose sugars (ribose or deoxyribose)

All of the above mentioned reactions are given below

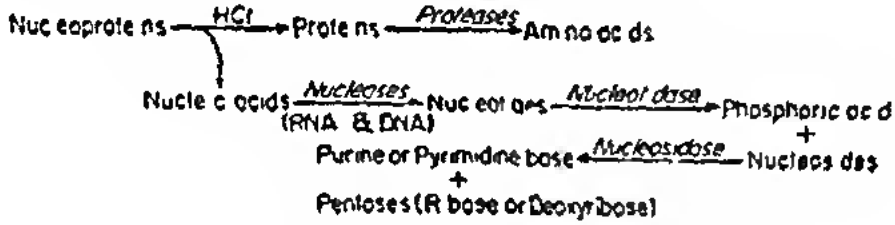


Fig 15 10 Digestion of nucleoproteins

Absorption

1 The purine or pyrimidine base and the pentoses are absorbed in the small intestine

2 Certain amounts of nucleosides and pentose phosphates may also be absorbed Most of the tissues contain nucleosidases which hydrolyze nucleosides absorbed from the intestines

GASTROINTESTINAL HORMONE

INTRODUCTION

1 Many hormones are liberated more by the gastrointestinal tract than any other single organ

2 The gastrointestinal hormones assist in digestive processes of the foodstuffs moving the digested products into the extracellular space through the intestinal mucosal cells, moving those products to distant cells through the circulation and expelling waste products

BIOMEDICAL IMPORTANCE

1 Excessive production of several of the gastrointestinal hormones cause disease syndrome The physicians cannot diagnose accurately unless they are aware of these syndromes

2 These hormones have also close link to neuropeptides

Acronyms Used in this Chapter

BLI	Bombesinlike immunoreactivity
CCK	Cholecystokinin.
GIP	Gastric inhibitory polypeptide.
GRP	Gastrin-releasing peptide.
PP	Pancreatic polypeptide
VIP	Vasoactive intestinal polypeptide

FEATURES OF GASTROINTESTINAL HORMONES

A. Diversity of actions

1 The classic definition of hormone is satisfied by many of the gastrointestinal peptides Examples include gastrin, secretin, GIP, CCK, motilin, PP and enteroglucagon

2 Other gastrointestinal peptides have *paracrine* actions or to act in a neurocrine fashion Examples include VIP, somatostatin, substance P, BLI, Enkephalins, and neurotensin Although these substances are found in high concentration in neurons, they are either not found in the circulation under normal conditions or have such short plasma half-lives that they would not be effective

Table 15.1. Gastrointestinal hormones

+E=Endocrine, N=Neurocrine, P=Paracrine, ( )=Suggested but not proved, +=yes, −=No

Hormones	Mechanism of action			Major Action
	E	N	P	
Gastrin	+	(+)	−	Gastric acid and pepsin secretion
CCK	(+)	(+)	−	Pancreatic amylase secretion.
Secretin	+	−	−	Pancreatic bicarbonate secretion
GIP	+	−	−	Enhances glucose-mediated insulin release, inhibits gastric acid secretion
VIP	−	+	(−)	Smooth muscle relaxation, stimulates pancreatic bicarbonate secretion
Motilin	(+)		−	Initiates interdigestive intestinal motility
Somatostatin		+	(+)	Numerous inhibitory effects
PP	(+)	−	(+)	Inhibits pancreatic bicarbonate and protein secretion
Enkephalins	−	+	(+)	Opiatelike actions

Substance P	–	+	(+)	Physiologic action uncertain
BLI		+	(+)	Stimulates release of gastrin and CCK
Neurotensin		+	(–)	Physiologic actions unknown
Enteroglucagon	(+)	(+)	(+)	Physiologic actions unknown

B. *Location of Gastrointestinal Peptide-producing cells.*

1 Many of the gastrointestinal peptides are found in the nerves in gastro intestinal tissues and most of them are also present in the central nervous system

2 Synthesis of the peptides by central nervous system tissue is difficult to prove, but new techniques of molecular biology should establish whether genes coding for these substances are active

3 The function of these peptides in the central and peripheral nervous system is under investigation

4 The distribution of the gastrointestinal hormones is mentioned below

Table 15.2 Distribution of gastrointestinal hormone

EC=Enterochromaffin cell =yet to be named

Hormones	Endocrine cell †	Localization	Localized in Gut Nerves
Gastrin	G	Gastric antrum, duodenum	(?)
CCK	I	Duodenum, jejunum	Yes
Secretin	S	Duodenum jejunum	No
GIP	K	Small bowel	No
VIP	D_1	Pancreas	Yes
Motilin	EC_2	Small bowel	No
Substance P	FC_1	Entire gastrointestinal tract	Yes
Neurotensin	N	Ileum	(?)
Somatostatin	D	Stomach, duodenum pancreas	Yes
Enkephalins		Stomach, duodenum, gall bladder	Yes

BLI	P	Stomach, duodenum	Yes
PP	D_2F	Pancreas	No
Enteroglucagon	A	Pancreas	No
	L	Small Intestine	

C. Precursors and Multiple Forms

1 Secretin only exists in a single form.

2 The presence of multiple forms of gastrointestinal peptides in gastrointestinal tissues and in the circulation impeded the definition of the number and nature of these molecules

D. Overlapping structure and function of Gastrointestinal peptides

1 In accordance with the sequence and functional similarity many of these hormones can be placed in one of two families These are the *gastrin family* and *secretin family*

2 The gastrin family consists of gastrin and CCK.

3 The secretin family includes secretin, glucagon, GIP, VIP, and glicentin (which has glucagonlike immunoreactivity but is a distinct peptide)

4 The neurocrine peptides neurotensin, bombesinlike peptides, substance P, and somatostatin bear no structural similarity to any other gastrointestinal peptide. They have very short plasma half lives and may play no physiologic role in plasma.

E. Mechanism of Action

1 Pancreatic acinar cells have got six different classes of receptors. These

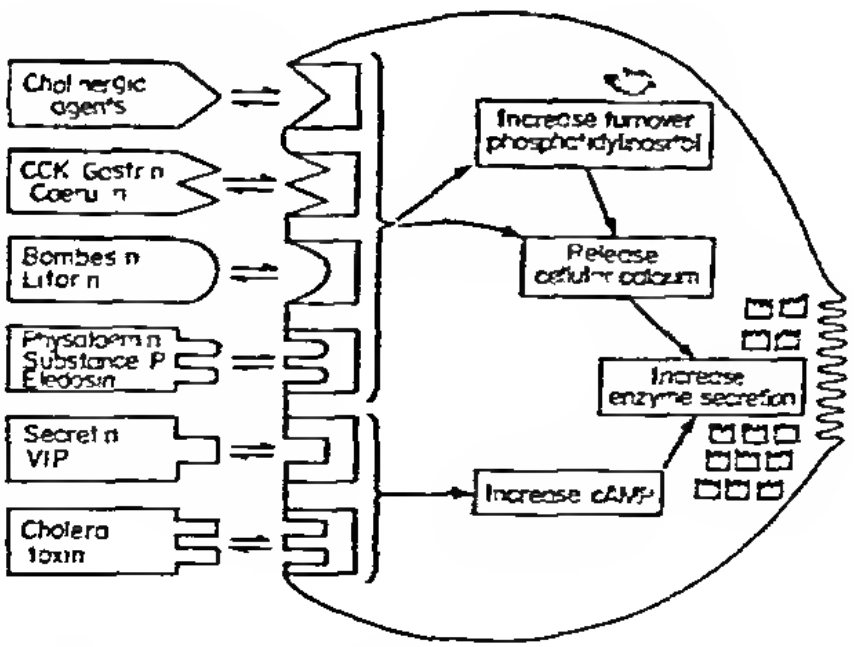


Fig 15 11 Mechanism of action of secretagogues on enzyme secretion from pancreatic acinar cells.

are for (a) muscarinic cholinergic agents. (b) The gastrin CCK family. (c) Bombesin and related peptides. (d) The physalaemin-substance P family. (3) Secretin and VIP. (f) Cholera toxin.

2. The peptide-receptor complexes activate two distinct intracellular mechanisms. One involves the mobilization of intracellular calcium stores, and the other involves the activation of adenylate cyclase and the generation of cAMP.

3. The mechanisms do not cross over, i.e., gastrin does not change cAMP levels, nor does secretin affect the intracellular Ca++ level. These two systems converge at some point.

4. The peptides causing Ca++ mobilization in the pancreatic acinar cells also affect the metabolism of phosphatidylinositol and enhance its conversion to diacylglycerol and various inositol phosphates. These effects are associated with depolarization of the acinar cell which may be involved in amylase secretion.

5. The mechanism of the actions of CAMP, Ca++, and phospholipids on amylase secretion is similar in many respects.

THE SECRETIN FAMILY

A. Secretin

1. Secretin is constituted of 27 amino acid.

2. It is synthesized and released by the S cells in the duodenum and proxi jejunum in response to duodenal acidification.

3. It contains 4 arginine residues and one histidine and is basic.

4. Its structure is identical to that of glucagon in 14 of 27 amino acids.

5. It is structurally quite similar to that of GIP and VIP.

6. Its entire structure is required for stimulation of bicarbonate and water secretion from the pancreas.

7. It causes lipolysis.

8. It is administered to assess the pancreatic exocrine function.

B. Gastric Inhibitory Polypeptide (GIP)

1. GIP consists of 43 amino acids and is released from the duodenal and jejunal mucosa in response to glucose.

2. It stimulates insulin release.

3. It is the physiologic B cell stimulating hormone of G.I.T.

4. Hyperglycemia appears to be required for its action.

C. Vasoactive Intestinal Polypeptide (VIP)

1. VIP is a basic 28-amino acid peptide whose physiologic function has not been defined.

2. It exists in the nerves of the submucosal plexus, the myenteroplexus, and blood vessels

3. It causes gut motility, sphincter relaxation, and blood flow.

4. Highly concentrated VIP stimulates secretion by the pancreas and small intestine.

5. Tumors that produce this peptide, VIPomas, cause a syndrome of watery diarrhea, hypokalemia and achlorhydria.

THE GASTRIN–CHOLECYSTOKININ FAMILY

A. Gastrin

1 Gastrin is produced by G cells of the antral gastric mucosa and to a small extent in the duodenal mucosa

2 It shows more heterogeneity in size than any other gastrointestinal hormone

3 Its each form has sulphated and nonsulphated form

4 The carboxy-terminal 14 amino acids of G_{34}, G_{17} and G_{14} are identical G_{34} is more abundant in the circulation than G_{17}, because its plasma half-life (15 minutes) is 5 to 7 times that of G_{17}

5 G_{17} is the main stimulus for gastric acid secretion which is under negative feedback control, since acidification of the antral region of the stomach decreases gastrin release

6 Gastrin also stimulates the secretion of pepsin and intrinsic factor from gastric mucosa

7 The carboxyl end of gastrin is responsible for biologic activity

8 The carboxyl terminal pentapeptide has the full range of physiologic action of G_{17}

9 Pentagastrin is a synthetic pentapeptide and is used clinically.

10 Increased gastrin release is caused by vagal stimulation (e g , caused by insulin-induced hypoglycemia), by acetyl-choline and by food intake particularly protein or amino acids, of the amino acids glycine is the most potent

11 It stimulates secretin release and can delay delivery of gastric contents into the duodenum by reducing the rate of gastric emptying

12 *Zollinger Ellison* syndrome is characterized by gastrin secreting tumors, *gastrinomas*, which cause excessive gastric acid production and intractable peptic ulcer disease

13 Pentagastrin causes calcitonin release and is used as a provocative test in the diagnosis of medullary thyroid cancer

B. Cholecystokinin (CCK)

1 Cholecystokinin is present in I cells in the mucosa of the duodenum and proximal jejunum

2 It is constituted of 39 mino acids

3 It exists in at least 5 molecular forms of which CCK 8 is the most abundant and potent form in the circulation

4 A 7-amino acid C terminal fragment with a sulphated tyrosine is required for maximal CCK activity (stimulation of pancreatic enzyme secretion and contraction of gall bladder)

5 It is released in response to peptides, amino acids, long chain fatty acids, calcium, and acid

6 It effectively stimulates the release of both insulin and glucagon from pancreas

7 Its physiologic actions are to cause gall bladder contraction and to stimulate pancreatic enzyme secretion

8 It performs many of the actions of gastrin and secretin on water, bicarbonate and acid changes

9 It represents as one of the "Gut factors" influencing insulin release during oral administration of glucose

OTHER GASTROINTESTINAL PEPTIDES

A. Substance P

1 Substance P is found in both the gut and the brain

2 This peptide contains 11 amino acids

3 The 5 carboxy-terminal amino acids of this peptide are required for its action which causes stimulation of smooth muscle contraction in the intestine

B. Bombesin

1 *Bombesin* is found in frog skin, but a similar peptide, often called *gastrin-releasing peptide (GRP)* has been isolated from endocrine cells in the gut, gut neurons and brain

2 The amino acids at positions 5 – 14 of bombesin are identical to those at positions 18– 27 of gastrin-releasing peptide except at one residue

3 It stimulates gastric and pancreatic secretion and increases mobility of the gall bladder and intestine

4 It has a growth-promoting autocrine action in small cell carcinoma of the lung

C. Motilin

1 This peptide contains 22 amino acids and is found in the intestinal mucosa

2 It stimulates acid and pepsin secretion by gastric mucosa

3 It is a stimulator of intestinal smooth muscle contraction

D. Somastatin

1 This peptide is formed in gastric D cells

2 It inhibits (by paracrine action) the release of gastrin, secretin, CCK, motilin, and GIP

E. Glucagon

1 This is made in the gastric mucosa A cells and is thought to contribute to the metabolic action of pancreatic glucagon

2 Other peptides with glucagon like immunoreactivity *(GLI)* have been isolated from the L cells of the ileum and colon

3 The major component of GLI is a large 100 amino acid peptide called *glicentin* which contains the exact sequence of pancreatic glucagon

4 Glicentin may mimic the actions of glucagon

F Neurotensin (a 13 amino acid peptide), Met- and Leu-enkephalins, and serotonin are found in intestinal cells and may be active in these tissues

Some 40 peptides have been found in neural tissues It is highly expected that more gastrointestinal peptide will be discovered

Enterocrinin

1. It is released from the duodenal mucosa

2. This polypeptide stimulates the secretion of intestinal juice by the small intestine

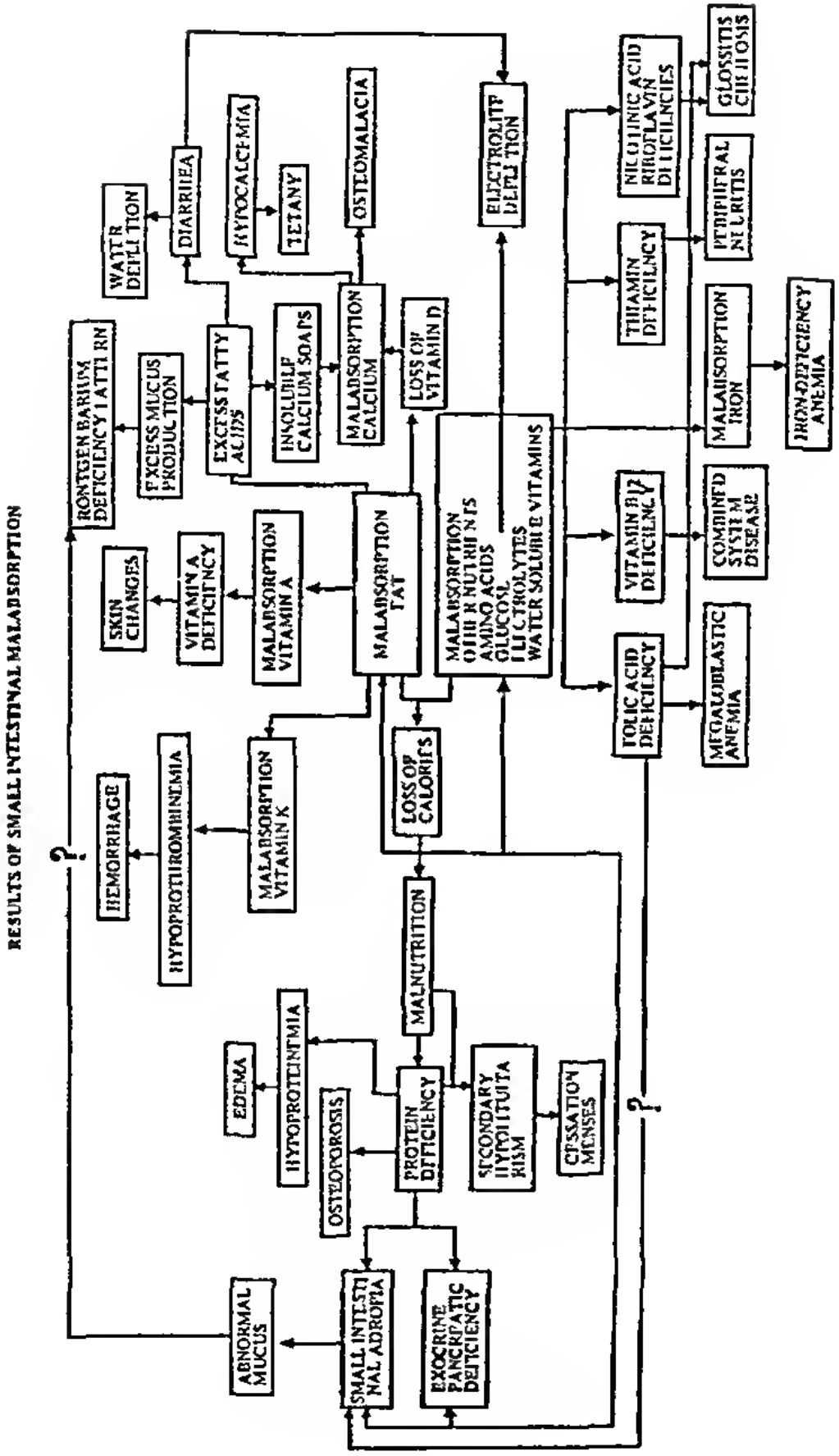


Fig. 15.12 Gastrointestinal Malabsorption Syndromes

Enterogastrone

1 Fat in the diet influences the secretion of this polypeptide by the duodenal mucosa

2 It inhibits gastric secretion and gastric motility

3 It inhibits the secretion of HCl and pepsin

Hepatocrinin

1 This polypeptide is liberated from the duodenum

2 It stimulates liver to secrete more bile

Exercise

1 How are foodstuffs digested in the Gastrointestinal tract ? (P U 69A , R U 74A)

2 Discuss the mechanism of absorption of the foodstuffs after digestion (P U 62A , 64S)

3 Write short notes on

(a) HCl formation (P U 68A)
(b) Gastro-intestinal Hormones (R U 70A)
(c) CCK-PZ (B U 74A)
(d) Intrinsic factor (Bh U 72S)
(e) Pancreatic juice (R U 73A)

CHAPTER 16

METABOLISM OF CARBOHYDRATE

Fate of absorbed carbohydrate

Carbohydrate after absorption into the portal blood passes to the systemic circulation through the liver Certain hepatic mechanisms are involved in the withdrawal of carbohydrates from the blood The hepatic mechanisms are :

1 Uptake of hexoses like fructose and galactose for conversion to glucose by the liver cells
2. Conversion of glucose to glycogen for storage in the liver
3 Utilization of glucose for energy production by oxidation
4 Utilization of glucose for the synthesis of other compounds such as fatty acids and certain amino acids

Others which oppose these above mechanisms lead to the release of glucose by the liver to the blood by the following means

1 Formation of blood sugar from hexoses other than glucose by the liver.
2. Conversion of liver glycogen to blood glucose
3 Synthesis of blood glucose by the liver and kidney from non-carbohydrate sources

The amount of glucose reaching the systemic circulation is the resultant of the operations of these two groups of opposing processes After that the blood glucose is utilized by the extrahepatic tissues

Utilization of glucose

A Storage

1 In the absence of urgent physiological demands, excess glucose may be deposited as glycogen in the liver, muscles and other tissues (Glycogenesis)

2 Since the amount of glucose stored in the liver is limited excess of this upper limit is converted to fatty acids and stored as triglycerides in the fat depots.

B Oxidation

1 Glucose is completely oxidized in all tissues to Co_2 and H_2O for physiological demands for energy

2. Sometimes under special circumstances in muscle, there is only the partial degradation of glucose (Glycolysis) forming lactic acid notably by liver

C Conversion to fat

Glucose is converted to fatty acids when glycogen storage is exceeded The conversion of glucose to fatty acids is irreversible but the transformation of glucose to glycerol is reversible

D Conversion to other carbohydrates and other substances -

1 Mannose, glucosamine and galactosamine form parts of the mucopolysaccharides and glycoproteins

2 Glucuronic acid is involved in the mucopolysacoharides and in 'detoxication' reactions

3 Galactose is required as a component of the glycolipids and lactose

4 Ribose and deoxyribose are required for the synthesis of the nucleic acids

E Conversion to amino acids

Certain amino acids are also formed from glucose These amino acids are said to be "glucogenic"

Metabolism Metabolism consists of anabolism (synthesis) and catabolism (break down)

INTERMEDIARY METABOLISM OF CARBOHYDRATE

In the mammalian organism the metabolism of carbohydrate is subdivided as follows

1 *Glycogenesis* The synthesis of glycogen from glucose in the liver and muscle

2 *Glycogenolysis* The breakdown of glycogen to glucose in the liver and pyruvate and lactate in the muscle

3 *Glycolysis* The breakdown of glucose and glycogen to pyruvate and lactate under anaerobic conditions

4 *Citric acid cycle* The oxidation of pyruvate to Co_2 and H_2O under aerobic conditions

5 *The hexose monophosphate shunt* (direct oxidative pathway, pentose phosphate cycle, phosphogluconate oxidative pathway) The oxidation of glucose by an alternative pathway to glycolysis and citric acid cycle

6 *Gluconeogenesis* Formation of glucose and glycogen from non-carbohydrate sources Citric acid cycle and glycolysis are involved in this process

GLYCOGENESIS

1 Glucose is first phosphorylated to glucose-6 phosphate by the enzyme hexokinase and glucokinase in presence of the coenzyme ATP and the activator Mg^{++} ATP is converted to ADP Hexokinase has high affinity for glucose Glucokinase is to remove glucose from the blood following a meal

2 Glucose 6 phosphate is then converted to glucose 1 phosphate the reaction is catalyzed by the enzyme *phosphoglucomutase* with Mg^{++} and the reaction is reversible Glucose-1, 6 diphosphate is formed as an intermediate due to the phosphorylation of the enzyme

3 Glucose-1-phosphate reacts with uridine triphosphate (UTP) to form the active nucleotide (*uridine diphosphate glucose* (*UDPG*) The reaction is catalyzed by the enzyme *UDPG pyrophosphorylase* with the release of inorganic pyrophosphate

4 The C_1 of the activated glucose of UDPG forms a glycosidic bond with the C_4 of the terminal glucose residue of glycogen liberating UDP by the enzyme glycogen synthetase (glucosyl transferase)

5 By the successive 1, 4 linkages by glucose units to the preexisting glycogen chain, the branches of the glycogen 'tree' become elongated

When the chain has been lengthened to between 6 and 11 glucose residues, a second enzyme, the branching enzyme (amylo-1, 4→1, 6 transglucosidase) acts on the glycogen This enzyme transfers a part of the -1, 4 chain (minimum length of 6 glucose residues) to a neighbouring chain to form α-1, 6-linkage which establishes a branch point in the molecule

Storage of Carbohydrate in postabsorptive normal adult (70 kg)

Liver Glycogen	1 0% = 72 grams
Muscle glycogen	40 7% = 245 gms
Extra cellular glucose	0 1% = 10 gms
Total	327 gms

Liver wight	1800 gms
Muscle mass	35 kg
Total Volume	10 L

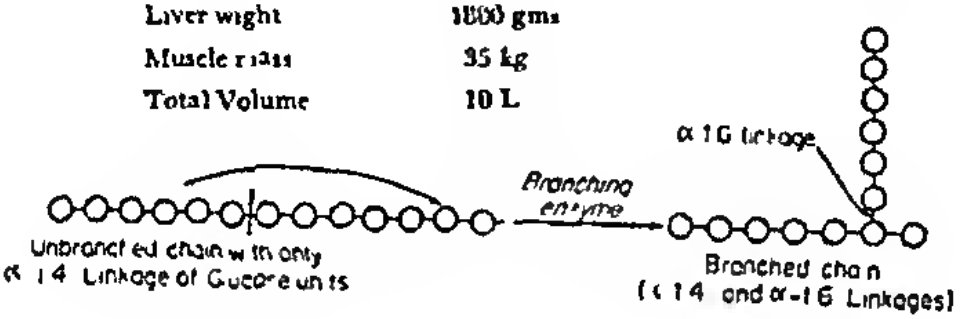


Fig 16 1 Action of branching enzyme

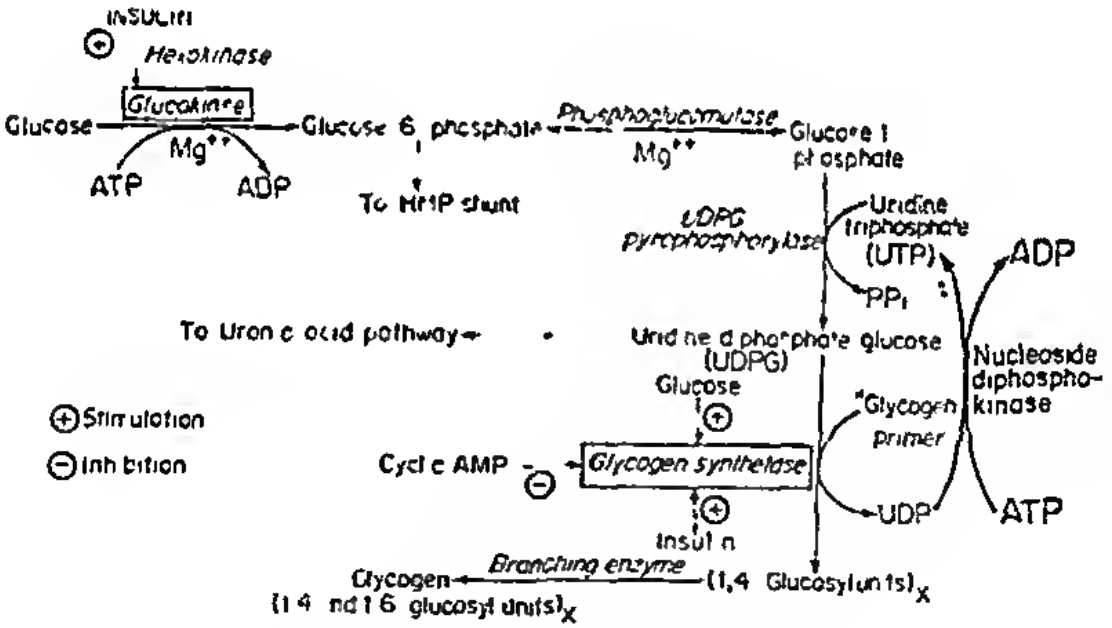


Fig 16 2 Pathway of glycogenesis in the liver and muscle

Glycogen

1 It is a branched polysaccharide consisting of D-D-glucose units These glucose units are connected to one another by glucosidic linkages between the first and fourth carbon atoms except at branch points

2 The molecular weight varies from 1 million to 5 million or more.

3. In case, some of the glucose chains terminate in the interior, a larger molecule is possible.

Glycogen synthetase

1 In muscle (and possibly liver) it is present in two inter-convertible forms—synthetase D (dependent) whose activity is dependent on the presence of glucose 6-phosphate and synthetase I (independeot) whose Km value for UDPG decreases in the presence of glucose 6 phosphate

2 Synthetase D is converted to synthetase I by *synthetase phosphatase*

3 Synthetase I is phosphorylated to form synthetase D by *synthetase kinase* (cAMP dependent protein kinase) which is active in the presence of 3 5 cyclic adenylic acid (cAMP) ATP acts as phosphate donor

4 Glycogen synthetase is stimulated by insulin and glucose but inhibited by cyclic AMP

Cyclic AMP

1 It is 3 5 adenylic acid (adenine ribose and phosphate) the 3rd aod 5th carbon atoms of ribose are attached to phosphate group The structure is given below

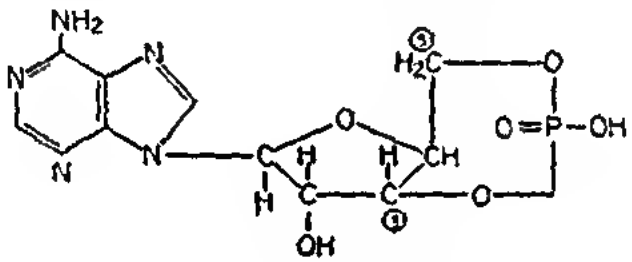


Fig 16 3 Structure of cAMP

2 It is the intracellular intermediate compound through many hormones act

3 *Adenylate cyclase* of cell membranes being activated by the hormones epinephrine norepinephrine and glucagon forms cAMP from ATP

4 cAMP is destroyed by *phosphodiesterase* The normally low level of it is maintained by the activity of phosphodiesterase In liver insulin increases the activity of cAMP

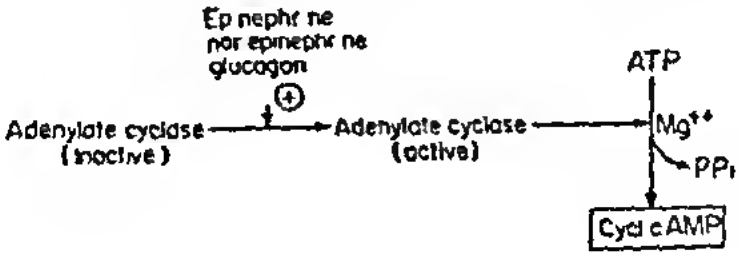


Fig 16 4 Formation of cAMP

Functions

1 cAMP causes inhibition (−) of glycogen synthetase

2 It stimulates (+) phosphorylase activity

3 Glycogen synthetase I is phosphorylated to form synthetase D by cAMP-dependent protein kinase and glycogen synthetase I is formed from synthetase D by syothetase phosphatase

Adenylate cyclase

1 It is the enzyme occurring in cell membrane

2 It is activated by epinephrine norepinephrine and glucagon and the activated eozyme forms cAMP from ATP

3 Thyroid hormones may increase tbe synthesis of adenylate cyelase

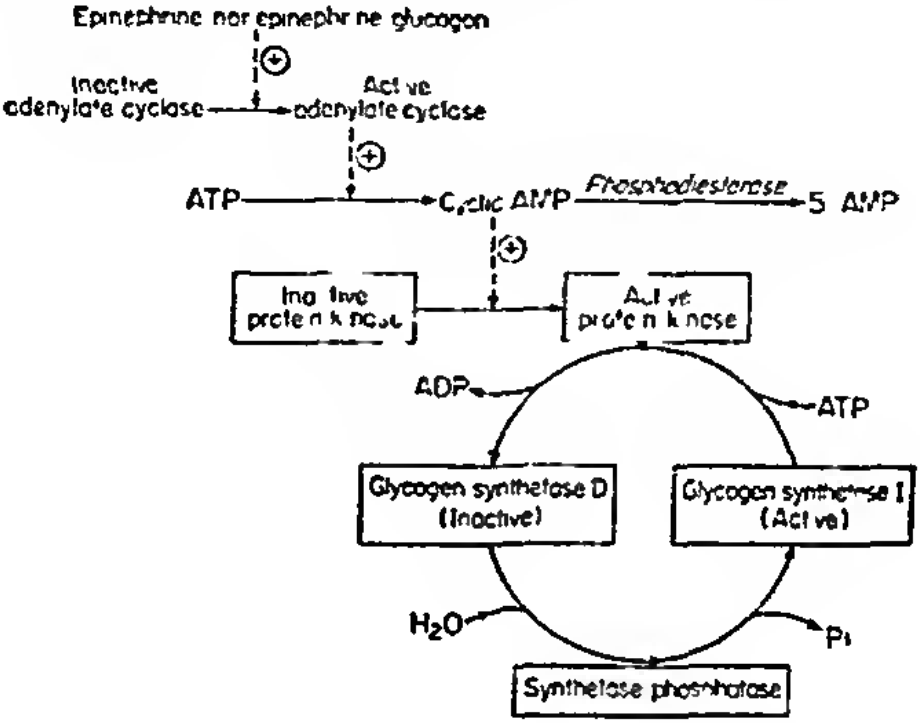


Fig 16 5 Control of glycogen synthetase in muscle

Cyclic GMP

1 Cyclic GMP is 3 5 guanylic acid (guaoine, ribose and phosphate), the 3rd and 5th carbon atoms of ribose are attached to phosphate

2 *Prolactin causes the elevation of cGMP in mouse mammary gland which* may he mediated by $PGF_{2}\alpha$ and PGF_{2} aod also raises the level of cGMP in the rat uterus

3 PGE_{2} at high concentratioo stimulates cGMP accumulation

4 PG endoperoxides also stimulate the production of cGMP in platelets and recent evideoce iodicates (hat PGG_{2} activates tbat soluble guanylate cyclase from platelets

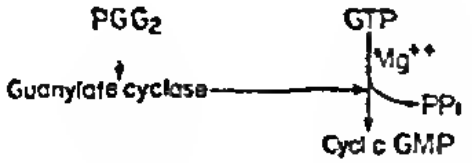


Fig 16 6 Formation of cyclic GMP.

UDPG :

1 UDPG has been isolated from animal tissues and from yeast.

2 Uridine diphosphate glucose is an intermediate product of glycogenesis formed from glucose-1-phosphate by the enzyme UDPG pyrophosphorylase in presence of the coenzyme UTP

3 It is essential for the synthesis of lactose in the mammary gland thus .

$$\text{UDPG} + \text{galactose-1-(P)} \xrightarrow[\text{Uridyl transferase}]{\text{Galactose-1-(P)}} \text{UDP-galactose} + \text{glucose 1-(P)}$$

$$\text{UDP-galactose} + \text{glucose} \xrightarrow{\text{Lactose synthetase}} \text{Lactose} + \text{UDP}$$

4 It can be enzymatically oxidized to UDP-glucuronic acid by UDPG-dehydrogenase and is required for the production of ascorbic acid (vitamin C) by the uronic acid pathway

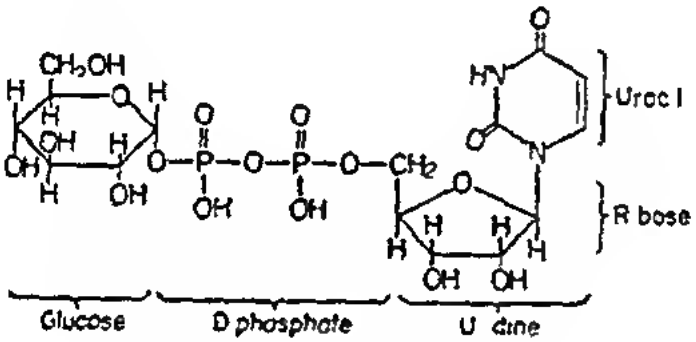


Fig 16 6 : Uridine diphosphate glucose (UDPG)

GLYCOGENOLYSIS

1 Glycogen breakdown in the liver and muscle is initiated by the enzyme *phosphorylase* This enzyme is specific for the phospborylytic breaking of the α-1, 4-linkages of glycogen to produce glucose-1-phosphate The removal of -1, 4 glucosyl residues continues until about 4 glucose residues remain on either side of a -1, 6-branch Cyclic AMP stimulates phosphorylase activity

The *debranching enzyme* (amylo-1, 6-glucosidase) causes the hydrolytic splitting of -1, 6-linkages

By the combined action of both these enzymes, glycogen is converted to glucose-1-phosphate

2 The action of phosphoglucomutase is reversible and glucose-6 phosphate is formed from glucose-1-phosphate

3 In liver and kidney (but not in muscle), there exists a specific enzyme, *glucose-6-phosphatase*, which removes phosphate from glucose 6-phosphate, enabling the free glucose to diffuse from the cell into the extracellular spaces including the blood The overall breakdown is shown on the next page

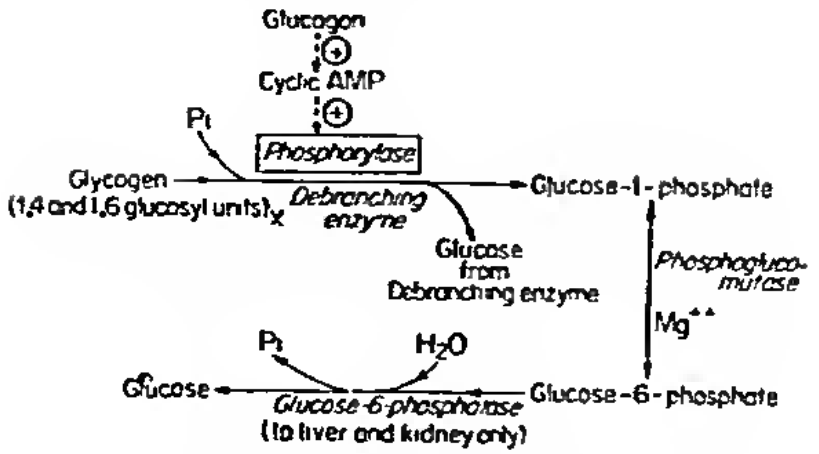


Fig 16.7 Pathway of glycogenolysis in the liver.

Phosphorylase :

1. In *liver*, phosphorylase exists in both active and inactive form. The active phosphorylase (phosphorylase a or phosphophosphorylase) can be inactivated by phosphorylase phosphatase to *dephosphophosphorylase*. Reactivation takes place by the enzyme *phosphorylase b kinase* or *dephosphophosphorylase kinase* in presence of ATP.

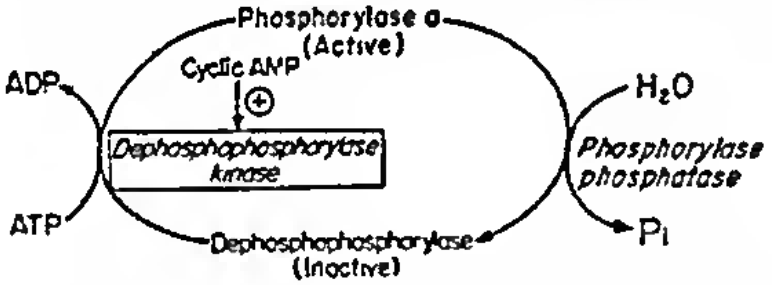


Fig 16.8 Inactivation and reactivation of liver phosphorylase

2. In *muscle*, phosphorylase is present in two forms : *phosphorylase a* (active in the absence of 5'-AMP) and *phosphorylase b* (active only in the presence of 5'-AMP).

Phosphorylase a is physiologically active. It is a tetramer containing 4 mol. of pyridoxal phosphate It is hydrolytically converted to *phosphorylase b*, a dimer, by *phosphorylase phosphatase*. Phosphorylase b contains 2 mol. of pyridoxal phosphate.

$$\text{Phosphorylase a} + 4H_2O \xrightarrow{\text{Phosphorylase phosphatase}} 2\ \text{phosphorylase b} + 4P_i$$
(active) (inactive)

Phosphorylase b recondenses to phosphorylase a by *phosphorylase b kinase*. Conversion of phosphorylase b to phosphorylase a signifies the mechanism for increasing glycogenolysis.

$$2\ \text{phosphorylase b} + 4ATP \xrightarrow[Mg^{++}]{\text{Phosphorylase b kinase}} \text{phosphorylase a} + 4ADP$$

3 Inactive muscle phosphorylase b kinase is converted to active phosphorylase by kinase by the activation of active protein kinase which is being stimulated by cyclic AMP Epinephrine is involved in cyclic AMP formation through adenylate cyclase

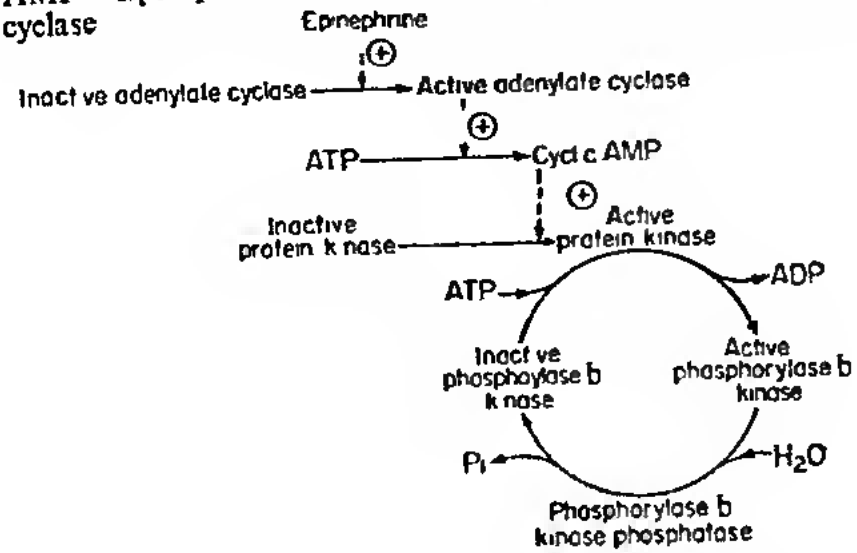


Fig 16 9 Control of phosphorylase in muscle

4 Difference is muscle phosphorylase and liver phosphorylase

(a) No cleavage in the structure in case of liver phosphorylase

(b) Muscle phosphorylase is not affected by glucagon

(c) Active phosphorylase inhibits liver synthetase phosphatase

GLYCOLYSIS

A muscle when contracts under anaerobic condition, pyruvate and lactate become the principal end product But if it contracts under aerobic condition, pyruvate and lactate disappear and these are further oxidized to Co_2 and H_2O Hence, carbohydrate metabolism is divided into two phases—anaerobic and aerobic When oxygen is in short supply, NADH is reoxidized by being coupled to the reduction of pyruvate to lactate

Sequence of reactions in glycolysis

All the enzymes of the Embden Meyerhof pathway are found in cytosol They catalyze the reactions involved in the glycolysis of glucose to lactate

1 Glucose is first phosphorylated to glucose 6 phosphate by the enzyme *hexokinase* and by an additional enzyme in the liver, *glucokinase* The activity of glucokinase is affected by nutritional state This reaction is accompanied by ATP and Mg^{++} This is an irreversible reaction

Hexokinase has a high affinity for glucose than glucokinase Its function is to supply glucose to the tissues even in the low blood glucose concentration It can catalyze the phosphorylation of other hexoses but at a slower rate than glucose But the function of glucokinase is to remove glucose from the blood following a meal Both the enzymes are stimulated by insulin

2 Glucose-6-phosphate is an important compound in the metabolic pathways (glycolysis, gluconeogenesis hexose-monophosphate shunt, glycogenesis, glycogenolysis) It is converted to fructose 6 phosphate by *phosphohexose isomerase*.

3 Fructose-6-phosphate is phosphorylated with ATP by phosphofructokinaset o form fructose-1, 6-diphosphate This is also an irreversible reaction The enzyme is also stimulated by insulin

4 The enzyme *Aldolase* splits fructose 1, 6-diphosphate into two triosephosphates · glyceraldehyde-3-phosphate and dihydroxy acetone phosphate

Both the triosphosphates are interconverted by *phosphotriosesisomerase.*

Dihydroxy acetone phosphate is also formed from glycerol which is phosphorylated to glycerol-3-phosphate then to dihydroxy acetone phosphate.

5 Glyceraldehyde-3-phosphate is oxidized to 1, 3-diphosphoglycerate by *glyceraldehyde-3-phosphate dehydrogenase* with the help of the coenzyme NAD Energy is released in the course of the reaction

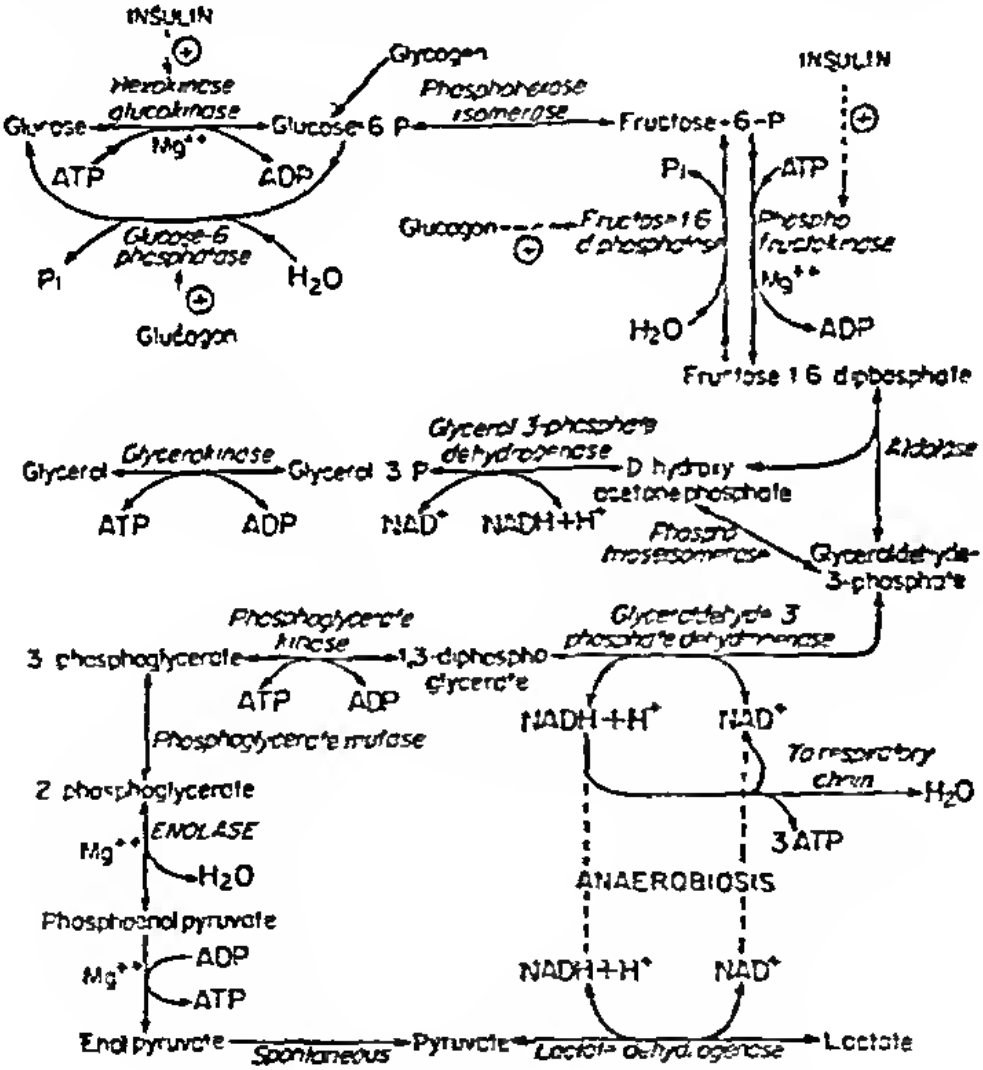


Fig 16 10 Embden-Meyerhof pathway of glycolysis

6 3-phosphoglycerate is formed from 1, 3-diphosphoglycerate by phosphoglycerate kinase with the production of ATP from ADP

Since two molecules of triosephosphates are formed from one molecule of glucose, 2 molecules of ATP are generated in this stage The reaction is reversible.

7. 3-phosphoglycerate is converted to 2-phosphoglycerate hy phosphoglycerate mutase.

8. 2-phosphoglycerate is catalyzed hy *enalase* to produce phosphoenolpyruvate io presence of Mg^{++}.

9. The high-energy phosphate of phosphoenolpyruvate is transferred to ADP hy pyruvate kioase to form ATP.

10. Enolpyruvate formed is cooverted spontaoeously to the keto form of pyruvate. This is ao irreversihle step.

11. If aoaerohic conditions prevail, the reoxidation of NADH through the respiratory chain to oxygen is preveoted. Pyruvate then is reduced to lactate hy the NADH. The reaction is catalyzed hy *lactate dehydrogenase.*

Glycolysis in erythrocytes even uoder aerohic condition forms lactate hecause of the ahsence of enzymatic machinery for the aerobic oxidation of pyruvate.

Total number of ATP formed hy glycolysis onder aoaerobic conditions opto pyruvic acid :

From one molecule of glucose, two molecules of glyceraldehyde-3-phosphate are formed. After that io the reactions of glycolysis each product is 2 molecules.

Reactions	*ATP formed*
1. Glyceraldehyde-3-phosphate→1, 3-diphosphoglycerate ...	6
2. 1, 3-diphosphoglycerate→3-phosphoglycerate	2
3 Phosphoenolpyruvate→Enol pyruvate	2
Total ..	10
ATP consumed In	
1. Glucose→glucose-6-phosphate	1
2. Fructose-6-phosphate→Fuctose-1, 6-diphosphate ...	1
Net ATP Synthesized ..	8

But in aoaerobic conditions, the total number of ATP will be only 2 upto lactic acid.

Because the reduced NAD (NADH) io glyceraldehyde-3-phosphate-dehydrogenase is utilized in lactate dehydrogenase. So NADH is not oxidized in mitochondria.

The total calories io this case is $2 \times 7600 = 15{,}200$ calories.

Effect of hormooes In glycolysis :

1. *Insulin* stimulates hexokioase aod glucokinase which catalyze the conversion of glucose to glucose-6-phosphate.

2. *Insulin* stimulates phosphofructokinase which catalyzes the conversion of fructose-6-phospbate to fructose-1, 6-diphosphate.

3. *Glucagon* stimulates liver glucose-6-phosphatase which is iovolved io the conversion of glucose-6-phosphate to glucose and also fructose-1, 6-diphosph a-tase involved io the cooversioo of fructose-1, 6-diphosphate to fructose-6-phosphate.

Inhibitors :

1 *Iodoacetate* is the inhibitor of glyceraldehyde 3-phosphate dehydrogenase involved in the conversion of glyceraldehyde-3-phosphate to 1, 3-diphosphoglycerate.

2. *Arsenate* inhibits the synthesis of ATP by accomplishing uncoupling of oxidation and phosphorylation in the conversion of 1, 3-diphosphoglycerate to 3-phosphoglycerate.

3 *Fluoride* inhibits enolase involved in the conversion of 2-phosphoglycerate to phosphoenolpyruvate

OXIDATION OF PYRUVATE TO ACETYL-CoA

1 Pyruvate is oxidatively decarboxylated to acetyl-CoA ("active acetate") before entering the citric acid cycle.

2. The reaction is catalyzed by the multienzyme complex consisting of several different enzymes. This complex is known as pyruvate dehydrogenase complex.

3 Pyruvate is decarboxylated in the presence of thiamine pyrophosphate (TPP) to a hydroxymethyl derivative which reacts with oxidized lipoate to form S-acetyl lipoate being catalyzed by the enzyme *pyruvate dehydrogenase.*

4 S-acetyl lipoate reacts with coenzyme A to form acetyl-CoA and reduced lipoate in presence of *dihydrolipoyl transacetylase*

5 The reduced lipoate is reoxidized by FAD in presence of *dihydrolipoyl dehydrogenase.*

6 Finally, the reduced FAD is oxidized by NAD^+ The reduced NAD ($NADH+H^+$) enters the respiratory chain producing 3 ATP.

7 The pyruvate dehydrogenase complex consists of about 29 mol. of pyruvate dehydrogenase and 8 mol of dihydrolipoyl dehydrogenase distributed around 1 mol of transacetylase.

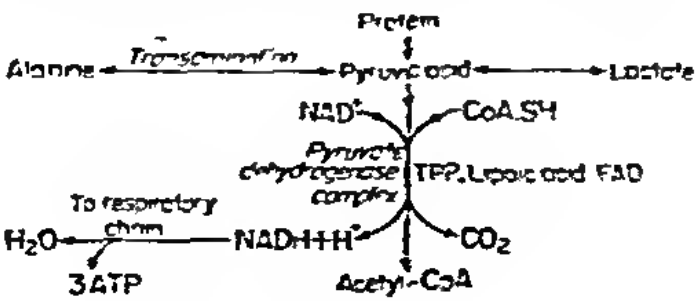


Fig 16.11 Oxidation of pyruvic acid to acetyl-CoA.

Inhibitors : Arsenite inhibits pyruvate dehydrogenase and dietary deficiency of thiamine also allows pyruvate to accumulate.

THE CITRIC ACID CYCLE
(KREBS OR TRICARBOXYLIC ACID CYCLE)
(IN MITOCHONDRIA)
(IN AEROBIC CONDITIONS)

This cycle consists of a series of reactions in mitochondria which catabolyzes the oxidation of acetyl CoA to CO_2 and H_2O in aerobic condition The acetyl CoA combines with a 4 carbon dicarboxylic acid, oxaloacetate to form 6-carbon tricarboxylic acid, citrate In the course of the reactions 2 molecules of CO_2 are lost and oxaloacetate is regenerated Oxaloacetate plays an important catalytic role

Reactions of the citric acid cycle

1 Acetyl CoA first combines with oxaloacetate to form citrate being catalyzed by *citrate synthetase*

2 Citrate is converted to isocitrate by the enzyme *aconitase* (aconitate hydratase) which contains Fe^{++} This conversion takes place in two steps—dehydration to cis aconitate and rehydration to isocitrate The reaction is inhibited by fluoroacetate

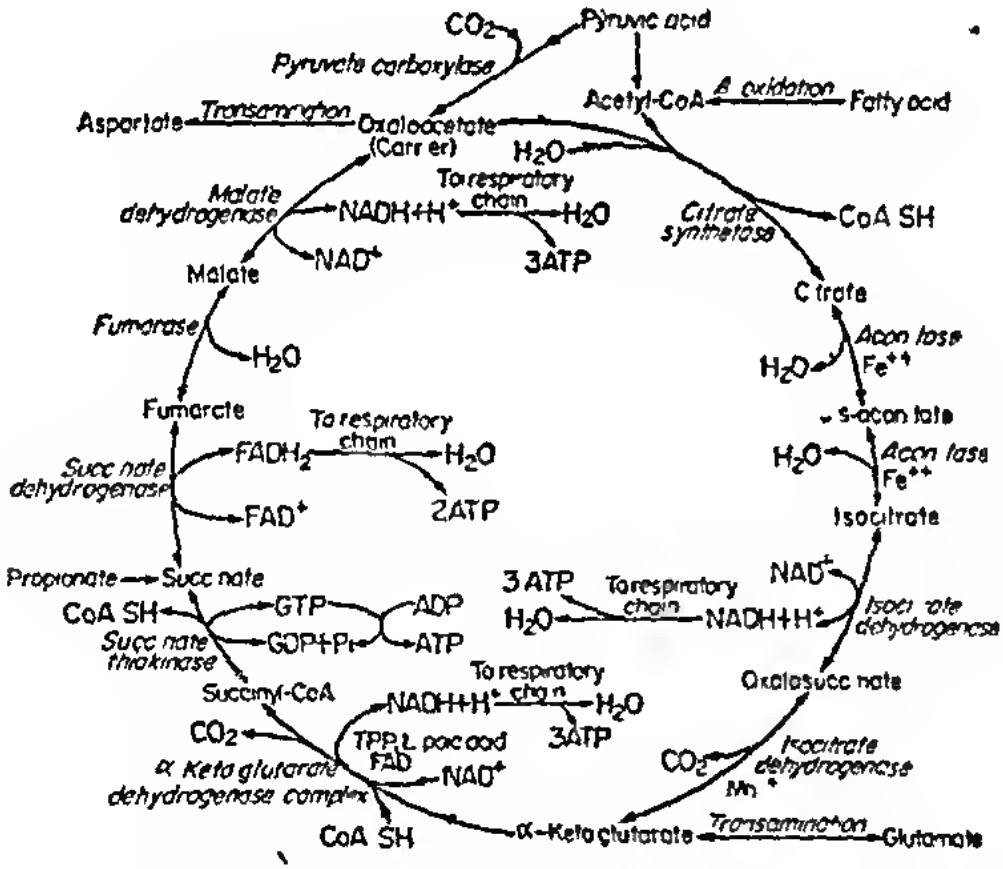


Fig 16 12 Citric acid cycle in mitochondria starting from pyruvic acid

* According to the Committee of Editors of Biochemical Journals Recommendations (1975) the standard biochemical convention the ending-ate in e g citrate denotes mixture of free citric acid and the ionized form in which the cations are not specified

3. Isocitrate undergoes dehydrogenation in the presence of *isocitrate dehydrogenase* to form oxalosuccinate.

4. The decarboxylation of oxalosuccinate produces α-ketoglutarate by the catalytic action of isocitrate dehydrogenase in presence of Mn^{++}.

5. α-ketoglutarate undergoes oxidative decarboxylation in the similar manner as that occurred in pyruvate. The reaction is catalyzed by α-ketoglutarate dehydrogenase complex which requires coenzymes TPP, lipoate, NAD^+, FAD^+ and CoA forming succinyl-CoA.

6. Succinyl-CoA is then converted to succinate by the enzyme *succinate thiokinase* (succinyl-CoA syathetase). The reaction reqnires GDP or IDP which is converted to GTP or ITP by the presence of inorganic phosphate.

7. Snccinateis further metabolized undergoing dehydrogenation catalyzed by succinate dehydrogenase. The reaction requires FAD. Fumarate is formed as a resnlt of water.

8. Fumarate nnder the influence of fumarase is converted to malate by the addition of the reaction.

9. Malate is converted to oxaloacetate by *malate dehydrogenase* which requires NAD^+.

Energetics of the citrie acid cycle :

Reaction	*No. of ATP formed*
Isocitrate→Oxalosuccinate	3
α-ketoglutarate→Succinyl-CoA	3
Succinyl-CoA→Succinate	1
Succinate→Fumarate	2
Malate→Oxaloacetate	3
Total ...	12

Total number of ATP in the complete oxidation of one moledule of glucose :

One molecule of glucose forms 2 molecules of pyruvic acid by glycolysis.

Number of ATP formed in glycolysis	8
„ „ „ „ in the oxidation of pyruvate to acetyl-CoA ...	3×2=6
„ „ „ „ in the citric acid cycle ...	12×2=24
Total ...	38

Number of molecules of CO_2 and H_2O formed in the oxidation of pyruvic acid :

Pyruvate→acetyl-CoA	1 molecule of CO_2
Oxalosuccinate→α-ketoglutarate	1 „ „ „
α-ketoglutarate→Succinyl-CoA	1 „ „ „
Total ...	3 molecules of CO_2

The oxidation of reduced NAD & FAD by respiratory chain produces	$5H_2O$
Utilized in TCA cycle	$3H_2O$
Net ...	$2H_2O$

Inhibitors

1 *Fluoroacetate* inhibits the enzyme aconitase and prevents the conversion of citrate to isocitrate

2 *Arsenite* inhibits α-ketoglutarate dehydrogenase and causes α ketoglutarate to accumulate

3 *Malonate or oxaloacetate* inhibits succinate dehydrogenase competitively resulting in succinate accumulation

Significance of the citric acid cycle

1 The major significance of the citric acid cycle is that it acts as the common metabolic pathway for the oxidation of carbohydrate, lipids and proteins because glucose fatty acids and many amino acids are metabolized to acetyl CoA which is finally oxidized in the citric acid cycle

2 The reducing equivalents in the forms of hydrogen or of electrons are formed by the activity of specific dehydrogenases during the oxidation of acetyl CoA in the cycle These reducing equivalents then enter the respiratory chain, where large amounts of high-energy phosphate are generated by the oxidative phosphorylation

3 The enzymes of the citric acid cycle are located in the mitochondrial matrix either free or attached to the inner surface of the inner mitochondrial membrane which facilitates the transfer of reducing equivalents to the adjacent enzymes of the respiratory chain which is also situated in the inner mitochondrial membrane

4 The citric acid cycle is *amphibolic* (dual) in nature which is the source for anabolic processes such as fatty acid and amino acid synthesis and gluconeogenesis

GLUCONEOGENESIS or NEOGLUCOGENESIS

1 The formation of glucose from non-carbohydrate substances such as lactic acid, amino acids and glycerol is called gluconeogenesis or neoglucogenesis

2 When the carbohydrate is insufficient in the diet gluconeogenesis meets the needs of the body for glucose

3 A continued supply of glucose is necessary as a source of energy and glucose is the only fuel which supplies energy to skeletal muscle under anaerobic conditions

4 There is always a certain basal requirement for glucose even when fat is supplied to the caloric requirement of the organism

5 In mammals, the liver and the kidney are the principal organs responsible for gluconeogenesis Gluconeogenesis is essentially a reversal of glycolysis Therefore, the glycolytic activity of liver and kidney is low when there is active gluconeogenesis

Metabolic pathways in gluconeogenesis

1 The metabolic pathways in connection with gluconeogenesis are the modifications of the Embden Meyerhof pathways and the citric acid cycle

2 They are concerned with the conversion of glucogenic amino acids, lactate, glycerol propionate (in ruminants) to glucose or glycogen

3 The energy barriers obstruct a simple reversal of glycolysis (i) between pyruvate and phosphoenolpyruvate, (ii) between fructose-1, 6-diphosphate and fructose 6-phosphate, (iii) between glucose 6-phosphate and glucose, (iv)

between glucose-1 phosphate and glycogen. These barriers are overcome by the following reactions

(i) The enzyme *pyruvate cardoxylase* present in mitochondria converts pyruvate to oxaloacetate in presence of ATP, biotin and CO_2. A second enzyme *phosphoenolpyruvate caboxykinase* present in the extramitochondrial part of the cell converts oxaloacetate to phosphoenolpyruvate in presence of GTP. Lactate with the help of these two enzymes and lactate dehydrogenase is converted to phosphoenolpyruvate.

In Mitochondria

$$\text{Pyruvate} + \text{ATP} + Co_2 \xrightarrow[\text{Biotin}]{\textit{Pyruvate carboxylase}} \text{Oxaloacetate} + \text{ADP} + \text{Pi}$$

Fig. 16.13

In the extramitochondrial part of the cell

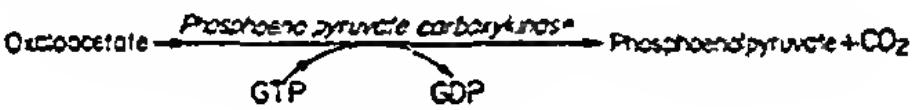


Fig. 16.14

But oxaloacetate does not diffuse readily from mitochondria. Alternative means are applied to convert oxaloacetate to malate which is readily diffused from mitochondria. Malate is then converted to oxaloacetate in the extramitochondrial portion of the cell

(ii) The conversion of fructose-1, 6-diphosphate to fructose-6-phosphate catalyzed by another enzyme fructose 1, 6-diphosphatase. This enzyme is present in liver, kidney and striated muscle but absent from adipose tissue, heart muscle and smooth muscle.

(iii) Glucose-6-phospbate is converted to glucose by glucose-6-phosphatase which is present in intestine, liver and kidney but absent from muscle and adipose tissue.

(iv) The conversion of glucose-1 phosphate to glycogen is through UDPG and glycogen synthetase.

Glucogenic amino acids after transamination or deamination form either pyruvic acid or members of the citric acid cycle. Therefore, the glucogenic amino acids and lactate can be converted to glucose or glycogen. *Propionate in ruminants* enters the main glucogenic pathway via the citric acid cycle being converted to succinyl CoA.

Conversion of propionate to succinyl-CoA

1. Propionate is first activated by thiokinase with ATP and CoA to form propionyl CoA.

2. Propionyl CoA undergoes CO_2 fixation reaction to form D-methyl malonyl CoA catalyzed by propionyl-CoA carboxylase and biotin is required as a coenzyme.

3 D methylmalonyl-CoA is converted to L-methylmalonyl-CoA by methylmalonyl-CoA racemase

4 L-metbylmalonyl-CoA is isomerised to succinyl-CoA by methylmalonyl-CoA isomerase which requires vitamin B_{12} as a coenzyme

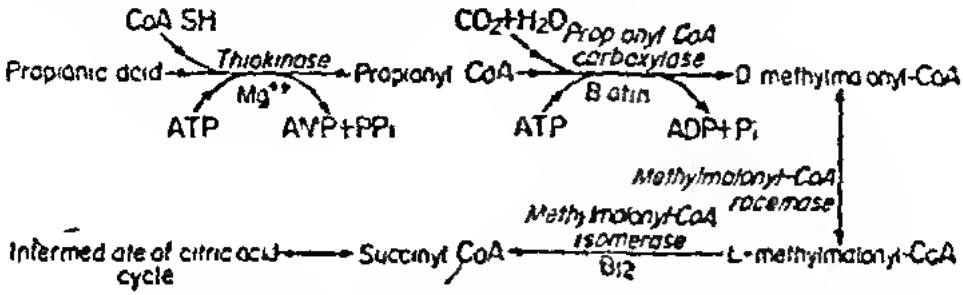


Fig 16 15 Propionic acid to succinyl-CoA

Conversion of glycerol

1 Glycerol is first converted to glycerol 3 phosphate by *glycerokinase* with ATP in liver and kidney

2. Glycerol-3-phosphate is oxidized to dihydroxyacetone phosphate by glycerol-3-phosphate dehydrogenase in presence of NAD^+ Dihydroxyacetone phosphate is then converted to glucose

Conversion of lactate to glucose :

1 Lactic acid is the major end product in muscle in anaerobic glycolysis Muscle tissue is incapable of resynthesizing glucose from lactate This conversion takes place entirely in the liver

2 Muscle lactate is transported to the liver by the blood In the liver, it is converted to glucose and glycogen by the enzymes concerned in gluconeogenesis

3 Liver glycogen is converted to glucose which is carried back to muscle by blood

This conversion of muscle lactate to glucose in liver and its re-entry into muscle is called "*Cori cycle*"

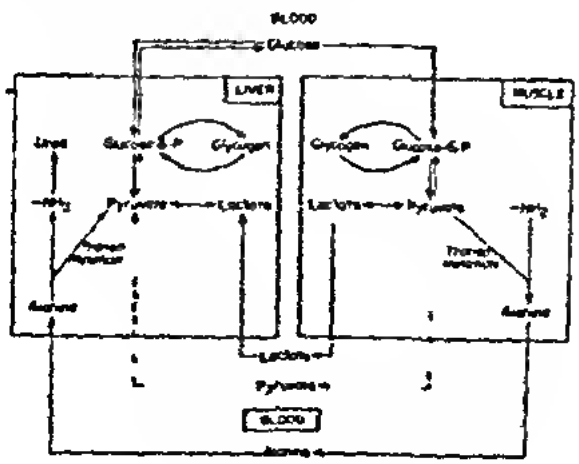


Fig 16 16 Lactic acid (Cori) cycle and Glucose-alanine cycle.

Conversion of amino acids to glucose :

1. The glucogenic amino acids are converted to the intermediates of citric acid cycle either by transamination or deamioation which is given in the figure below

2 These intermediates are converted to malate and finally converted to glucose by the enzymes involved in glucooeogenesis

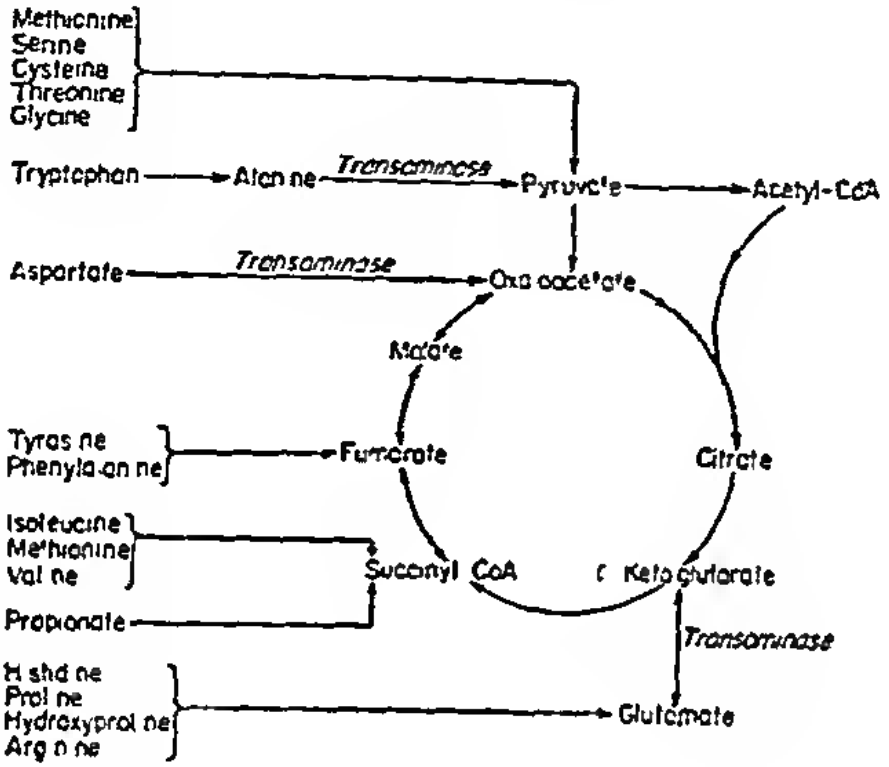


Fig 16 17 Conversion of amino acids to the intermediates of citric acid cycle by transamination or deamination.

Conversloo of fatty acids to glucose

Fatty acids ore metabolized to acetyl CoA by β-oxidation Acetyl CoA enters the citric acid cycle and theo converted to malate Malate is diffused from the mitochondria to the extramitochondrial portioo of the cell where it is finally converted to glucose by the enzymes involved io gluconeogenesis

Acetyl-CoA is not permeable to pass from the mitochondria to the cytosol through the mitochondrial membrane But citrate is permeable through mitochoodrial membrane to pass to the cytosol where it is splitted to acetyl CoA aod oxaloacetate

THE HEXOSE MONOPHOSPHATE SHUNT (HMP SHUNT) OR PENTOSE PHOSPHATE PATHWAY

1 This is an alternate aerobic pathway for the oxidation of glucose io the liver, lactating mammary glaod aod adipose tissue in additioo to the Embden-Meyerhof pathway for glycolysis

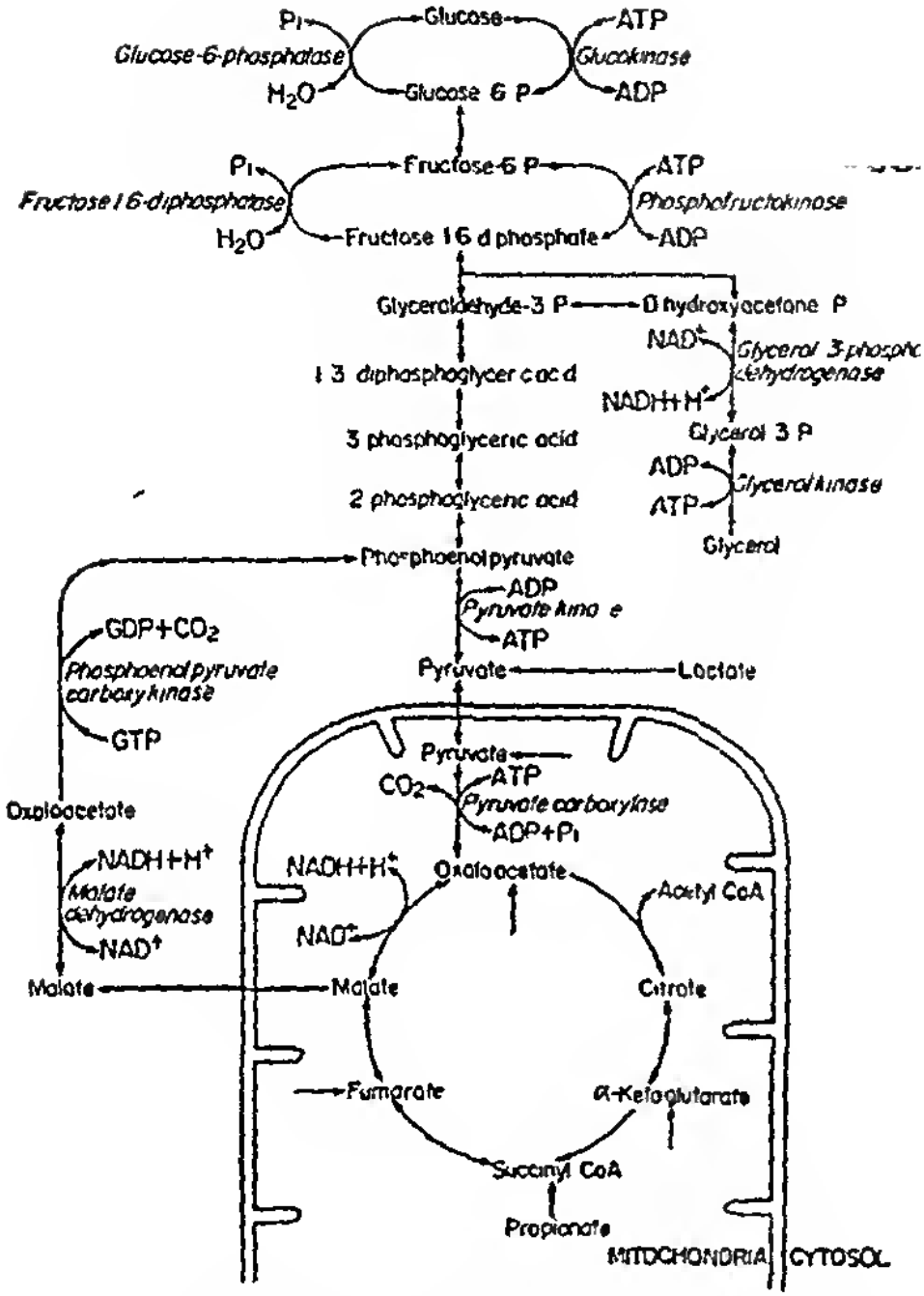


Fig 16 18 Major pathway of gluconeogenesis in the liver Entry points of glucogenic amino acids are shown by arrows

2 The enzymes of this pathway are present in the extramitochondrial portion of the cell This pathway is active in liver, adipose tissue, adrenal cortex, thyroid, erythrocytes, testis and lactating mammary gland.

3 In this pathway, 3 molecules of glucose-6-phosphate yield 3 molecules of CO_2 and 3 molecules of 5 carbon residues (pentose sugars) The latter are converted ultimately to 2 molecules of glucose-6-phosphate and one molecule of glyceraldehyde-3 phosphate

4 NADP instead of NAD is used as a hydrogen acceptor in this pathway.

Metabolic reactions

The reaction takes place in two phases -

(i) Glucose-6-phosphate by dehydrogenation and decarboxylation gives rise to ribulose-5-phosphate.

(ii) Ribulose-5-phosphate is converted back to glucose-6-phosphate by *transketolase* and *transaldolase*

1 Glucose-6-phosphate is dehydrogenated to 6-phosphogluconate via 6 phosphogluconolactone by glucose-6-phosphate dehydrogenase in presence of NADP and the cofactors Mg^{++}, Mn^{++} or Ca^{++} 6-phosphogluconolactone is acted by gluconolactone hydrolase in presence of Mg^{++}, Mn^{++} or Ca^{++}. Genetically, the deficiency of glucose-6-phosphate dehydrogenase in erythrocytes is associated with a tendency to hemolysis by primaquine and sulfonamide

2. 6-phosphogluconate is oxidized by 6-phosphogluconate dehydrogenase in the presence of the coenzyme NADP and cofactors Mg^{++}, Mn^{++} or Ca^{++} to 3-keto 6-phosphogluconate which is decarboxylated to form ribulose-5-phosphate

3 Ribulose-5-phosphate is acted on by *ribulose-5-phosphate epimerase* which changes the configuration about carbon 3 forming xylulose 5-phosphate and also by the enzyme *ribose-5 phosphate ketoisomerase* which converts ribulose-5 phosphate to ribose-5-phosphate

4 *Transketolase* with the help of TPP and Mg^{++} transfers carbons 1 and 2 of xylulose 5-phosphate to the ribose-5-phosphate forming sedoheptulose-7 phosphate and glyceraldehyde-3 phosphate

5 *Transaldolase* allows the transfer of a 3-carbon moiety from sedoheptulose-7-phosphate to the glyceraldehyde-3-phosphate to form fructose-6-phosphate and erythrose-4-phosphate

6 *Transketolase* with the help of TPP and Mg^{++} transfers carbon 1 and 2 from xylulose-5-phosphate to erythrose-4-phosphate producing fructose-6-phosphate and glyceraldehyde-3 phosphate

The overall reaction of the hexose monophosphate shunt is mentioned below.

$$3\,\text{Glucose 6 P} + 6\,\text{NADP}^+ \longrightarrow 3CO_2 + 6\,\text{NADPH} + 6H^+ + \text{Glyceraldehyde 3 P} + 2\,\text{Glucose-6-P}$$

The overall metaholism is given below.

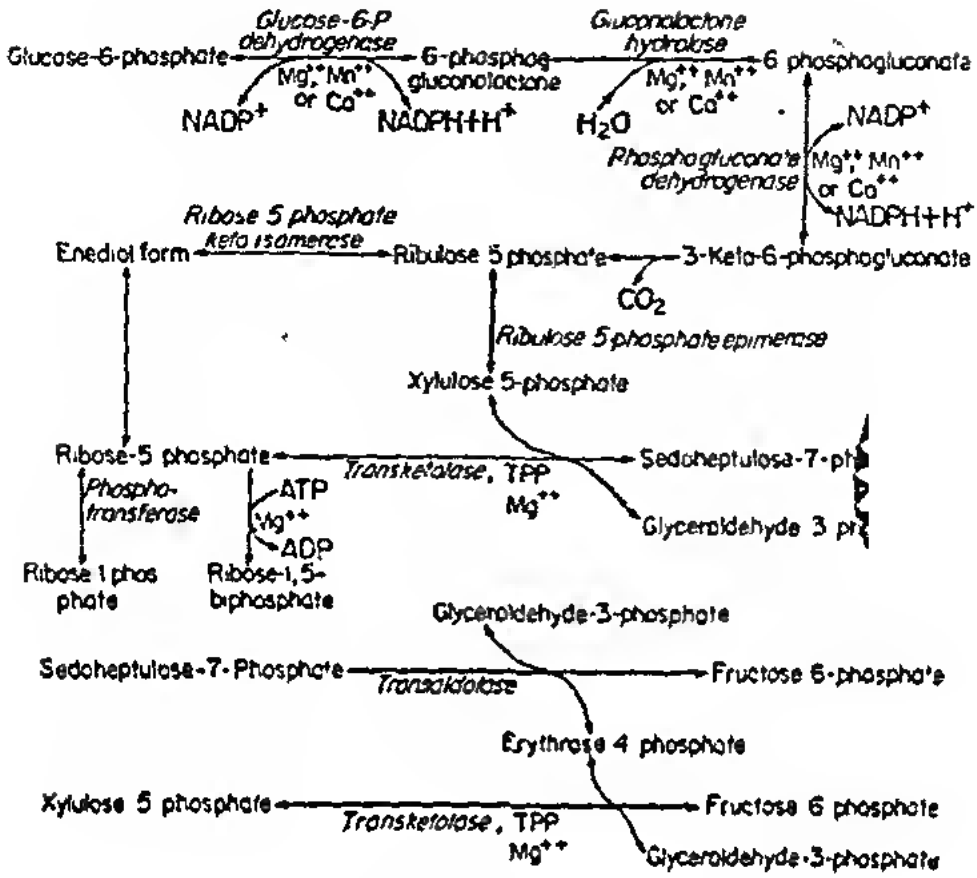


Fig 16.19. The hexose monophosphate shunt
or
Pentose phosphate pathway.

Metabolic sigalficaace of the HMP shunt :

1. CO_2 is the characteristic product in this pathway which is not formed in Embden-Meyerhof pathway. This product is utilized for the synthesis of fatty acid aad puriae hases etc.

2. The reduced NADP (NADPH) formed ia this pathway is used in the synthesis of fatty acids, cholesterol or steroids and in the synthesis of amiao acids via glutamate dehydrogenase outside the mitochondria. The reduced NADP is also required in uroaic acid pathway for the synthesis of ascorbic acid and xylulose-5-phosphate. Ascorbic acid is not synthesized in man and other primates including guineapig. The system which utilizes reduced NADP stimulates an active degradation of glucose via this shunt pathway. The reduced NADP functions in the operation of the shunt pathway in red blood cells and it has a direct correlation between the glucose-6-phosphate dehydrogenase and the fragility of red cells (susceptibility to hemolysis) when the cells are subjected to certain drugs such as primaquine and sulfonamide.

3. The peatose sugars produced in this pathway are utilized for the synthesis of nucleic acids aad nucleotides.

Skeletal muscle is incapable of synthesizing ribose This is probably accomplished by a reversal of the shunt pathway utilizing fructose-6-P, glyceraldehyde-3 P and the enzymes transketolase and transaldolase.

4 The products fructose-6-P and glyceraldebyde-3-P are utilized in Embden-Meyerbof pathway for glycolysis

5 This pathway in the erythrocytes provides reduced NADP (NADPH+H$^+$) for the reduction of oxidized glutathione (G—S—S—G) to the reduced glutathione by the enzyme glutathione reductase The reduced glutathione then removes H_2O_2 from the erythrocytes by glutathione peroxidase. This reaction is important because accumulation of H_2O_2 may decrease the life span of erythrocytes by increasing the rate of oxidation of hemoglobin to methemoglobin

THE URONIC ACID PATHWAY

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

2 In man and other primates as well as in guineapigs, ascorbic acid cannot be synthesized and gulonic acid is ultimately converted to L-xylulose

Metabolic reactions

1 Glucose-6-phosphate is converted to glucose-1-phosphate by phosphoglucomutase The product then reacts with UTP to form UDPG by the help of the enzyme UDPG pyrophosphorylase

2 UDPG is oxidized to uridine diphosphoglucuronic acid by UDPG dehydrogenase in presence of NAD$^+$ UDP glucuronic acid is the "active" form of glucuronic acid for reactions involving incorporation of glucuronic acid into chondroitin sulfate

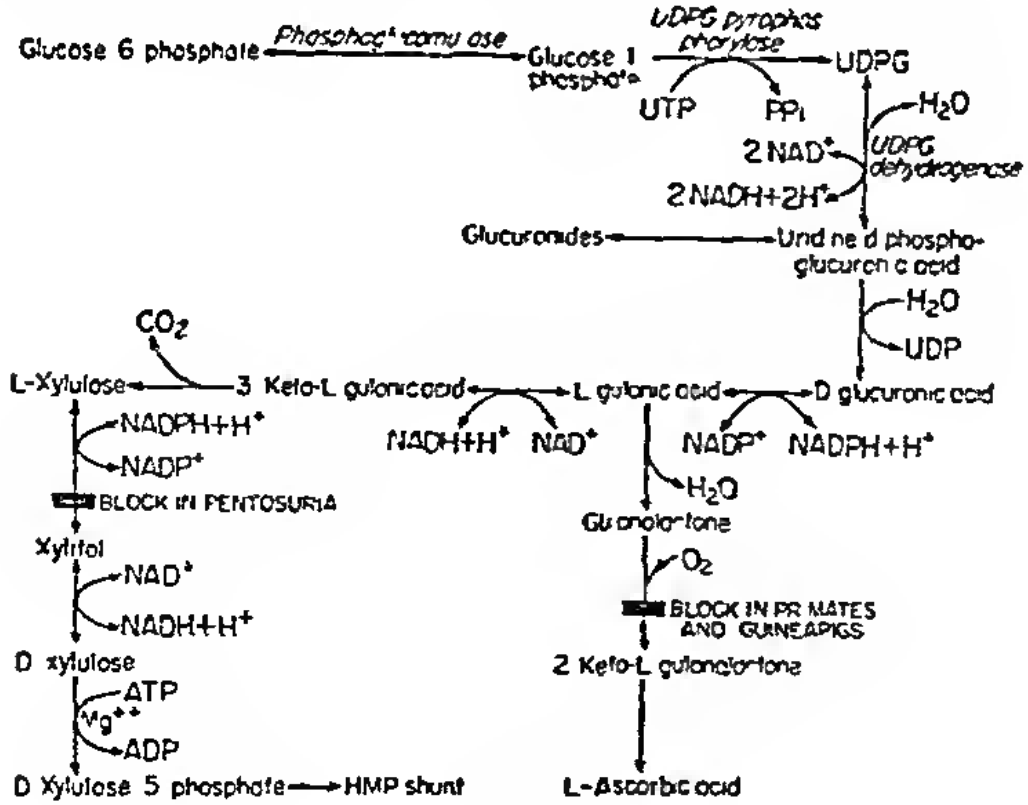


Fig 16 20 Uronic acid pathway

3 Glucuronic acid is reduced to L-gulonic acid in the presence of reduced NADP L-gulonic acid is the precursor of ascorbic acid

4 In man and other primates as well as in guineapigs L-gulonic acid is oxidized to 3 keto L-gulonic acid which is then decarboxylated to L-xylulose L-xylulose is cooverted to D-xylulose in presence of reduced NADP D xylulose being converted to D xylulose-5-phosphate by ATP is further metaholized in the HMP shunt

5 In the rare hereditary disease called "*essential pentosuria*" large quantities of L-xylulose appear io the urine due to the absence of the enzyme which can cause reduction of L-xylulose to xylitol

METABOLISM OF FRUCTOSE

1 *Hexokinase* can phosphorylate fructose to form fructose 6 phosphate but the affinity of the enzyme for fructose is very small compared with its affinity for glucose Hence, this is not the major pathway for fructose utilization

2 *Fructokinase* present in liver converts fructose to fructose-1-phosphate hy ATP This takes place in kidney aod intestioe also The activity of this enzyme is not affected *by fasting or by insulin* That is why fructose disappears from the hlood of diahetic patients at a normal rate This is the major pathway for the phosphorylation of fructose The Km for fructose of the enzyme in liver is very low, indicating a very high affinity of the enzyme for its suhstrate

3 *Aldolase B* splits fructose 1 phosphate ioto glyceraldehyde and dihydroxyacetooe phosphate The absence of this enzyme leads to a *hereditary fructose intolerance* Glyce-aldehyde again enters the glycolysis *Triokinase* present in human liver converts glyceraldehyde to glyceraldehyde-3 phosphate This is the major pathway for the further metaholism of glyceraldehyde

4 There is the additional possibility that fructose-1-phosphate may he phosphorylated to fructose-1, 6-diphosphate hy the catalyzing effect of 1-phosphofructokinase which has been fouod in muscle and in liver

5 *Fructosuria* is a very rare condition in which fructose is found in the urine This is a congenital defect—essential fructosuria—due to a defect in its metabolism This condition is harmless and is due to a deficiency of fructokinase

The overall metabolism is given helow

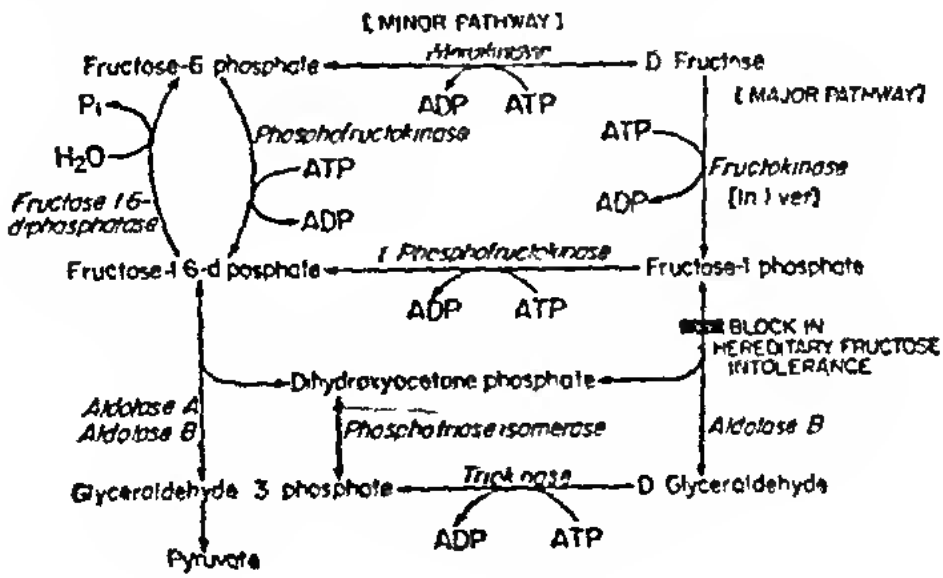


Fig 16.21 Metabol sm of fructose

METABOLISM OF GALACTOSE

Galactose is available by the hydrolysis of lactose of Milk

Metabolic reactions

1 Galactose is phosphorylated by galactokinase in presence of ATP to form galactose-1-phospbate

2 Galactose-1-phosphate reacts with UDPG to form UDP galactose and glucose-1-phospbate by *galactose-1 phosphate uridyl transferase* UDP galactose may be converted to UDP glucose by *uridine diphosphate galactose epimerase* UDP glucose can form glycogen

3 UDP galactose condenses with glucose to form lactose in the mammary gland by *lactose synthetase*

Galactose is required in the body not only in the formation of milk but also as a constituent of glycolipids, mucoproteins Galactokinase shows increased activity upon the feeding of galactose

Galactosemia, an inherited metabolic disease occurs owing to the inability to metabolize dietary galactose for which galactose accumulates in the blood and passes to the urine There is also high accumulation of galactose-1 phospbate in the red blood cells of the galactosemic individual, which shows no deficit of galactokinase *Recently*, it is suggested that an inherited lack of galactose-1-pbosphate uridyl transferase in the liver and red blood cells is responsible for galactosemia Still in galactosemic individual there is the formation of UDP galactose from UDP glucose by epimerization In case it is untreated it may lead to cataracts, mental retardation and death The condition may be treated by eliminating milk from the diet

The overall metabolism is schemed below

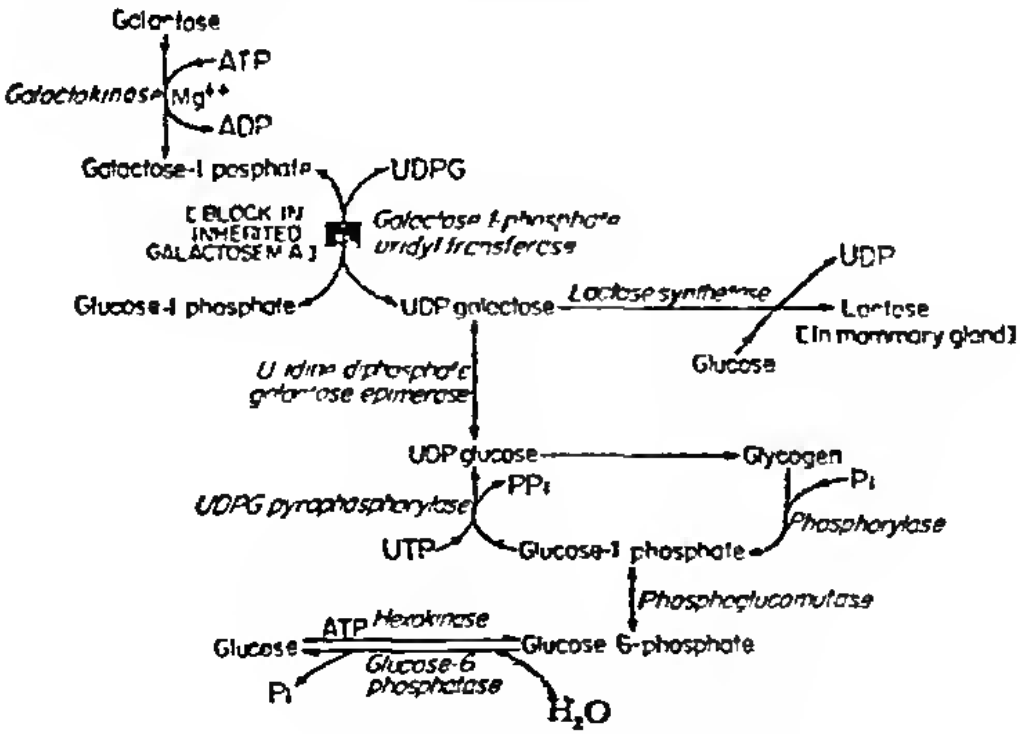


Fig 16 22 Metabolism of galactose

GLYCOGEN STORAGE DISEASES

The term "glycogen storage disease is a genetic one in which abnormal quantities of glycogen are deposited in the liver kidoey, heart and muscle This is due to the enzyme deficiency Seven main types have been recognized

Type I glycogenosis (Von Gierke s Disease)

1 The disease is due to the deficiency of glucose 6-phosphatase for which glycogen cannot be broken down to liberate glucose and glucose-6 phosphate promotes glycogen synthesis

2 Children with this disease tend to develop hypoglycemia

3 They use fat mostly as an energy source and this leads to lipemia, acidemia and ketosis

4 There is fatty infiltration of the liver

5 The hypoglycemia inhibits insulin secretion which in turn also inhibits protein synthesis and growth is ceased

6 Hypoglycemia stimulates epinephrine production which causes the breakdown of muscle glycogen forming lactate This lactate competes with urate for excretion by the kidney So blood urate level is increased

Type II glycogenosis (Pompe s Disease)

1 The disease is due to the deficiency of a lysosomal enzyme acid maltase

2 There is excessive amounts of glycogen in all tissues

3 The heart is enlarged and there is extreme muscle weakness

4 Death occurs usually before the ninth month of life

Type III glycogenosis (*Limit Dextrinosis*)

1 In this condition, there is an accumulation of limit dextrin in liver and muscles This substance is produced by the action of phosphorylase on glycogen

2 Debrancher enzyme system is deficient in this disease

3 Patients with this disease are known to survive well into adult life

Type IV glycogenosis (*Amylopectinosis*)

1 This disease is due to the deficiency of the branching enzyme in the liver

2 Amylopectins are formed in the liver, heart kidney and muscle

3 The disease is fatal and survival being four years

Type V glycogenosis (MC Ardle's Disease)

1 In this disease there is a defect of muscle phosphorylase

2 Cramps occurs in muscle after moderate exercise and recovery is attained during rest

3 The exercised muscles can metabolize fructose

Type VI glycogenosis

1 There is always a deficiency of liver phosphorylase

2. Liver glycogen concentration is usually increased

Type VII glycogenosis

1 This disease is due to the deficiency of phosphofructokinase

2. There is marked accumulation of glucose-6-phosphate and fructose-6-phosphate and a marked diminution of fructose-1, 6-diphosphate

LACTOSURIA

Lactosuria occurs in women during the period of lactation Small to moderate amounts of lactose may be found in the urine of most of the pregnant women, the amount increases as pregnancy advances It appears more frequently in the afternoon

MALTOSURIA

This is a rare condition of no known clinical significance

FRUCTOSURIA

Fructose may appear in the urine under the following circumstances

1 The patients with hepatic insufficiency excrete fructose in urine when large quantities of fructose are ingested

2 Essential fructosuria is a rare congenital disorder in which there is the deficiency of fructokinase and characterized by inability to utilize fructose completely Insulin has no influence upon this condition

There are two varieties of this condition In one the metabolism of other carbohydrates is undisturbed and there are no clinical symptoms In another form the rise in blood fructose is accompanied by a sharp drop in the blood glucose concentration with severe symptoms of hypoglycemia This is due to the deficiency of aldolase B in the liver resulting in accumulation of fructose-1-phosphate which blocks important pathways of fructose utilization

PENTOSURIA

Pentose may appear in the urine under the following circumstances

1 After the ingestion of large quantities of fruits there is alimentary pentosuria occurring in normal individuals It has no clinical significance except the wrong idea for glycosuria

2 Essential pentosuria is due to 'inborn errors of metabolism' This occurs owing to the lack of the enzyme which causes the reduction of L-xylulose to xylitol in the liver As a result L xylulose is excreted in urine It occurs practically in males of jewish subjects The utilization of other carbohydrates is unimpaired

ENDOCRINE (HORMONAL) INFLUENCES ON CARBOHYDRATE METABOLISM

The supply of glucose to the blood by the liver and its utilization in the tissues is regulated and integrated with one another and also by the metabolism of proteins and lipids Endocrine organs play a very important role in the metabolism of carbohydrate which is discussed below

A Insulin

1 Causes glucose transport across cell membrane by increasing the permeability of the cells

2 Increases glycogen formation in liver and muscle by stimulating hexokinase and glycogen synthetase

3 Accelerates the conversion of glucose to fat

4 Stimulates protein synthesis

5 Inhibits ketogenesis

6 Inhibits gluconeogenesis

7 Increases glycolysis by stimulating hexokinase and phosphofructokinase in liver and muscle

8 Throws effect on muscle ion balance

9 Causes initial phosphorylation (hexokinase reaction) of glucose for further oxidation

The sulfonylureas—e g tolbutamide—are the stimulators of insulin release Insulin is destroyed by *insulinase* which separates the A and B chain of it *Peptidases* also play a part in the destruction of insulin

B Adrenocortical hormones

The adrenalectomized animal exhibits the following changes in carbohydrate metabolism

1 Decrease in liver glycogen

2 Less marked decrease in muscle glycogen

3 Hypoglycemia

4 Decreased intestinal absorption of glucose

The changes are prevented by glucocorticoids The glucocorticoid hormones release gluconeogenic precursors such as amino acids from muscle The hormones also release FFA from adipose tissue resulting in inhibition of hepatic glycolysis The increase in FFA in the liver results in an increase of acetyl CoA which activates pyruvate carboxylase and so stimulates gluconeogenesis

C Anterior pituitary factors

Growth hormone produces the following changes in carbohydrate metabolism

1 Hyperglycemia and aggravation of diabetes in depancreatized animals

2 Inhibition of insulin action with decreased utilization of carbohydrate and lowering of the respiratory quotient

3 Diminution of the breakdown of muscle protein

4 Increase in the mobilization of fat in the form of FFA from adipose tissue with the increase in lipase activity The increase in FFA and its use as the source of energy, results in the excessive production of ketone bodies

5 ACTH enhances the release of free fatty acids from adipose tissue and inhibits glucose utilization It causes the increase in blood sugar level by stimulating the secretion of adrenal cortex hormones

D Epinephrine

1 Causes an increase in blood sugar and in blood lactate due to the acceleration of glycogenolysis in the liver and muscles

2 Diminishes the uptake of glucose by tissue cells thus interfering with its utilization

3 Causes diminution in the amount of insulin released from the pancreas.

E Thyroid hormone -

1 Thyroxine accelerates hepatic glycogenolysis with consequent rise in blood sugar This is due to increased sensivity to epinephrine

2 Thyroxine also increases the rate of absorption of hexoses from the intestine and accelerates gluconeogenesis

3 It has diabetogenic action

F Glucagon -

1 Promotes glycogenolysis in liver stimulating phosphorylase via cyclic AMP

2 Causes breakdown of glycogen to lactic acid in muscle The lactic acid is again converted to glucose by Cori cycle"

3 Stimulates gluconeogenesis in liver

G Sex hormones

Estrogens stimulate increased insulin secretion and thus reduce blood sugar level Testosterone also decreases blood sugar level

REGULATION OF BLOOD GLUCOSE CONCENTRATION

In the resting postabsorptive state (overnight fast) the true glucose present in the venous blood is 60 100 mg/100 ml and the value is higher i e 80-120 mg/100 ml including nonsugar reducing substances After the ingestion of a carbohydrate meal it may rise to 120-130 mg/100 ml The normal blood glucose level of sheep is 40 mg/100 ml and of cattle 60 mg/100 ml These lower values are due to the fact that ruminants ferment virtually all dietary carbohydrate to lower fatty acids which replace glucose as the main metabolic fuel The condition in which the blood sugar is raised above the normal range is called *hyperglycemia* and when it goes below the normal level it is called *hypoglycemia* The concentration of glucose in the blood depends on two general factors (i) the rate of its entrance into the blood (ii) the rate of its removal from the blood stream

Sources of blood glucose

A From carbohydrates of the diet

1 Most carbohydrates in the diet after digestion form glucose galactose or fructose which are absorbed into the portal vein

2 In the liver, galactose and fructose are converted to glucose

B From various glucogenic compounds -

1 In the skeletal muscle glucose is oxidized to lactic acid which is transported by blood to the *liver and kidney* where glucose is reformed and undergoes oxidation in the tissues via the circulation This process is known as the ' Cori cycle or lactic acid cycle'

2 In the adipose tissue, the synthesis of triacylglycerol takes place from glycerol which is derived initially from blood glucose Acylglycerols of adipose tissue continually undergo hydrolysis to form free glycerol which diffuses

out of the tissue into the blood. This is converted haek to glucose in the liver and kidney by gluconeogenesis. Hence, a continuous cycle exists in which glucose is transported to adipose tissue from the *liver and kidney* and glycerol is returned to be synthesized into glucose by the liver and kidney.

3. During starvation, the amino acid, alanine, is transported from muscle to liver with an effect of a net transfer of amino nitrogen from muscle to liver and of free energy from liver to muscle. The energy required for the synthesis of glucose from pyruvate in the liver is derived from fatty acid oxidation.

C. From liver glycogen by glycogenolysis :

Glucose is formed in the liver from glycogen by phosphorylase and debranching enzyme and also by glucose-6-phosphatase by the effect of the hormone epinephrine and glucagon.

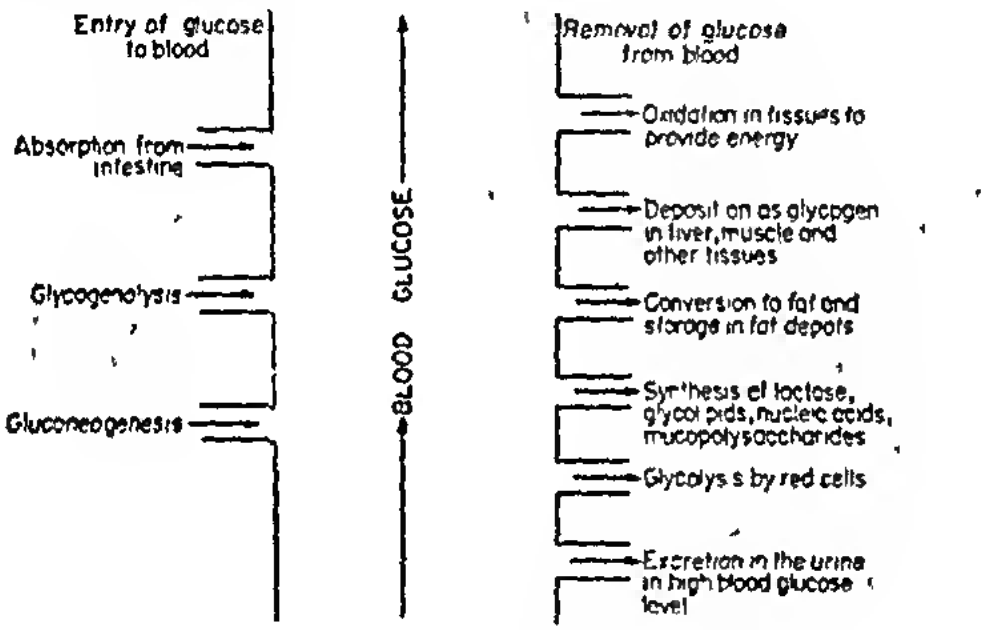


Fig. 16.23. Entrance and removal of blood glucose.

Regulation of the blood glucose :

The stable blood glucose level is maintained by the role of liver, skeletal muscle, kidney, muscular exercise and hormones.

Role of liver :

1. Liver is the pivot of carbohydrate metabolism of the whole body. The presence of glucose-6-phosphatase in the liver converts glucose-6-phosphate to glucose which diffuses into the blood stream to form the constant and the only source of glucose of blood unless and until glucose is available from the intestine from carbohydrate diet.

2. Muscle glycogen cannot be converted to glucose due to the lack of the enzyme glucose-6-phosphatase. Therefore, glycogen is converted to lactic acid which by "Cori cycle or lactic acid cycle" is converted to glucose in the liver and the glucose is diffused to the blood stream.

3 The liver cells, like other cells, require the oxidation of organic substances to maintain their own vital functioning. In the absence of fuel, glucose, glycogen is diminished and the oxidation of fat occurs forming keto acids. Some of the keto acids are utilized for cellular energy But if the concentration of keto acids is increased, the keto acids diffuse into the blood stream and accumulate producing ketosis

4 When the glycogen reservoir diminishes, the amino acids of the body proteins are utilized by the liver for gluconeogenesis.

Role of skeletal muscle

1 Extrahepatic tissues are relatively impermeable to glucose and therefore, insulin is required for the uptake of glucose to these cells

2. Increased blood glucose promotes glycogenesis and oxidation of glucose in muscles Muscle glycogen does not serve directly as a source of glucose during hypoglycemia. But glucose is supplied to the blood from muscle glycogen by "Cori cycle or lactic acid cycle"

Role of kidney :

1 There is considerable evidence that the kidney is able to form glucose from a number of carbohydrate intermediates and also from amino acids.

2. It possesses some capacity for gluconeogenesis, although the capacity is minor as compared to that of the liver

3 When the blood glucose level exceeds the *renal threshold* level (160-180 mg/100 ml) renal tubules are incapable of reabsorbing all the filtered sugar in glomeruli and the excess glucose is excreted in urine This results in the decrease of blood glucose concentration

Role of muscular exercise :

Muscular exercise promotes the entry of glucose into muscle cells and the glucose is utilized by the muscle Thus, it lowers blood glucose level

Role of hormones

A. Insulin :

1 It is produced by the β-cells of the islets of Langerhans in the pancreas and is liberated into the blood by the direct response to hyperglycemia. Insulin is released by *amino acids, free fatty acids, ketone bodies, glucagon secretin* and *tolbutamide* *Epinephrine* and *norepinephrine* block the release of insulin.

2 It increases the rate of uptake of glucose to tissues.

3 It promotes glycogenesis by stimulating hexokinase and glycogen synthetase and oxidation of glucose by stimulating phosphofructokinase

4 It decreases hepatic glycogenolysis and gluconeogenesis.

5 It stimulates lipogenesis and protein synthesis.

6 It inhibits ketogenesis.

B Anterior pituitary hormones :

1 The anterior pituitary gland secretes hormones that elevate the blood sugar level by antagonizing the action of insulin They are growth hormone, ACTH and other 'diabetogenic' principles.

2. Growth hormone secretion is stimulated by hypoglycemia It decreases glucose uptake in certain tissues, e g muscle

3 Growth hormone mobilizes free fatty acids from adipose tissue which themselves inhibit glucose utilization

4 It produces hyperglycemia which stimulates secretion of insulin causing β-cells exhausted.

5. ACTH enhances the release of free fatty acids from adipose tissue and inhibits glucose utilization It also increases blood glucose level by stimulating the secretion of adrenal cortex hormones

C. Adrenal cortex hormones :

1 Glucocorticoids (11-oxysteroids) are important in carbohydrate metabolism. They lead to gluconeogenesis by the increased protein catabolism in the tissues, increased hepatic uptake of amino acids and increased activity of transaminases in the liver.

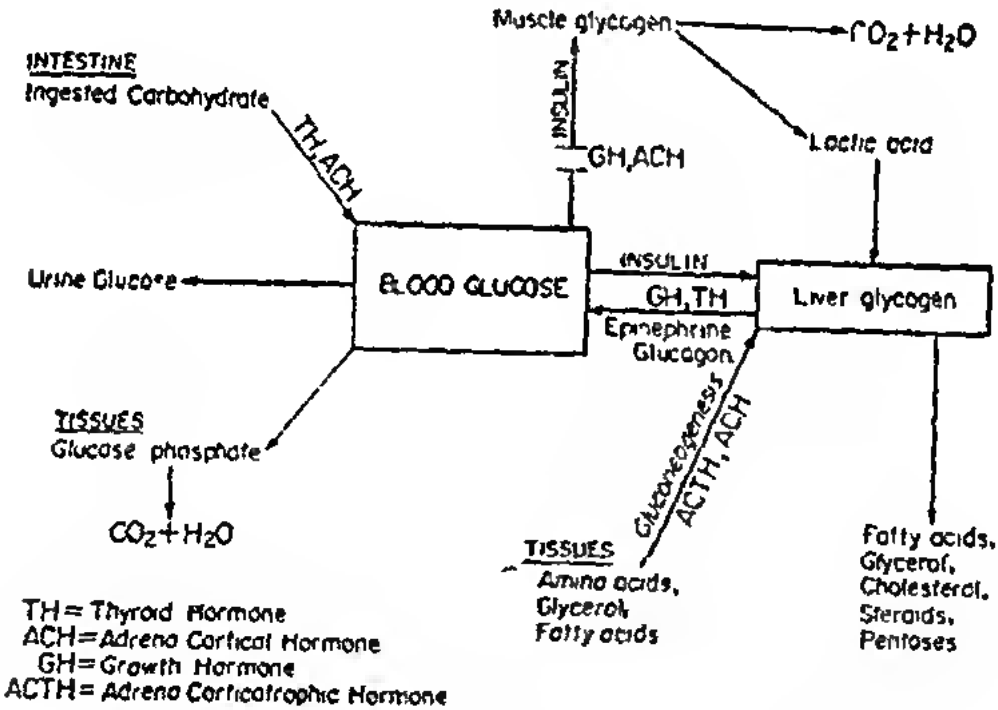


Fig 16.24 Factors controlling blood glucose concentration

2. Glucocorticoids inhibit the utilization of glucose in extrahepatic tissues They are antagonists to insulin

3. Glucocorticoids also increase the formation of glucose in the liver by stimulating glucose-6-phosphatase and fructose-1, 6-diphosphatase.

D. Epinephrine :

1. Epioephrine, secreted by the adrenal medulla, stimulates glycogen breakdown in muscle by increasing phosphorylase activity

2. In muscle, glycogen is converted to lactic acid instead of glucose due to the lack of glucose-6-phosphatase. This lactic acid is converted to glucose in the liver by "Cori cycle" and diffuses into the blood.

3. It diminishes the release of insulin from pancreas.

E. Glucagon :

1. Glucagon is produced by the alpha cells of the islets of Langerhans of the pancreas being stimulated by hypoglycemia It causes glycogenolysis by activating phosphorylase in the liver.

2. It stimulates glucose-6-phosphatase in the liver to form glucose from glucose-6-phosphate.

3 It enhances gluconeogenesis from amino acids and lactate.

F. Thyroid hormones :

1. Thyroxine has diabetogenic action.

2. It increases the rate of absorption of hexoses and accelerates gluconeogenesis.

3. It stimulates hepatic glycogenolysis with the consequent rise in blood sugar. This is due to increased sensitivity to epinephrine.

G. Sex hormones :

Estrogens cause increased liberation of insulin and thus decrease blood sugar level. Testosterones also decrease blood sugar level.

THE RENAL THRESHOLD FOR GLUCOSE

Glucose is continually filtered by the glomeruli when the blood sugar rises to a high level But it is completely returned to the blood by the renal tubular reabsorption The reabsorption is influenced by phosphorylation by enzymes. The capacity of the renal tubules to reabsorb glucose is limited which is at the rate of about 350 mg/minute If the filtrate contains more glucose than can be reabsorbed, the excess passes into the urine to produce *glycosuria.*

Glycosuria occurs in the individuals when the venous blood sugar exceeds 160-180 mg/100 ml This level of the venous blood sugar is said to be the *renal threshold* for glucose.

The maximal rate of reabsorption of glucose by the tubule (*TmG*—the tubular maximum for glucose) is constant Hence, it is a more accurate measurement than the renal threshold which varies with the change of glomerular filtration rate.

DIABETES MELLITUS

Diabetes mellitus means the hyperglycemia due to lack of insulin. This also takes place due to the over production of other hormones, e g glucagon, hormones of the anterior pituitary, adrenal and thyroid which are antagonists to insulin or due to increased production of insulinase which inactivates insulin.

Classification :

1. Primary, Idiopathic or essential diabetes :

Primary diabetes is classified into the following subgroups depending on the degree of severity of the disease.

(a) Potential diabetic (prediabetic) The individual with normal glucose tolerance test but with a family history of the disease

(b) Latent diabetic (suspected diabetic) The individual with a normal glucose tolerance test but he had a diabetic type of glucose tolerance curve after cortisone administration, pregnancy or severe infection

(c) Asymptomatic diabetic (chemical diabetic) The individual with a diabetic glucose tolerance curve but without signs of diabetes

(d) Clinical diabetic (overt diabetic) (i) Juvenile—onset type, (ii) Adult or maturity onset type

Juvenile—onset type .

(i) This condition develops during the first 40 years of life in normal or less than normal body weight

(ii) The symptoms become severe and insulin treatment if delayed, Keto-acidosis and diabetic coma develop

(iii) Insulin secretion is very low

(iv) The β-cells of pancreas are exhausted and the cells become atrophied resulting in diabetes mellitus

Adult or Maturity—onset type

(i) This condition appears after 40 years of age who are obese

(ii) This is a mild type of diabetes which can be controlled by diet and oral hypoglycemic drugs

(iii) Insulin level are lower than normal because the β-cells secrete less amount of insulin

2 Secondary diabetes :

Damage to pancreas In chronic pancreatitis and pancreatic carcinoma, the pancreatic destruction results in the absolute insulin deficiency

Presence of insulin antagonists Excess growth hormone secretion (acromegaly) or excess glucocorticoid secretion (Cushing s syndrome) act as antagonists to insulin

Inhibition of insulin secretion The secretion of insulin by β-cells is inhibited by the excess secretion of epinephrine and thyroxine resulting in the breakdown of liver glycogen

A diabetes like condition can be produced in experimental animals by injecting alloxan which is a pyrimidine derivative containing 4 0×0 groups Alloxan destroys the β-cells of pancreas for which insulin production is inhibited But the α-cells remain unaffected and the glucagon production remains intact Dehydroascorbic acid if injected in large doses also destroys β-cells of pancreas and produces alloxan type diabetes

Growth hormone if repeatedly injected into experimental animals the β-cells of the pancreas are stimulated to secrete insulin The cells become hypertrophied and insulin secretion finally stops resulting in permanent diabetes mellitus in the animals

Diabetic coma

1 In case of diabetes, if the insulin is not taken and the diet is not controlled, diabetes becomes severe leading to diabetic coma.

2. In diabetic coma, there is ketonemia and ketonuria.

3 The patient becomes semi-conscious or fully unconscious with debydration

4 The patient should be treated with soluble insulin (50 units) immediately followed by normal saline containing 10 per cent glucose intravenously

5 The treatment should be controlled by frequent estimation of glucose in blood and urine every 30 minutes to make sure that blood glucose does not fall below normal level

Treatment of diabetes mellitus

1 Administration of oral antidiabetic drugs like diabenese which are not polypeptides in moderate diabetes and insulin in severe diabetes

2. High protein and low carbobydrate and fat diet are advisaole High protein meal serves as a prolonged source of carbobydrate without rapid hyperglycemic effect and it has protective effect on liver

Changes in carbohydrate, fat and protein metabolism in diabetes *mellitus*.

Carbohydrate metabolism

1 Diminished entry and oxidation of glucose in muscle and other tissues.

2 Decreased glycogen formation in liver and muscle.

3 Decreased synthesis of fat and increased breakdown of glycogen in liver

Fat metabolism

1 Fats are mobilized from adipose tissue and fatty acids are oxidized producing large amounts of acetyl CoA Due to lack of carbobydrate metabolism, acetyl CoA is not oxidized via TCA cycle. Hence, two molecules of acetyl CoA condense to form ketone bodies

2 The ketone bodies in large amounts to blood produces ketosis which leads to coma in severe diabetes mellitus.

4 Administration of insulin corrects the condition

Protein metabolism

1 Since the energy is not available by oxidation of carbobydrate and fat, the breakdown of tissue protein occurs leading to negative energy balance. The synthesis of tissue protein from dietary proteins requires insulin. Due to lack of insulin, tissue protein is not formed from dietary protein. The concentration of amino acids in blood and liver increases The amino acids are deaminated in liver and glucose is produced from ketoacids by gluconeogenesis. The urea excreted more in urine The body will be in negative nitrogen balance.

2 Administration of insulin corrects the condition Insulin promotes oxidation of glucose to meet the energy needs and also the synthesis of tissue protein from dietary protein Insulin also suppresses gluconeogenesis.

Comparision between Hypoglycemic coma and Diabetic coma

	Hypoglycemic coma	*Diabetic coma*
Cause	No food, overdose of insulin	Normal food intake and no insulin.
Onset	Sudden and in good health	The patient suffers from ill-health for several days
Symptoms	Sweating, dizziness, tremor, mental confusion, semi consciousness, headache, seeing double images	Dehydration, abdominal pain and vomiting
Signs	Moist skin, full pulse, normal or raised blood pressure, normal breathing	Dry skin weak pulse, low blood pressure, air hunger
Urine	Ketone bodies absent, glucose absent	Ketone bodies and glucose present.
Blood	Hypoglycemia, normal blood bicarbonate Sugar level comes down to 30 mg/100 ml	Hyperglycemia, reduced blood bicarbonate Sugar level reaches to 550 mg/ 100 ml

GLYCOSURIA

The condition in which abnormal quantities of glucose are excreted in urine is called glycosuria

Normal urine contains traces of glucose which cannot be detected by Benedict's test. Beyond the renal threshold value (160-180 mg/100 ml), the tubules cannot reabsorb glucose which escapes reabsorption is excreted in urine Some individual have low renal threshold value Glycosuria occurs in two conditions (A) In normal blood glucose level and (B) In hyperglycemia

A Glycosuria in normal blood glucose level

1 Alimentary glycosuria Some individuals excrete glucose in urine after the intake of large amounts of sugar or carbohydrate-rich meal in spite of their normal blood sugar level They have normal renal threshold value for glucose but their blood glucose level shoots up (200-220 mg/100 ml) for a short period Hence, a transitory glycosuria occurs

2. Emotional glycosuria Glycosuria occurs during periods of excessive nervous strain and emotional excitement such as intense fear, anger and severe anxiety due to increased secretion of epinephrine This condition is termed "emotional or psychic' glycosuria

Emotional glycosuria has been observed in college students sitting for the final examination worried athletes and candidates for competitive examinations

Glycosuria results in the use of ether and chloroform as anaesthetics due to hypersecretion of epinephrine

3 Glycosuria in pregnancy and lactation Glycosuria occurs in normal pregnant women, particularly in the later months due to temporary reduction in maximum tubular reabsorption capacity of glucose and partly due to decreased glucose tolerance caused by temporary hypertrophy of the pituitary gland

Lactose is also excreted in urine during late in pregnancy and lactation which should not be mistaken for glucose

4 Renal glycosuria (Hereditary glycosuria) Some individuals have low renal threshold value for glucose which may be below 150 mg/100 ml This condition is harmless and is known as "diabetes innocens' or 'benign glycosuria." These individuals have an impaired tubular reabsorption for glucose and it is a hereditary defect.

The following are the standards for the diagnosis of true renal glycosuria

(i) Normal blood sugar level in fasting

(ii) Glucose is present in every sample of urine either in fasting state or after a meal

(iii) Carbohydrate utilization is normal

(iv) No disturbance of fat metabolism Ketosis may develop when the patient fasts than when he overeats

(v) Moderate doses of insulin have little or no effect upon the glycosuria

Practical danger in making the diagnosis of renal glycosuria lies in the confusion of this condition with mild diabetes mellitus No metabolic disturbance occurs in subjects with renal glycosuria as long as the carbohydrate intake is adequate to compensate for the amount lost in the urine

Artificially, a severe renal glycosuria can be produced by poisoning the kidney with phlorrhizin which prevents reabsorption of glucose from the tubules As a result, there is a continuous loss of glucose and the blood sugar level becomes abnormally low This hypoglycemia causes the secretion of epinephrine and glucagon which accelerate liver glycogenolysis Due to the exhaustion of liver glycogen glucose is formed from the tissue protein to maintain the blood sugar level The fasting urine on analysis has been found that the ratio glucose excreted/N excreted (*the G/N or D/N ratio*) is constant

B Glycosuria in hyperglycemia

Glycosuria occurs due to increased blood glucose level in diabetes mellitus The blood glucose level in this condition become very high and glucose is excreted in urine

GLUCOSE TOLERANCE TEST (GTT)

Glucose tolerance test (GTT) gives the assessment of the degree of severity of diabetes mellitus This can be performed in the following manner

1 The individual is kept fasting for at least 12 hours after the last meal

2 Samples of venous blood and urine are collected in the fasting state to find out the initial blood and urine glucose level

3 Immediately after this, 50 grams of glucose is administered to that subject. Samples of blood and urine are collected at an interval of 30 minutes for a period of 2 to 3 hours The results of blood glucose analysis are plotted as a graph against time

Normal person

The main features of GTT curve in normal persons are as follows

1 Fasting blood sugar is between 80 to 120 mg/100 ml

2 Blood glucose level reaches its peak to about 130 mg/100 ml in 1 hour

3 The blood glucose level returns to the fasting level at the end of 2 2½ hours after glucose meal

4 The urine does not contain any glucose

Prediabetic persons

The main features of GTT curve in persons who are developing diabetes (Prediabetic) are as follows

1 Fasting blood sugar level is between 105 and 110 mg/100 ml

2. The peak level is attained in 1 hour and the value ranges from 150 to 160 mg/100 ml

3 The blood glucose level returns to the normal level only after 3 hours

4 Urine does not contain glucose

Mild diabetes

The main features of the GTT curve of mild diabetics are as follows

1 Fasting blood sugar is between 115 and 125 mg/100 ml

2. The speak level is attained in 1 hour and ranges from 190 to 200 mg/100 ml

3 Urine con ains 1 to 2 per cent glucose

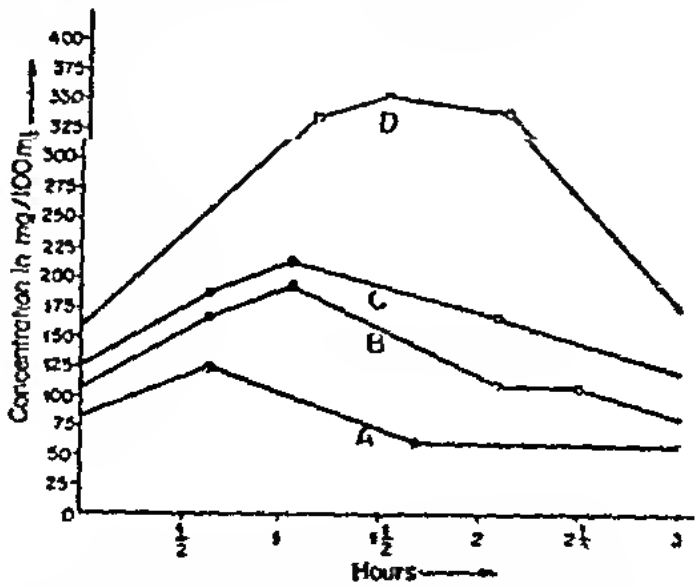
The main features of GTT curve of severe diabetics are as follows

1 Fasting blood glucose level is about 150 to 160 mg/100 ml

2. The peak level is attained in 1 hour and ranges from 320 to 350 mg/100 ml The peak level is maintained for 1 to 2 hours

3 The blood sugar returns to the fasting level after 4 hours

4 Urine contains large amounts of glucose (more than 2 per cent) when collected at 1 hour, 1½ hour and 2 hours intervals



A=Normal subject B=Prediabetes C=Mild diabetes D=Severe

Fig. 16.25 Glucose tolerance test curve (GTT curve)

CONVERSION OF CARBOHYDRATES INTO FAT

1 Glucose and the intermediates such as pyruvate and acetyl-CoA are converted to fat in the body by anabolic processes

2. The rate of conversion is high in relation to the diet containing high proportion of carbohydrate It is depressed in restricted caloric intake, on a high-fat diet or in the deficiency of insulin as in diabetes mellitus. Increased free fatty acids inhibits lipogenesis

3 Hexose monophosphate shunt (HMP shunt) is closely associated with the lipogenesis because this shunt supplies reduced NADP (NADPH) for the synthesis of fat

4 At present, it is recognized that *acetyl CoA carboxylase* step is the rate-limiting reaction in the pathway of lipogenesis Long chain acyl-CoA molecules produced from lipolysis inhibit acetyl-CoA carboxylase and thus inhibits the synthesis of new fatty acids

5 Microsomes catalyze the esterification of acyl CoA with glycerol 3-phosphate to form triacylglycerols and phospholipids.

6 Acyl-CoA also inhibits the transport of citrate from mitochondria into the cytosol and thus changes the availability of citrate for lipogenesis.

7 *Insulin* stimulates lipogenesis by several mechanisms :

(i) It increases the transport of glucose into the cell and thus increases the availability of pyruvate and glycerol 3-phosphate.

(ii) It can convert the inactive pyruvate dehydrogenase into the active form

(iii) It depresses cyclic AMP (cAMP) and thus inhibits lipolysis and thereby reduces the concentration of long chain acyl-CoA, which is an inhibitor of lipogenesis

8 Oxidation of fatty acids, due to lack of insulin or increased levels of free fatty acids, causes the increased lipolysis of triacylglycerols and thus increases the concentrations of acetyl CoA and NADH in mitochondria inhibiting pyruvate dehydrogenase As a result, the formation of acetyl CoA from carbohydrate via pyruvate is blocked.

The pathway of conversion of carbohydrate into fat is shown below :

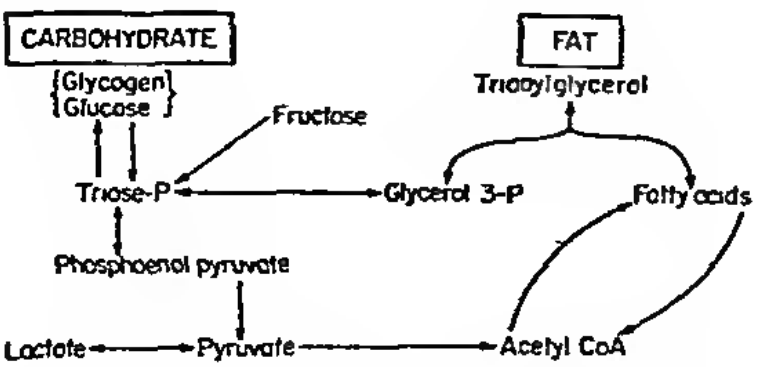


Fig ' 16 26 Pathway of conversion of carbohydrates into fat.

HEXOKINASE

1 The molecular weight of the enzyme hexokinase is 96 600

2. This enzyme exists in plant and animal tissues

3 Liver contains two hexokinase enzymes—glucokinase is probably formed in the non parenchymal cells and hexokinase is formed in the parenchymal cells

4 Glucose enters the metabolic stream through phosphorylation as glucose-6-phosphate by the catalytic action of glucokinase in the hexokinase reaction Insulin regulates the activity of glucokinase and glucose phosphorylation Liver hexokinase appears to be unaffected by insulin.

5 Hexokinase has a high affinity for glucose

6 This enzyme catalyzes the phosphorylation of glucose, fructose, mannose and glucosamines etc

A. T. P.

It is adenosine triphosphate or high energy phosphate bond

Discovery Lohmann isolated ATP from muscle The phosphate groups are consecutively removed from ATP to ADP and AMP Lohmann reaction is mentioned below

$$ATP + H_2O \longrightarrow ADP + Phosphate$$

$$Creatine\ phosphate + ADP \rightleftharpoons ATP + Creatine$$

This is the first energy production reaction which occurs in muscle contraction Creatine phosphate serves as a reservoir of active phosphate groups to maintain the concentration of ATP

Function

1 It acts as a basic unit of biological energy

2 It acts as a phosphate transfer reaction

3 It helps photosynthesis

4 It is involved in the activation of fatty acids and amino acids

5 It causes phosphorylation of glucose, galactose and fructose

6 It acts as a coenzyme in the energy metabolism.

Effects of shock Shock causes the rapid decrease of high energy phosphates in the shocked tissue Severe shock causes collapse and anoxia. Electric shock and cyanide poisoning decrease brain glycogen, phosphocreatine and ATP

Formation It is formed by the catabolism of food stuff where the reduced equivalents are oxidized in the respiratory chain

GLYCOGEN

Chemistry

1 It is a polysaccharide consisted of many glucose units by 1, 4 and 1, 6 linkages

2. It forms a colloidal solution

3 Its structure is just like a tree having numerous branches Each branching is at an interval of 12 18 glucose units

4 Its molecular weight is very high of the order of several millions.

Tissue glycogen

Glycogen is the chief carbohydrate of tissues just as glucose of blood and other body fluids The followings are the contents of glycogen in the tissues

Liver—5% (average) , 10-15 per cent on carbohydrate diet

Skeletal muscle—0 5%.

Skin and brain—0 1% or less

Liver glycogen is reversibly convertible to blood glucose and maintain the blood sugar level in the impairment of intestinal absorption of carbohydrate The energy is liberated when glycogen is broken down completely to CO_2 and H_2O The formation of fat from carbohydrate is from the breakdown products of glycogen or glucose

Glycogen is formed from glucose by glycogenesis and glycogen is broken down to glucose in the liver by glycogenolysis

Factors affecting tissue glycogen

1 *Dietary state* The glycogen content of tissues is markedly altered by changes in the dietary condition

2 *Exercise* Heavy exercise greatly accelerates the utilization of carbohydrate in the tissues and ultimately leads to drastic reduction of muscle and liver glycogen

3 *Insulin* It is required for both the oxidation of glucose and its conversion to glycogen It also accelerates the activity of hexokinase and glycogen synthetase responsible for glycogen formation

4 *Epinephrine and glucagon* Both these hormones cause glycogenolysis by stimulating phosphorylase and produce glucose in the liver and lactic acid in the muscle

Glycogen storage disease Already discussed (Page 273)

ACETYL-CoA

Acetyl CoA can be regarded as a focal point in metabolism It is a common high energy substance produced by the oxidation of fatty acids, amino acids and pyruvate from carbohydrate It is utilized in many ways and referred to as the "building stone" of fatty acid synthesis

Formation Acetyl CoA is formed from coenzyme A (CoA) The components of CoA are adenine-phosphoribose-phosphate-phosphate-pantothenic acid mercapto-ethanolamine The 'SH is the functional group, combining with acyl substances having the carboxyl group (R COOH) to form thioesters (R CO S CoA) In respect of this linkage the factor is designated as CoA SH This linkage is a high energy bond and is formed at the expense of ATP

Utilization It is utilized in cells in various ways

1 In the complete oxidation of glucose by the TCA cycle

2 In the fatty acid synthesis

3 In the acetylation of choline (in nerve) and of aromatic amines

4 In the formation of acetoacetyl-CoA in ketone body formation

5 In the synthesis of cholesterol steroid hormones, bile acids etc

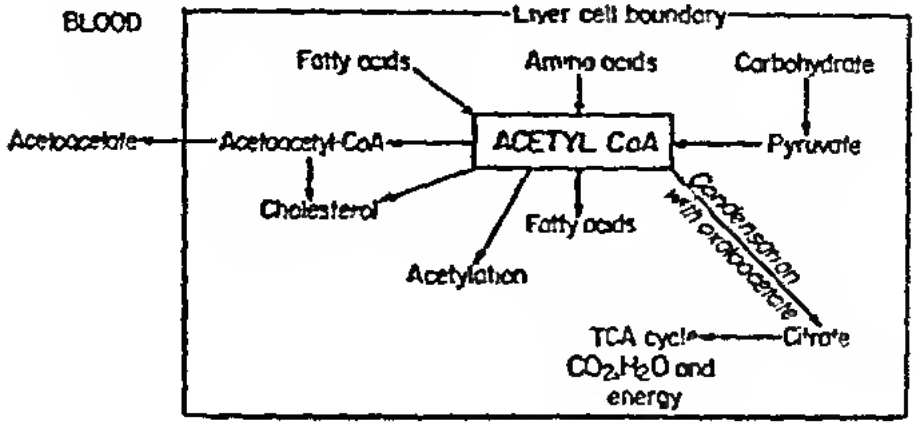


Fig 16 27 Formation and utilization of acetyl-CoA

CO-CARBOXYLASE

Thiamine pyrophosphate (TPP) is said to be the co-carboxylase This is the coenzyme formed from thiamine It is a group transferring coenzyme

Function

1 TPP as coenzyme is involved in the conversion of pyruvate to acetyl CoA along with pyruvate dehydrogenase complex Pyruvate is decarboxylated in the presence of TPP to a hydroxyethyl derivative of the thiazole ring of enzyme bound thiamine diphosphate which reacts with oxidized lipoate to form S-acetyl lipoate

2 Similar reaction occurs in the conversion of α ketoglutarate to succinyl-CoA where TPP acts as coenzyme

3 TPP also acts as coenzyme in the conversion of pentose phosphates to sedoheptoluse-7 phosphate and glyceral-dehyde-3 phosphate catalyzed by trans-ketolase in the HMP shunt

Exercises

1 Draw a flow diagram showing the reactions of Kreb s cycle Discuss its importance (M U 76A)

2. What is Kreb s cycle ? Why is it so called ? What is the alternate name of the cycle ? Justify the name State very briefly the steps of the Kreb's cycle (R U 76A)

3 What do you mean by intermediary metabolism ? Explain why glucose-6-phosphate is regarded as the key intermediate metabolic product of carbohydrate metabolism (P U 75S)

4 Discuss the various steps of TCA cycle and point out where energy water and CO_2 are blasted ? How many molecules of ATP are produced in the cycle (P U 71S Mar 75S)

5 Describe schematically the citric acid cycle (Mith 66S 71S, R U 70S, P U 68S)

6. Describe the pentose phosphate pathway of carbohydrate metabolism and the significance of it. (Cal. U 75A)
7. Give an account of the citric acid cycle and explain why it is called a common metabolic pathway (Mad. 74A, Mith. 62A)
8. Discuss the oxidation of glucose in the tissues upto the stage of pyruvic acid with particular reference to the generation of energy rich phosphate bonds. (Mith. 68S)
9. Describe the pathway of conversion of carbohydrates into fat. (Mith. 67S)
10. Describe briefly the mechanism by which the body derives energy from glucose. (Bh. U 75A)
11. Discuss the formation of glucose from non-carbohydrate substances. (P U 73A, R. U 76S, Bh. U 71S)
12. Describe the hormonal influence on carbohydrate metabolism. (P U 76A)
13. What is the normal blood sugar level? How is it maintained at a constant level? (P U 65A, 69A, Luck. 67S)
14. Describe the mechanism of regulation of blood sugar level. (Mith. 75A, P U. 73A)
15. Name the reducing sugars that may be found in Pathological urine. Describe a method for the estimation of the concentration of reducing sugar in urine. (R. U 78S)
16. Discuss the glucose tolerance test. (R. U 73A)
17. Write notes on
    (i) Synthesis of lactose (Bh. U 74S, Mad. 74A)
    (ii) Glycogen (M. U 73A, Mith. 62A)
    (iii) Neoglucogenesis (M. U 74S, R. U 71S, Mith. 62A, 65A)
    (iv) Glycogenesis (Mad. 75S, Mith. 65S, 75S)
    (v) Glycogenolysis (Bh. U 74S, R. U 70A, Mith. 62S 64A)
    (vi) Benedict's test. (Mith. 73A, Bh. U 76S, P U 68S)
    (vii) Glucose tolerance test. (Mith. 62A, 66S, P U 72S)
    (viii) Glycosuria. (R. U 65S, Luck. 64A)
    (ix) Renal glycosuria. (Mith. 75A, P U. 63A)
    (x) A. T. P (P U 75S, M. U 73A, R. U 71A, 73A, Mith. 71A, 75A)
    (xi) High energy phosphate bond. (Mith. 62A, 64S)
    (xii) UDPG (R. U 72S, P U 72S)
    (xiii) Hexokinase (R. U 73S)
    (xiv) Co-carboxylase (Mith. 60S)
    (xv) Conversion of glyceraldehyde-3-phosphate to 1, 3-diphosphoglycera. acid. (R. U 72S)
    (xvi) Fructosuria. (Bh. U 71A)
    (xvii) Galactosemia. (M. U 72S)
    (xviii) Glycogen storage disease (R. U 67S, Mad. 70A)
    (xix) Diabetes mellitus (P U 68A, R. U 66S)

8 Write in short the process of neoglucogenesis Mention the specific enzymes necessary for the above process (C.U. 1981)

9 Describe with diagram the metabolic pathways of glycogenesis and glycogenolysis in muscle indicating the hormonal influences on them

Describe how will you perform and interprete an oral glucose tolerance test (C U 1983)

10 Describe with diagram how acetyl CoA is formed from pyruvate and long chained fatty acids Enumerate the fate of acetyl-CoA in the body

What will happen to acetyl CoA if there is a lack of insulin in the body (C.U 1983)

CHAPTER 17

METABOLISM OF LIPIDS

The lipids of metabolic significance include triacylglycerol (triglycerides, neutral fat), phospholipids, steroids together with long chain fatty acids (free fatty acids), glycerol and ketone bodies

Advantages of lipids over carbohydrate or protein

1 The caloric value of lipid is over twice as great (9 3 Kcal/g) and it is associated with less water in storage

2 Triacylglycerol is the most concentrated form in which potential energy can be stored

3 Fatty acids on oxidation provide more metabolic water than other metabolic fuels This is helpful to mammals accompanying dry environments

4 Less amount of lipid in the diet can supply adequate amnunt of certain polyunsaturated fatty acids (the essential fatty acids) and it is essential nlso for fat soluble vitamins Lipid is also necessary for their efficient absorption from the gastrointestinal tract

OXIDATION OF TRIACYLGLYCEROL

1 Triacylglycerol is first hydrolyzed to fatty ncids and glycerol mostly in adipose tissue

2. The free fatty acids are released into the plasma where they combine with serum albumin

3 Long chain fatty acids are oxidized in liver, heart, kidney, muscle, lung testis, brain and adipose tissue

4 Glycerol is utilized by liver, kidney, intestine and lactating mammary gland where the nctivating enzyme *glycerokinase* is present ahundantly

Oxidation of fatty acids

Fatty acids are oxidized hy β-, α and ω-oxidation Quantitatively, β-oxidation is the most important pathway The term β-oxidatinn means the oxidation takes place in the β-carhon of the fatty acid with the removal of 2 carbon atoms at a time from the carhoxyl end of the molecule

The fatty acids containing even number and odd number of carhon atnms ns well as the unsaturated fatty acids are oxidized hy β-oxidation

β-oxidation of fatty acids

1 Several enzymes collectively known as "*fatty acid oxidase*" are found in the mitochondrial matrix adjacent to the respiratory chain (which is found in the inner membrane)

2 Long chain fatty acids are first activated to "active fatty acid ' or acyl-CoA in the *cytosol* hy the enzyme *thiokinase* in the presence of ATP, coenzyme A nnd Mg^{++} But activation of lower fatty acids occurs within the mitochondria Thiokinases are found hoth inside and outside the mitochondria.

3. Long chain acyl-CoA does not penetrate mitochondria without the presence of carnitine The enzyme *carnitine-palmityl acyl transferase* associated with the mitochondria membranes allows long chain acyl groups to penetrate the mitochondria The mechanism is shown below :

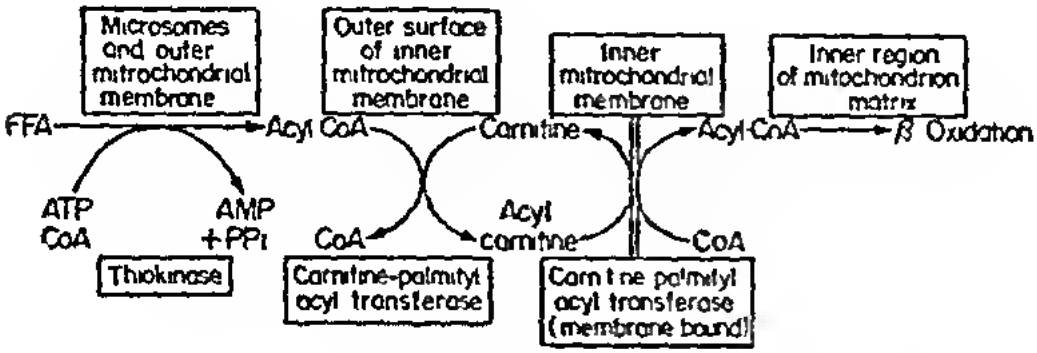


Fig 17 1 Role of carnitine in the metabolism of long chain fatty acids

Another enzyme, *carnitine-acetyl acyl transferase*, present in mitochondria, catalyzes the transfer of short chain acyl groups between CoA and carnitine

$$\text{Acetyl CoA} + \text{Carnitine} \xleftrightarrow{\text{Carnitine-acetyl acyl transferase}} \text{Acetyl carnitine} + \text{CoA}$$

4 Acyl CoA is then converted to α, β unsaturated acyl-CoA by the enzyme *acyl-CoA dehydrogenase* in presence of the coenzyme flavoprotein which contains FAD as prosthetic groups The reduced coenzyme is reoxidized by the respiratory chain

5 Water is added to saturate the double bond and form β-hydroxy acyl-CoA, catalyzed by the enzyme *enoyl CoA hydratase* (crotonase)

6 The β-hydroxy acyl CoA undergoes dehydrogenation on the β-carbon forming β-keto acyl CoA The reaction is catalyzed by *β hydroxy acyl-CoA dehydrogenase* in presence of NAD The reduced coenzyme (NADH) is reoxidized by the respiratory chain

7 Finally, β-keto acyl-CoA is split at the β-position by *thiolase* (β-ketothiolase) with the addition of one molecule of CoA The products of this reaction are acetyl CoA (C_2-units) and an acyl-CoA containing 2 carbon less than the original acyl CoA molecule which undergoes further oxidation by β-oxidation In this way, a long chain fatty acid is degraded completely to acetyl-CoA which is completely oxidized to CO_2 and water via the citric acid cycle within the mitochondria.

Fatty acids with an odd number of carbon atoms are oxidized by the pathway

of β-oxidation until *propionyl-CoA* is formed. This compound is converted to succinyl-CoA which is a constituent of the citric acid cycle.

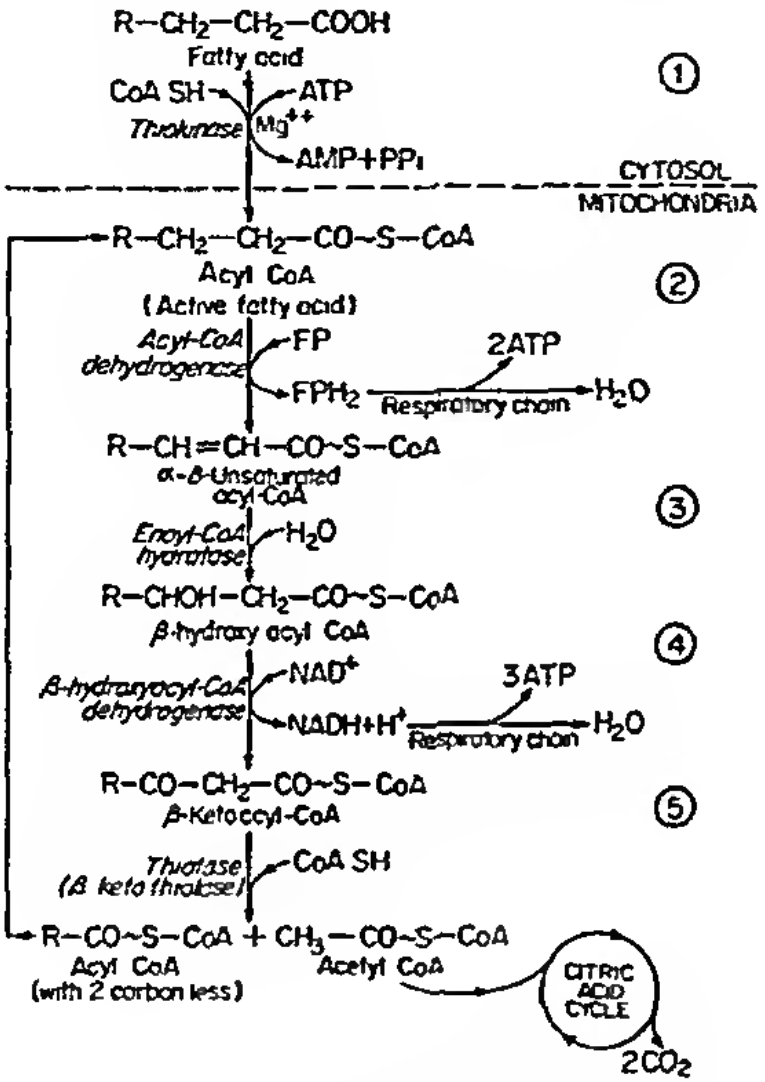


Fig 17.2 β-oxidation of fatty acids

Unsaturated fatty acids are also oxidized following β-oxidation. The reactions are given on the next page

α- and ω-oxidation of fatty acids :

α-oxidation takes place by the removal of one carbon at a time from the carboxyl end of the molecule. It does not require CoA intermediates and does not lead to the generation of high-energy phosphates.

ω-oxidation takes place by hydroxylase enzymes involving cytochrome P-450 in microsomes. The — CH_3 group is converted to a — CH_2OH group which is ultimately oxidized to — COOH group forming a dicarboxylic acid.

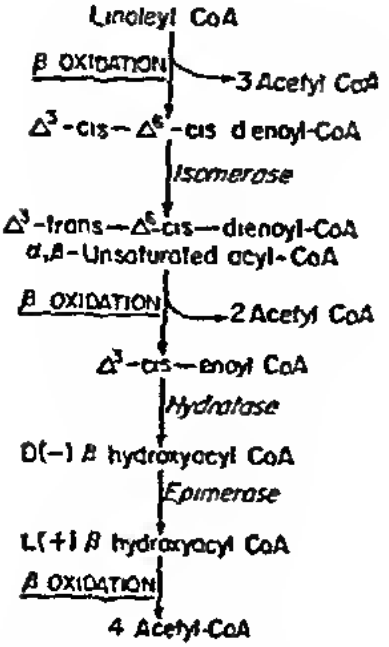


Fig. 17.3 Sequence of reactions in the oxidation of unsaturated fatty acids, e.g. linoleic acid

Energetics of fatty acid oxidation :

Palmitic acid (16 carbons) undergoes 7 times β-oxidation and produces 8 molecules of acetyl CoA. Each time, β-oxidation produces 5 ATP.

∴ Total number of ATP formed through β-oxidation	$(7\times5) = 35$
Total number of ATP formed on oxidation of acetyl-CoA through citric acid cycle	$(8\times12) = 96$
Total	= 131
2 ATP utilized for initial activation of fatty acid	−2
Net total yield	= 129 ATP.

$129\times7.6 = 980$ Kcal/mol

The caloric value of palmitic acid is 2340 Kcal/mol

∴ The process captures as high-energy phosphate as $\frac{980\times100}{2340} = 41\%$

BIOSYNTHESIS OF LIPIDS

The process of synthesis of triacylglycerol (neutral fat) and fatty acids from acetyl CoA in adipose tissue and to a lesser extent in other tissues is said to be *lipogenesis.*

Synthesis of fatty acids

Formerly, it was considered that fatty acid synthesis was the reversal of its oxidation. Now, it is clear that the *mitochondrial* system for fatty acid synthesis is responsible only for elongation of existing fatty acids of moderate chain length. The synthesis of palmitate from acetyl-CoA is completed only by the highly active *extramitochondrial* system. Three different systems are mentioned below.

A. Mitochondrial system

1. Mitochondria catalyze the incorporation of acetyl-CoA into long chain fatty acids under *anaerobic* conditions.
2. The enzymes are mostly the same as those involved in β-oxidation excepting α, β-*unsaturated acyl-CoA reductase* which converts α, β-unsaturated acyl-CoA to a saturated compound, requiring NADPH.
3. Pyridoxal phosphate (B_6—PO_4) as a coenzyme is required for the enzyme condensing acetyl-CoA with acyl-CoA; thus, thiolase is not used in this synthetic pathway.
4. Since this system takes place under anaerobic condition the physiologic significance of this pathway is uncertain.

B. Microsomal system for chain elongation

1. This is the main pathway for the elongation of existing fatty acid molecules.
2. Acyl-CoA compounds are converted to higher fatty acids by means of malonyl-CoA along with NADPH. The acyl groups include the saturated series from C_{16}—C_{18} as well as some unsaturated C_{18} fatty acids.
3. Fasting prevents chain elongation.

C. Extramitochondrial system for De NOVO synthesis

1. This system is found in the soluble fraction of many tissues, such as liver, kidney, lung, brain, mammary gland and adipose tissue. ATP, NADPH, CO_2 and Mn^{++} are required in this system. The end product is the free palmitate.
2. CO_2 is required in the initial carboxylation of acetyl-CoA to malonyl-CoA by *acetyl CoA carboxylase* in presence of ATP and biotin. If biotin is bound to the protein avidin of egg white, acetyl-CoA carboxylase is inhibited.
3. The multienzyme complex contains two types of —SH groups, "central" and "peripheral". Acetyl-CoA reacts with the peripheral —SH groups and malonyl-CoA reacts with the central —SH group.
4. The acetyl group attacks the methylene group of the malonyl residue to liberate CO_2 and form acetoacetyl enzyme attached to the central —SH group.
5. Acetoacetyl enzyme is then reduced, dehydrated and reduced again to

form the saturated acyl enzyme compound NADPH acts as the hydrogen donor in both the reductions with the mediation of FMN in the reaction

6 In mammalian systems, free palmitate is liberated from the enzyme complex by hydrolysis The multi-enzyme functions efficiently and is free from interference

The overall reaction for the synthesis of palmitate from acetyl CoA and malonyl-CoA is as follows

$$\text{Acetyl-CoA} + 7\ \text{malonyl-CoA} + 14\ \text{NADPH} + 14\text{H}^+ \longrightarrow \text{Palmitate} + 7\text{CO}_2 + 6\text{H}_2\text{O} + 8\text{CoA SH} + 14\text{NADP}^+.$$

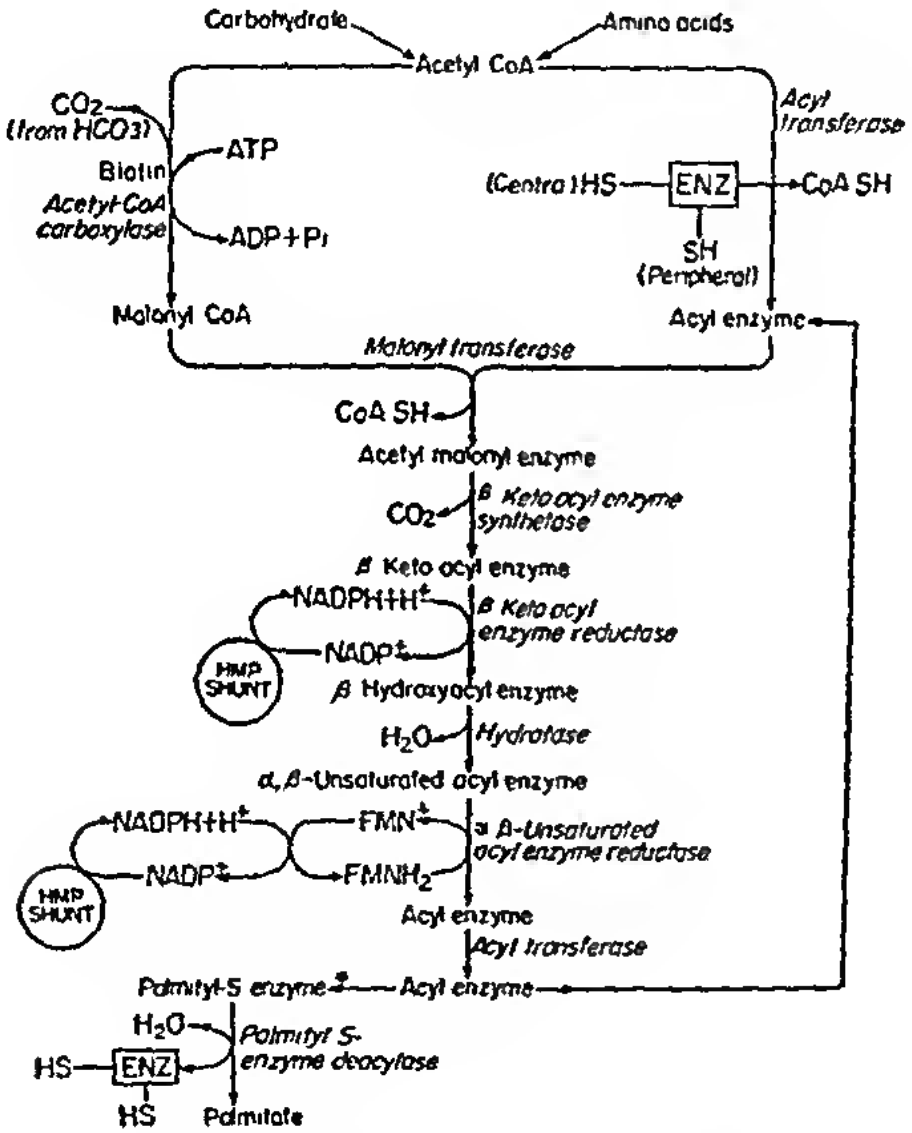


Fig 17 4 Extramitochondrial synthesis of palmitate

Recently, it is suggested that butyryl-CoA is the primer molecule in mammalian liver and mammary gland In ruminants, propionyl CoA acts as a primer to form long chain fatty acids with odd number of carbon atoms

Acetyl CoA is formed from carbohydrate via the oxidation of pyruvate within the mitochondria. But acetyl CoA does not diffuse readily into the extramitochondrial compartment Citrate is diffused Citrate is splitted by ATP-citrate lyase (citrate cleavage enzyme) and then acetyl-CoA is involved in fatty acid synthesis in the extramitochondrial compartment. Pyruvate undergoes oxidative decarboxylation within the mitochondria to form acetyl CoA which combines with oxaloacetate to form citrate Malate is transported to the mitochondria to form oxaloacetate Oxaloacetate also is converted into malate in the extramitochondrial compartment. The fact is given below

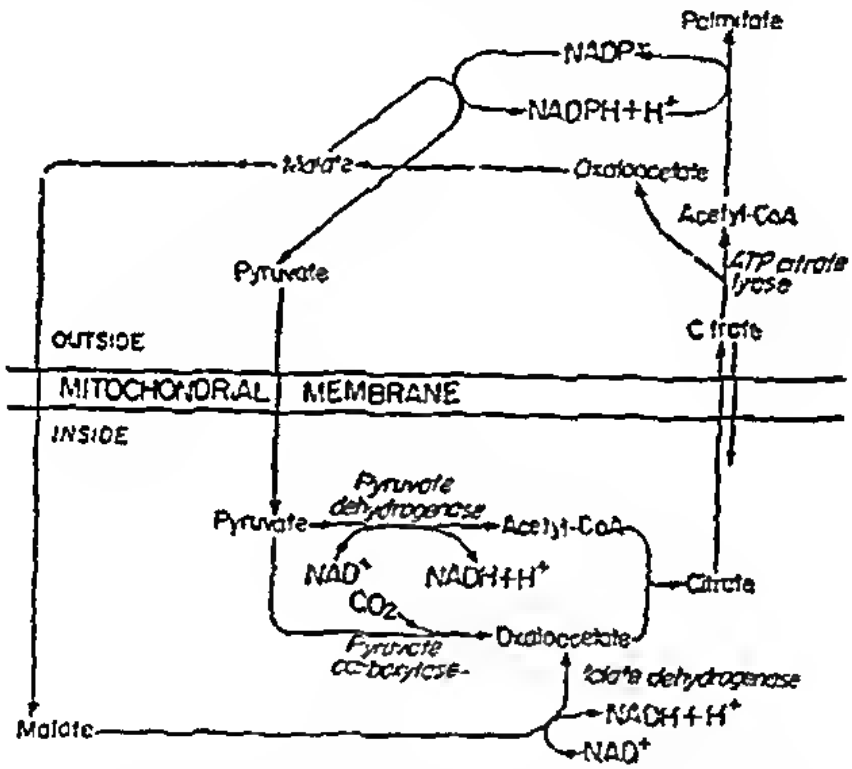


Fig 17.5 The provision of citrate and NADPH for lipogenesis from carbohydrate.

BIOSYNTHESIS OF ACYLGLYCEROLS (Glycerides)

1 Fatty acids are activated to acyl-CoA by the enzyme *thiokinase* using ATP and CoA

2 Two molecules of acyl CoA combine with glycerol 3 phosphate to form 1, 2-diacylglycerol phosphate by *glycerol 3-phosphate acyl transferase*

In intestinal mucosa, a monoacylglycerol pathway exists by which monoacylglycerol is converted to 1, 2-diacylglycerol by *monoacylglycerol acyl transferase*

3 Another molecule of acyl CoA is esterified with the diacylglycerol to form a triacylglycerol by diacylglycerol acyl transferase

The overall reaction is given below

$$\text{Fatty acid} + \text{ATP} + \text{CoA} \xrightarrow{\text{Thiokinase}} \text{Acyl—CoA (Fatty acid CoA)}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerokinase}} \text{Glycerol 3 phosphate} + \text{ADP}$$

$$2\ \text{Acyl CoA} + \text{glycerol 3 phosphate} \xrightarrow[\text{acyl transferase}]{\text{Glycerophosphate}} \text{1, 2-diacylglycerol phosphate}$$

$$\text{1, 2 diacylglycerol phosphate} \xrightarrow{\text{Phosphatase}} \text{1 2 diacylglycerol} + \text{phosphoric acid}$$

$$\text{1, 2 diacylglycerol} + \text{acyl CoA} \xrightarrow[\text{transferase}]{\text{Diacylglycerol acyl}} \text{Triacylglycerol (Triglyceride)}$$

BIOSYNTHESIS OF PHOSPHOLIPIDS

1 Formation of phosphatidic acid

By the reaction between 1, 2-diacylglycerol and ATP in the presence of a kinase enzyme phosphatidic acid is formed

$$\text{1, 2 diacylglycerol} + \text{ATP} \xrightarrow{\text{Kinase}} \text{1 2 diacylglycerol phosphate (phosphatidic acid)} + \text{ADP}$$

2 Formation of phosphatidyl choline (Lecithin)

Choline is activated by two stages first by ATP and then by CTP to convert it to CDP choline which reacts with 1 2 diacylglycerol to form phosphatidyl choline

$$\text{Choline} + \text{ATP} \longrightarrow \text{choline monophosphate}$$

$$\text{Choline monophosphate} + \text{CTP} \longrightarrow \text{CDP-choline} + \text{PP}_i \quad \text{(Active choline)}$$

$$\text{CDP choline} + \text{1, 2-diacylglycerol} \longrightarrow \text{phosphatidyl choline} + \text{CMP}$$

3 Formation of phosphatidyl ethanolamine (Cephalin)

Ethanolamine is actived to active ethanolamine in two stages It first reacts with ATP and then to CTP to form CDP ethanolamine which reacts with 1 2 diacylglycerol to form cephalin

$$\text{Ethanolamine} + \text{ATP} \longrightarrow \text{Ethanolamine monophosphate} + \text{ADP}$$

$$\text{Ethanolamine monophosphate} + \text{CTP} \longrightarrow \text{CDP-ethanolamine} + \text{PP}_i \quad \text{(Active ethanolamine)}$$

$$\text{CDP ethanolamine} + \text{1, 2 diacylglycerol} \longrightarrow \text{phosphatidyl ethanolamine} + \text{CMP}$$

The enzyme *phosphoetl anolamine acyl glycerol transferase* responsible for the formation of phosphatidyl ethanolamine is not present in liver

4 Formation of phosphatidyl serine

This can be formed directly from phosphatidyl ethanolamine by serine

$$\text{Phosphatidyl ethanolamine} + \text{serine} \longrightarrow \text{phosphatidyl serine}$$

5. Phosphatidyl Inositol :

$$\text{CDP-diacylglycerol} + \text{inositol} \xrightarrow{\text{Transferase}} \text{phosphatidyl inositol} + \text{CMP}.$$

6. Formation of sphingomyelin :

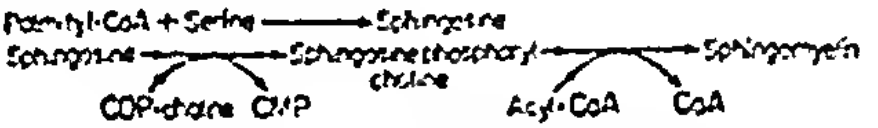


Fig. 17.6

7. Formation of glycerol ether phospholipids and plasmalogens :

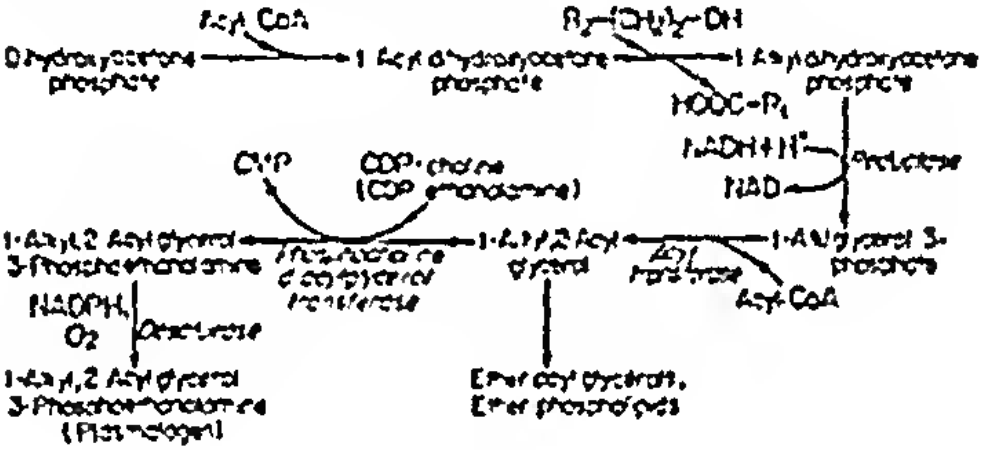


Fig. 17.7

8. Formation of cardiolipin :

Cardiolipin, a phospholipid is present in mitochondria. It is formed from phosphatidyl glycerol.

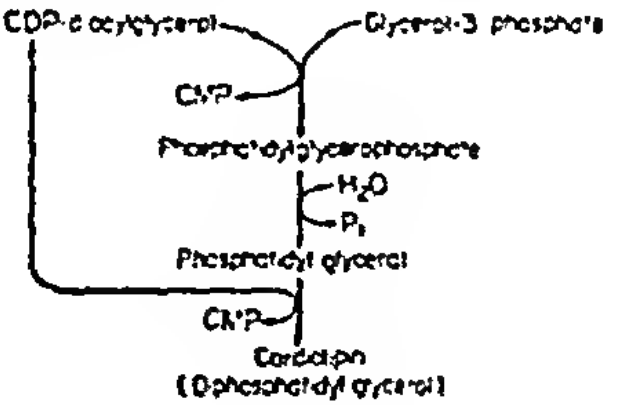


Fig. 17.8.

Degradation and turnover of phospholipids

Although phospholipids are actively degraded, each portion of the molecule turns over at a different rate The presence of enzyme allows partial degradation followed by resynthesis

Phospholipase A_2 catalyzes the hydrolysis of the ester bond in position 2 of glycerophospholipids to form lysophospholipids which in turn may be reacylated by acyl CoA in the presence of an acyltransferase *Phospholipase A_1* attacks the ester bond in position 1 of phospholipids *Phospholipase C* attacks the ester bond in position 3 releasing 1, 2-diacylglycerol and a phosphoryl base *Phospholipase D* is a plant enzyme which hydrolyzes the nitrogenous base from phospholipid

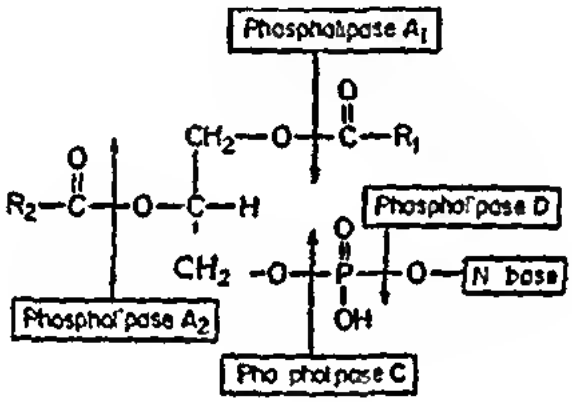


Fig 17.9

Physiological functions of phospholipids

1 Certain enzymes appear to require tightly bound phospholipids for their action
2 These are involved in blood coagulation
3 These help lipid absorption in intestine
4 These facilitate lipid transport between tissues
5 These assist in ion transport and secretion of hormones by cells
6 These help the oxidation of fatty acids

Synthesis of mono-unsaturated fatty acids

Liver is the main organ responsible for the interconversion with the saturated fatty acids An enzyme system catalyzes the conversion of stearyl CoA to oleonyl-CoA Oxygen, NADPH or NADH is necessary for the reaction. The

seqnence of reaction is given below It is specific for introducing a double bond in the Δ^9 position of saturated fatty acids, eg palmitic and stearic acid.

Stearyl CoA + Enzyme ⟶ Steary' Enz. + CoA

Stearyl Enz. + O_2 + NADPH$^+$ —[Hydroxylase]→ Hydroxystearyl Enz. + H_2O + NADP$^+$

Hydro vstearyl Enz. —[Hydratase]→ Oleyl Enz. + H_2O

Oleyl Enz. + CoA ⟶ Oleyl CoA + Enz.

Fig 17 10 Microsomal desaturase system

Synthesis of polyunsaturated fatty acids

Linoleic and linnlenic acid must be supplied in the diet to perform the synthesis nf the other members nf the ω-6 and ω-3 series of polyunsaturated fatty acids. Linoleate may be converted tn arachidonate. The pathway is first by dehydrogenation of the CoA ester through γ linoleate followed by the addition of a 2-carbon unit to give eicosatrienoate (homo γ linoleate) The latter forms arachidonate by a further dehydrogenation which is shown below

In the *fasting* state and in the absence of *insulin*, the desaturation and chain elongation system is greatly diminished.

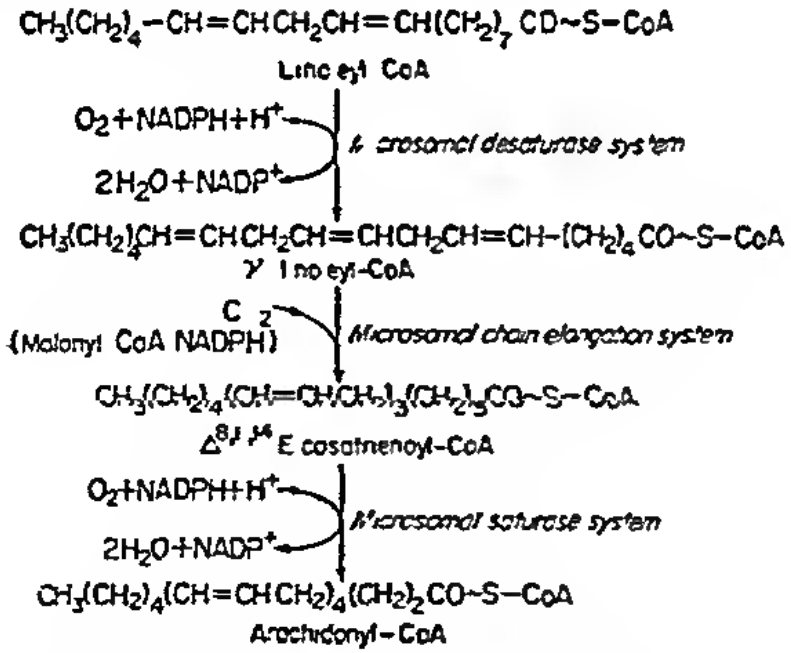


Fig 17 .1 Conversion of linoleate to arachidonate

PROSTAGLANDINS

Prostaglandins are a grnup nf naturally occurring substances synthesized primarily in the prnstrate They had been first isnlated from extracts of human seminal fluid and nf the vesicular gland of sheep They are widely distributed in mammalian tissues, e g , lung kidney, thyroid, spleen, brain iris, endometrium,

gastrointestinal mucosa, amniotic fluid and menstrual fluids The stimulation of adrenal glands and of nerves causes the liberation of prostaglandios in the circulation

Arachidonic acid and some related C_{20} fatty acids with methylene-interrupted bonds syntbesize a group of pbarmacologically aetive compounds known as prostaglandins

Chemistry :

1 Prostaglandins have a common structure based on prostanoic acid whlch contaios 20 carbon atoms

2 They are separated into four groups A, B, E and F depending on the variations in the double bonds and in the hydroxyl and ketone groups

3 The carbon chains are bonded at the middle of the chain by a 5 membered ring

4 A, B and E have an oxo grouping at position 9, whereas F has o hydroxyl group in tbis position

5 A hasa double bond between positions 10 and 11, whereas B has a double bond between positions 8 and 12 E and F do not have a double bond in the riog but possess a hydroxyl group at position 11

6 All active prostaglandins have at least one double bond between positions 13 ond 14 Some have two double boods, the second being between positions 5 ood 6 aod some prostaglandins have three double bonds, the additional bond being between positions 17 aod 18

7 The commoo siogle double bond has the trans eonfiguration, whereas tbe otber double bonds have the cis configuration

8 All prostaglandins have a hydroxyl group at position 15 and some have aoother hydroxyl group at position 19

9 Wben the 5 earbon ring bas two hydroxyl groups, their positions give rise to the possibility of α or β isomers

10 The *six primary prostaglandins* which occur in most cells, can be eonverted to tbe 8 secoodary prostaglandins that have been identified in natural materials

11 Tbe syntbesis of prostaglandins requires the consumptioa of 2 molecules of O_2 and 2 molecules of reduced glutathione The syntbesis is catalyzed by the prostaglandin synthetase complex Aspirin inhibits its synthesis

12 Fourteen prostaglandins bave been isolated from male reproductive tract. Tbese are PGA_1, PGA_2, PGB_1, PGB_2, PGE_1, PGE_2 PGE_3, $PGF_{1\alpha}$, $PGF_{2\alpha}$, $PGF_{3\alpha}$, 19 OH PGA_1, 19 OH PGA_2, 19 OH PGB_1, 19 OH PGB_2

[The number in subscript indicates the number of double bonds and the Greek letter tbe isomeric form]

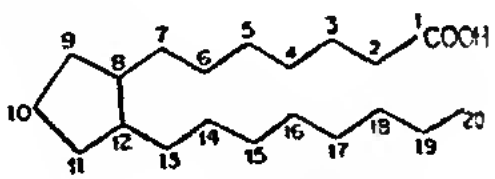


Fig 17 12 Prostanoic acid

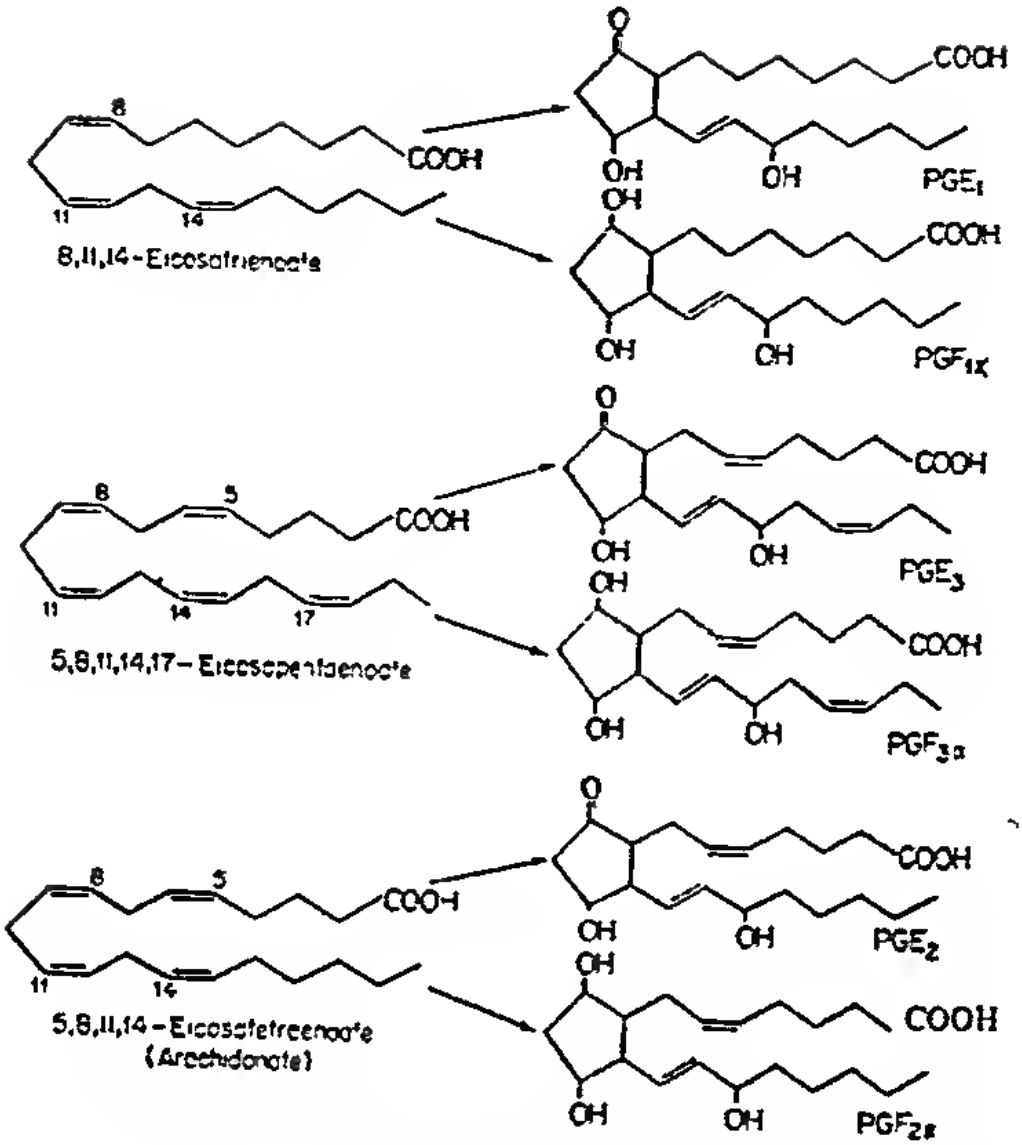


Fig 17.13 The six primary prostaglandins and their biosynthetic origin (PG, Prostaglandins)

Functions

1. Prostaglandins exhibit hormone-like activity. They are the most potent biologically active substance. As little as 1mg/ml causes contraction of smooth muscle in animal.

2. They increase cAMP in platelets, thyroid, corpus luteum, fetal bone, adenohypophysis and lung ; but lower cAMP in adipose tissue.

3. Although they are synthesized from the "essential fatty acids" they do not relieve symptoms of essential fatty acid deficiency, because they are too rapidly metabolized.

4. They promote the secretion of epinephrine and cause significant increase in the activity of phosphophosphorylase in liver. They produce a direct inhibitory effect on glycogen synthetase in liver.

5 They inhibit the formation of cAMP which is necessary for the activation of hormone sensitive lipase and thus decrease lipolysis adipose tissue Free fatty acids stimulate more prostaglandin secretion which ultimately inhibit lypolysis

6 They cause vasodilatation, decreased peripheral resistance, decreased blood pressure, enhanced capillary permeability

7 They inhibit gastric secretion,, increase intestinal motility and cause loose motion

8 They are involved in spermatogenesis, sperm maturat on and transport.

9 They are involved in the regulation of hypothalamic releasing factor release

10 Infusion of PGE_2 into the renal artery of dogs causes an increase in urinary volume and excretion of Na^+,K^+,Cl^-

Clinical importance :

1 Prostaglandins exert stimulatory effect on contractions of the human uterus PGE_1, PGE_2 $PGF_{2}\alpha$ are given intravenously to induce labor PGE_2 and $PGF_{2}\alpha$ are also effective orally to induce labor

2 They are also used as contraceptives to prevent conception

3 PGA_1 infused intravenously acts as a vasodilator and lowers the blood pressure

4 PGE_1 by inhalation produces improvement in asthmatic patients and becomes an inhibitor of gastric acidity when administered intravenously

5 PGA_1, PGE_1, PGE_2 and $PGE_{1}\alpha$ act as vasoconstrictor on the blood vessels of the nasal mucosa and PGE_1 is an effective nasal decongestant

6 Amniotic fluid during labor contains high concentration of $PGF_{2}\alpha$ which causes myometrial contractions $PGF_{2}\alpha$ is present in maternal venous blood immediately before uterine contractions in normal spontaneous labor The placenta is the major source of prostaglandins found in the amniotic fluid and maternal circulations

7 They are used to control inflammation

Side-effects :

1. PGE_2 causes uterine smooth muscle to contract when induction of labor is desired and also causes gastrointestinal smooth muscle to contract which leads to cramping and diarrhoea

2 PGE_2 irritates the mucosa lining of the throat causing pain and coughing

Metabolism

Prostaglandins are quickly metabolized by the enzyme *15-hydroxyprostaglandin dehydrogenase* which is present in most mammalian tissues This enzyme is blocked by the introduction of a methyl group at the C_{15} position Then only the half life of a prostaglandin is prolonged

Inhibitors :

Aspirin, indomethacin, phenazine, tranylcypromine

MOBILIZATION OF FAT FROM ADIPOSE TISSUE AND ITS METABOLISM

Adipose tissue was previously considered an inert storage depot for fat But according to recent investigations, this tissue is not static It performs important functions in the metabolism of lipids This tissue is under nervous control and conditions which cause sympathetic discharge result in the liberation of unesterified fatty acids and thus a loss of fat. Mobilization of fat is inhibited by denervation Different types of adipose tissue (e g white and brown) exhibit differences in metabolism

Both the glycolytic sequence of reactions and the pentose-shunt pathway are operative in adipose tissue Triacylglycerol is synthesized from acyl CoA and glycerol -3 phosphate in adipose tissue Since the activity of *glycerokinase* is low in adipose tissue, glycerol cannot be utilized to any great extent in the esterification of acyl CoA to form triacylglycerol For the provision of glycerol 3-phosphate, adipose tissue is dependent on a supply of glucose

Triacylglycerol is hydrolyzed by a *hormone-sensitive lipase* to form free fatty acids and glycerol But the tissue cannot utilize glycerol readily Therefore, glycerol diffuses out into the plasma and it is utilized by liver and kidney where active glycerokinase is present The free fatty acids can be resynthesized in adipose tissue to acyl CoA by *thiokinase* and re-esterified with glycerol 3 phosphate to form triacylglycerol Thus, there is a continued cycle of lipolysis and re-esterification within the adipose tissue When the rate of re-esterification is not sufficient to cope with the rate of lipolysis, free fatty acids accumulates and diffuses into the plasma Thus, the level of free fatty acid (FFA) raises in the plasma

In starvation or in diabetes mellitus, the availability of glucose in adipose tissue is reduced and less glycerol 3 phosphate is formed As a result, the rate of lipolysis exceeds the rate of esterification with the accumulation of free fatty acids which are released into the plasma

In adipose tissue glucose is oxidized to CO_2 via citric acid cycle, oxidized in the HMP shunt and converted to long chain fatty acids and can form acylglycerol via glycerol-3 phosphate When glucose utilization is reduced, the greater portion of the glucose is utilized to form glycerol 3-phosphate and acylglycerol

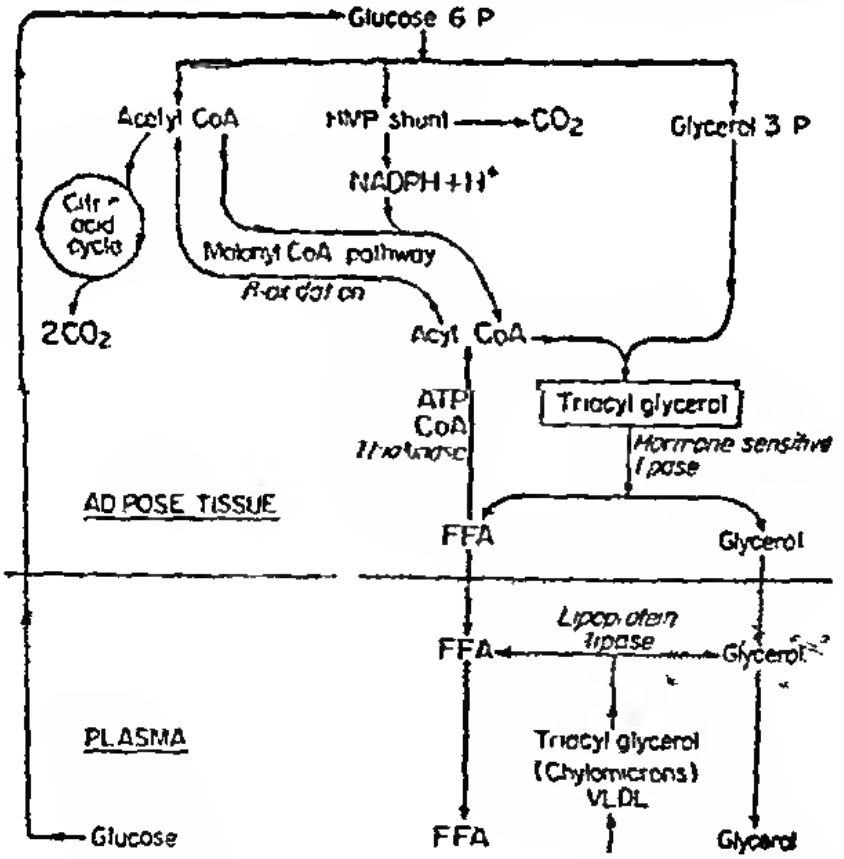


Fig 17 14 Metabolism and mobilization of fat in adipose tissue

Influence of hormones on adipose tissue

A Insulin :

1 It inhibits the release of free fatty acids from adipose tissue, enhances lipogenesis and the synthesis of acylglycerol and increases the oxidation of glucose to CO_2 via HMP shunt

2. It inhibits the activity of hormone-sensitive lipase and reduces the release of free fatty acids as well as glycerol

3 Insulin, nicotinic acid and prostaglandin E_1 inhibit the synthesis of cAMP depressing adenylate cyclase or stimulating phosphodiesterase Prostaglandins E_1 in low concentration causes the release of catecholamines resulting in the increase in free fatty acid mobilization

B ACTH, MSH, TSH, growth hormone, vasopressin, epinephrine, norepinephrine and glucagon .

1 These hormones accelerate the release of free fatty acids from adipose tissue by increasing the rate of lipolysis of the triacylglycerol stores

2 Many of these activate the hormone sensitive lipase and increase glucose utilization

3 Glucocorticoids and thyroid hormones do not increase lipolysis but the presence of these hormones in the lipolytic processes is essential Thyroid hormone inhibits phosphodiesterase activity The lipolytic effect of growth hormone in presence of glucocorticoids is slow

4 These hormones stimulate adenylate cyclase for the formation of cAMP from ATP and this cAMP stimulates the *protein kinase* which converts inactive hormone sensitive triacylglycerol lipase into the active lipase In addition to the hormone sensitive triacylglycerol lipase, the adipose tissue contains diacylglycerol and monoacylglycerol lipases which are more active than the hormone-sensitive triacylglycerol lipase and catalyze the rate limiting step in lipolysis.

Lipolysis is controlled by the amount of cAMP present in the tissue cAMP is degraded to 5′ AMP by the enzyme *cyclic 3 , 5 -nucleotide phosphodiesterase* This enzyme is inhibited by methyl xanthines such as caffeine and theophylline Therefore, drinking of coffee or the administration of caffeine causes the high elevation of plasma free fatty acids in humans.

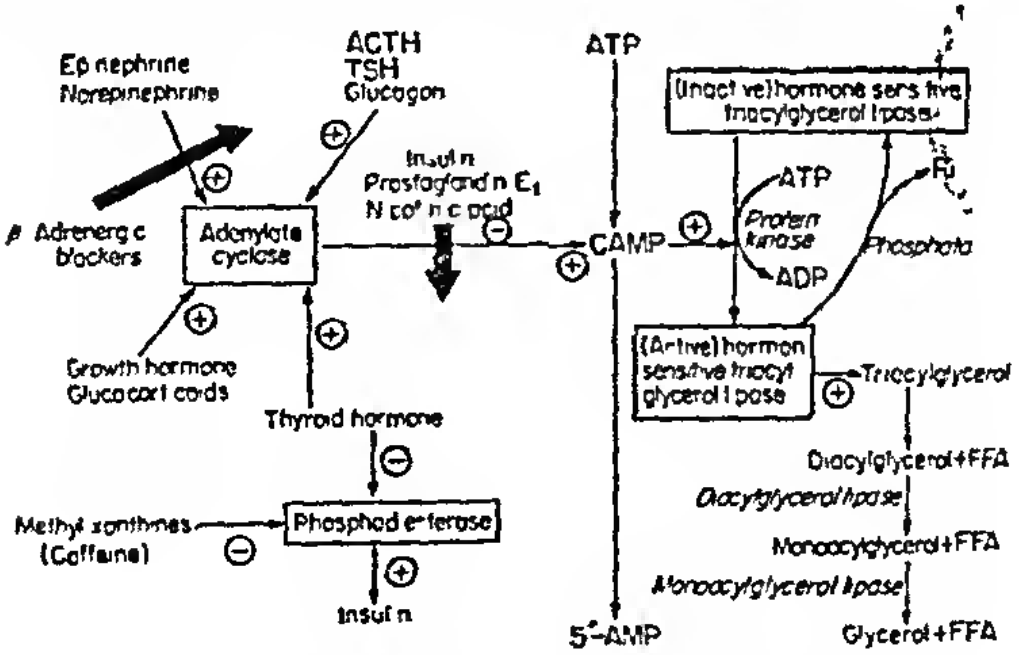


Fig 17 15 Influence of hormone on adipose tissue l polysis

The sympathetic nervous system, through liberation of norepinephrine, plays a central role in the mobilization of free fatty acids by exerting a tonic influence.

Role of brown adipose tissue :

1 The tissue is present in small amounts in newborn animal and in human infants around the neck. This is very active in newborn animals. It contains high content of mitochondria, cytochromes and a well-developed blood supply.

2 Its oxygen consumption is very high with the conversion of glucose and fatty acids to CO_2.

3 Lipolysis is active, but re-esterification takes place with glycerol because of the presence of significant amounts of *glycerokinase* in this tissue. *Norepinephrine* liberated from sympathetic nerve endings increases lipolysis in this tissue.

4 Oxidation produces much heat and little free energy is tapped in ATP.

5 Free glycerol from lipolysis is converted to glycerol 3 phosphate by glycerokinase and is oxidized directly in the tissue.

METABOLISM OF THE PLASMA LIPOPROTEINS

Five groups of lipoproteins present in plasma exhibit the important role in the transport and metabolism of lipids. These are the following :

1 Chylomicrons. Derived from intestinal absorption of triacylglycerol.

2. Very low density lipoproteins (VLDL or pre-β lipoproteins). Mainly derived from the liver for the export of triacylglycerol and also formed from dietary lipids.

3 Low density lipoproteins (LDL or β lipoproteins). These represent a final stage in the catabolism of VLDL and chylomicrons.

4 High density lipoproteins (HDL or α lipoproteins) : These are involved in VLDL, chylomicrons and cholesterol metabolism.

5 Free fatty acids. These are not classified with the other plasma lipoproteins as their structure is different. These consist of long chain fatty acids attached to serum albumin.

Apolipoproteins (Apoproteins) :

The lipoproteins contain one or more proteins or polypeptides known as apoprotein. The apoproteins in the lipoproteins are present as follows :

HDL — A I and A II, C I, C-II and C-III, arginine rich apoprotein
LDL — Apoprotein B
VLDL — Apoprotein B, C-I, C-II, C-III, arginine rich apoprotein
Chylomicrons — Apoprotein B, C-I, C II, C-III
Some lipoproteins are also glycoproteins.

Electrophoretic separation of lipoprotein and their normal concentration in human plasma :

1 α Lipoproteins. These occupy the α globulin region after electrophoresis. These contain 45% protein, 8% triacylglycerol, 20% cholesterol, 27% phospholipids.
Normal concentration — 300 mg%.

2 β Lipoproteins. These occupy the β globulin region after electrophoresis. They contain 46% cholesterol, 23% phospholipids, 10% triacylglycerol. Their concentration increases in atherosclerosis and coronary thrombosis.
Normal concentration — 300 mg%.

3 Pre-β lipoproteins. These occupy the region in between α and β-lipoproteins. These contain low protein, but contain 50% triacylglycerol, fair amo-

unt of cholesterol and phospholipids Their concentration is also increased in atherosclerosis and coronary thrombosis etc

Normal concentration 150 mg%

Formation of chylomicrons and VLDL

1 Both chylomicrons and VLDL are found in chyle formed by the lymphatic system draining the intestine VLDL is the vehicle of transport of triacylglycerol from the liver to the extrahepatic tissues

2 Apoprotein B which is essential for their formation is syothesized by ribosomes in the rough endoplasmic reticulum and is incorporated into lipopro teins in the smooth endoplasmic reticulum which is the main site of synthesis of triacylglycerol, phospholipids and cholesterol Carbohydrate is added to the lipoproteins found in the Golgi apparatus

3 Both are released from the intestine or hepatic cell by reverse pinocytosis

4 Chylomicrons pass into the spaces between the intestinal cells making their way into the lacteals draining the intestine VLDL are secreted by hepatic parenchymal cells into the space of Disse and then into the hepatic sinusoids

Catabolism of chylomicrons and VLDL

A Role of lipoprotein lipase (clearing factor lipase)

1 It is present in the walls of blood capillaries and also found in the extracts of heart, lung, spleen, lactating mammary gland

2 Its concentration in the normal blood is less It is released from the tissues into the circulation following injection of heparin

3 Phospholipids and apoprotein C-II are required as cofactors for its activity

4 It hydrolyzes triacylglycerol to monoacylglycerol through diacylglycerol The monoacylglycerol is finally hydrolyzed by monoacylglycerol hydrolase

B Role of the liver

Chylomicron remnants (about half the diameter of parent chylomicrons) are taken by the liver in vivo and by the perfused liver, in which the cholesteryl esters are hydrolyzed and the triacylglycerol fatty acids are metabolized

Metabolism of LDL

1 It is formed from VLDL and chylomicrons

2 It is removed from the circulation by the liver The half time of disappearance of apoprotein B in LDL from the circulation is about 2½ days

3 Fibroblasts and lymphocytes may degrade LDL in extrahepatic tissues

Metabolism of HDL

1 It is syothesized and secreted from liver and intestine

2 Nascent HDL from intestine does not contain apoprotein C but only apoprotein A Nascent HDL formed by the liver contains apoprotein and free cholesterol These lipoproteins are similar to the particles found in the plasma of patients with a deficiency of the plasma enzyme lecithin Cholesterol acyl transferase (LCAT) and in the plasma of patients with obstructive jaundice

3 The liver and the intestine are the final sites of degradation of HDL apoproteins

Plasma lipoprotein abnormalities :

1 In cases of abnormal hyperlipemia, the concentration of serum VLDL is increased and the concentration of serum HDL may be decreased, increased or normal

2 In cases of hyperlipemia with marked hypercholesterolemia, serum LDL is increased.

3 Serum LDL has been found to be increased in diabetes mellitus, hypothyroidism, obstructive jaundice, the nephrotic syndrome and in glycogen storage diseases

4 The concentration of serum LDL and of total serum cholesterol is significantly increased in *atherosclerosis*

5 Studies of serum lipoproteins are still of limited clinical value in myocardial infarction, cerebral thrombosis etc

6 LDL and also VLDL are possible risk factors in studies related to increased susceptibility to ischemic heart disease

Role of liver in lipid metabolism :

1 The liver has active enzyme systems for synthesizing triacylglycerols, phospholipids, cholesterol, plasma lipoproteins and for converting fatty acids to ketone bodies.

2 The fatty acids used in the synthesis of liver triacylglycerol are derived from two sources (a) from acetyl CoA derived from carbohydrate, (b) uptake of free fatty acids from the circulation

3 It is the site for the synthesis of bile acids from cholesterol

4 It is the major site for the oxidation of fatty acids

5 Feeding of diets high in carbohydrate containing sucrose or fructose, high levels of circulating free fatty acids, ingestion of ethanol and the presence of high level of insulin enhance the synthesis of triacylglycerol and the secretion of VLDL by the liver

6 It has the enzyme systems for lengthening and shortening of fatty acids and for saturating and desaturating fatty acids

7 This organ is chiefly concerned in removal of phospholipids, cholesterol and lipoproteins from the plasma

8 Hepatic glucokinase increases with the availability of carbohydrate in the diet This increases glucose incorporation into the liver and hence glycolysis and fatty acid synthesis The fatty acids are carried to the adipose tissue as triacyl glycerol in VLDL

Starvation diminishes glucokinase and leads to diminished fatty acid synthesis in the liver The hypoglycemia stimulates growth hormone production which in turn stimulates lipolysis The free fatty acids so liberated from adipose tissue can influence carbohydrate metabolism in the liver where they are broken down with the formation of acetyl CoA The lipolytic effect of growth hormone is also necessary for its protein anabolic action

Fatty liver and Lipotropic factors

Lipid (mainly as triacylglycerol) can accumulate in the liver for the following reasons causing fatty liver .

1 The increased levels of plasma free fatty acids resulting from mobilization of fat from adipose tissue

2. The hydrolysis of lipoprotein or chylomicron triacylglycerol by lipoprotein lipase in extrahepatic tissues

3 Increasing amounts of free fatty acids are taken up by the liver and esterified

4 The production of plasma lipoprotein does not keep pace with the influx of free fatty acids allowing triacylglycerol to accumulate

5 During starvation and the feeding of high fat diets, the quantity of triacylglycerol present in the liver is significantly increased

6 Uncontrolled diabetes mellitus and toxaemia of pregnancy cause fatty appearance and enlargement of the liver

7 The metabolic block in the synthesis of lipoproteins from lipid and apoprotein

8 The deficiency of *lipotropic factor* causes triacylglycerol to accumulate even though only a normal rate of fatty acid synthesis and uptake of free fatty acids take place

9 Carbon tetrachloride, chloroform, phospborus, lead, arsenic and ethionine (α amino γ-ethylmercaptobutyric acid) cause fatty liver These substances inhibit hepatic protein synthesis Orotic acid blocks apo B synthesis

10 Alcoholism also leads to fat accumulation in the liver, hyperlipidemia and ultimately cirrhosis

11 Protein deficiency, essential fatty acid and vitamin deficiencies (e g vitamin E, pyridoxine, pantothenic acid) cause fatty liver The deficiency of essential fatty acids depresses the synthesis of phospholipids and therefore cholesterol is involved in esterification causing fatty livers

Lipotrople factor

The substances that preveot the accumulation of fat in the liver are known as lipotropic factor The phenomenon is said to be lipotropism

Choline, methionine and betaine and β propiothetin act as lipotropic agents in curing fatty livers due to choline deficiency Diets poor in protein (containing methionine) or lecithin (containing choline) tend to favour the production of fatty liver Choline is synthesized using labile methyl groups donated by methionine in the process of transmethylation Vitamin B_{12} and folic acid which are important in hematopoiesis are also able to produce lipotropic effect Vitamin B_{12} is concerned in the biosynthesis of labile methyl groups and folic acid in transmethylation reactions Inositol exerts a limited lipotropic effect in fat free diets Casein nnd certain other proteins possess lipotropic activity

KETOSIS

The fatty acids undergo excessive oxidation in the *liver* under certain metabolic conditions producing large quantities of keto acids—acetoacetic acid and β hydroxybutyric acid, which pass into the blood by diffusion Acetoacetic acid then undergoes spontaneous decarboxylation to produce acetone These three substances—acetoacetate, β hydroxybutyrate and acetone—are collectively known as the *ketone bodies* (acetone bodies or ketones)

Normally, the blood of mammals contains ketone bodies not exceeding 1 mg / 100 ml. The concentration is little higher than this in ruminants Daily excretion of ketone bodies of normal person is less than 1 mg Higher than normal quantities in the blood or urine constitute *ketonemia* (hyperketonemia) or *ketonuria* respectively The condition in which there is a high concentration of ketone bodies in tissues and blood is called *ketosis*

Acetoacetic acid and β hydroxybutyric acid are moderately strong acids and are buffered in blood or tissues Their excretion in large quantities admits some loss of buffer cation (in spite of ammonia production by the kidney) which depletes the alkali reserve causing *ketoacidosis*

The process of formation of ketone bodies is termed *ketogenesis* and the process of breakdown of ketone bodies taking place in peripheral tissues is called *ketolysis*

Ketogenesis in the liver

Ketosis generally occurs in severe diabetes mellitus, prolonged starvation, glycogen storage diseases, Toxemia of pregnancy, infective hepatic disease and continued fever Experimentally, it occurs in the oral administration of fatty acids, high fat diet, low carbohydrate diet, pancreatectomy, administration of growth hormone or ACTH Under these conditions, there is diminished carbohydrate utilization and increased fat mobilization

In ruminants, the rumen converts butyric acid formed from fermentation to β-hydroxybutyrate which enters the blood stream The ruminant lactating mammary gland also produces ketone bodies But these ketone bodies do not cause ketosis in these species

Ketone bodies are formed in the liver but utilized in the extrahepatic tissue. Enzymes responsible for ketone body formation are associated mainly in the *mitochondria*

Acetyl-CoA (C_2 units) formed in β-oxidation of fatty acids is the basic unit for the formation of ketone bodies Two molecules of acetyl-CoA condense to form acetoacetyl CoA by a reversal of thiolase reaction Two pathways have been proposed for the formation of acetoacetate from acetoacetyl CoA

1. **First pathway (Minor pathway)**

The first pathway is by simple deacylation catalyzed by the enzyme *acetoacetyl CoA deacylase* which is shown below .

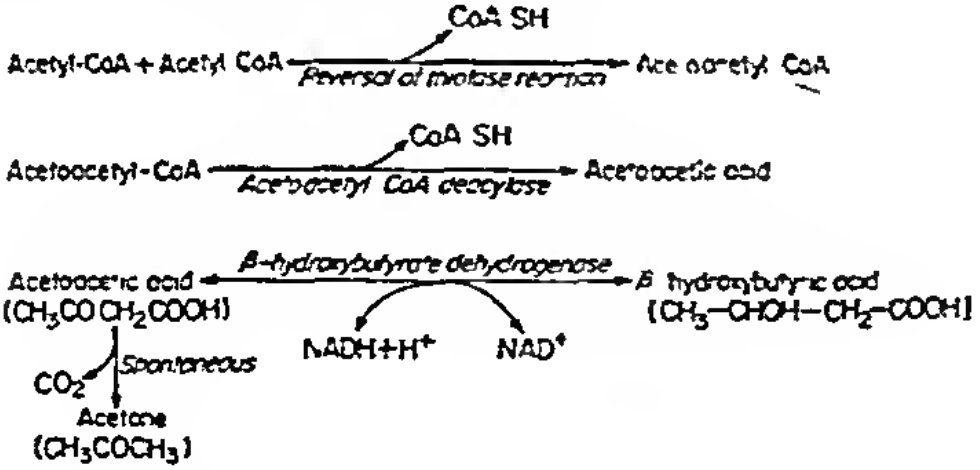


Fig. 17 16 Minor pathway of ketogenesis in liver

2. **The second pathway (The major route)** -

It involves the condensation of acetoacetyl-CoA with another molecule of acetyl-CoA to form β-hydroxy-β-methyl glutaryl CoA by HMG-CoA synthetase. HMG-CoA is splitted into acetoacetic acid and acetyl-CoA by *HMG-CoA lyase*

present in mitochondria From acetoacetic acid, acetone and β-hydroxybutyrate are\formed which are shown below :

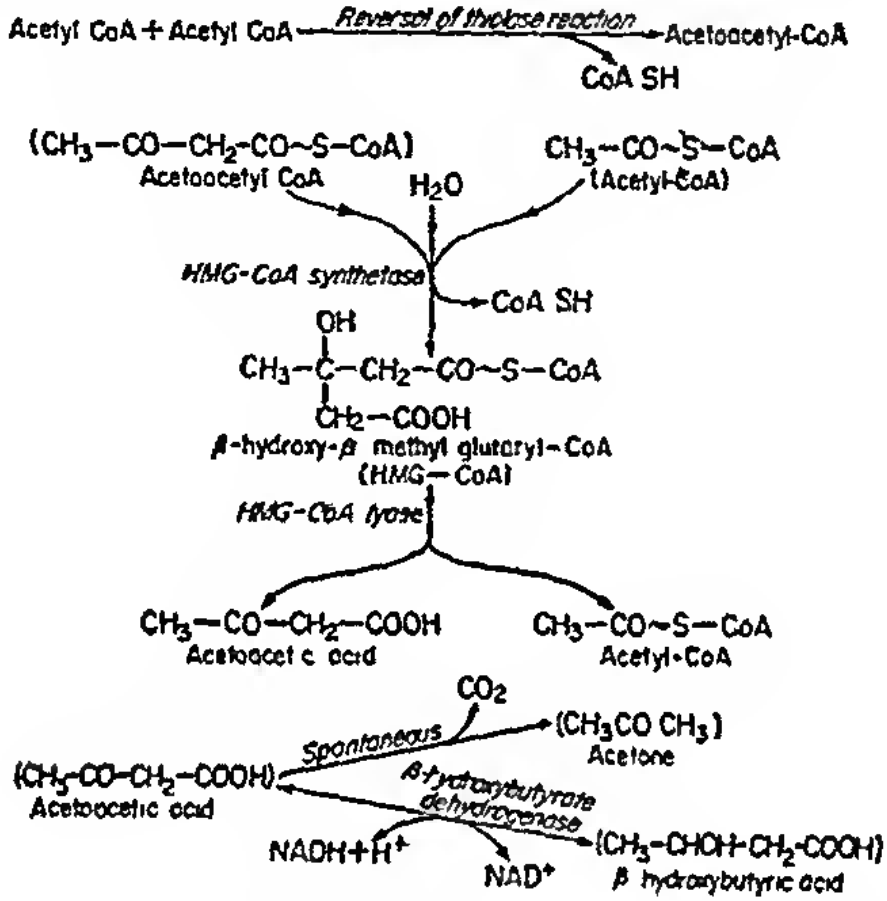


Fig 17 17 Major pathway for ketogenesis in liver

Metabolism of ketone bodies or utilization of ketone bodies in the extrahepatic tissues

Acetoacetic acid and β hydroxybutyric acids are carried from liver to extrahepatic tissues mainly kidney and muscle where they are oxidized for energy production after conversion to acetyl CoA The enzyme responsible for the activation of acetoacetate to acetoacetyl CoA is absent from liver for which liver cannot utilize these acids Two reactions take place in extrahepatic tissues for the activation of acetoacetate to acetoacetyl CoA

In the first reaction, succinyl CoA reacts with acetoacetic acid in presence of the enzyme *acetoacetate succinyl CoA transferase* (Thiophorase) to form acetoacetyl CoA and succinate

In the second reaction, acetoacetate is activated by ATP in presence of CoA catalyzed by *acetoacetic thiokinase* to form acetoacetyl CoA

Alternatively, β hydroxybutyric acid is activated directly by *thiokinase* in extrahepatic tissues to form acetoacetic acid which is then converted to acetoacetyl CoA by any of these above two reactions

The acetoacetyl CoA formed by these reactions is split to acetyl CoA by *thiolase* and oxidized in the citric acid cycle The reactions are given in Fig 16 18

Acetoacetate and β hydroxybutyrate are readily oxidized by extrahepatic tissues but acetone is oxidized with difficulty and its rate of utilization is also very slow Several pathways have been proposed for the utilization of acetone

1. First pathway :
   Acetone is converted to acetoacetate by reversal of decarboxylation.
2. *Second pathway :*
   Acetone is converted to propanediol which can form 1 carbon (formate) unit and 2 carbon (acetate) unit.

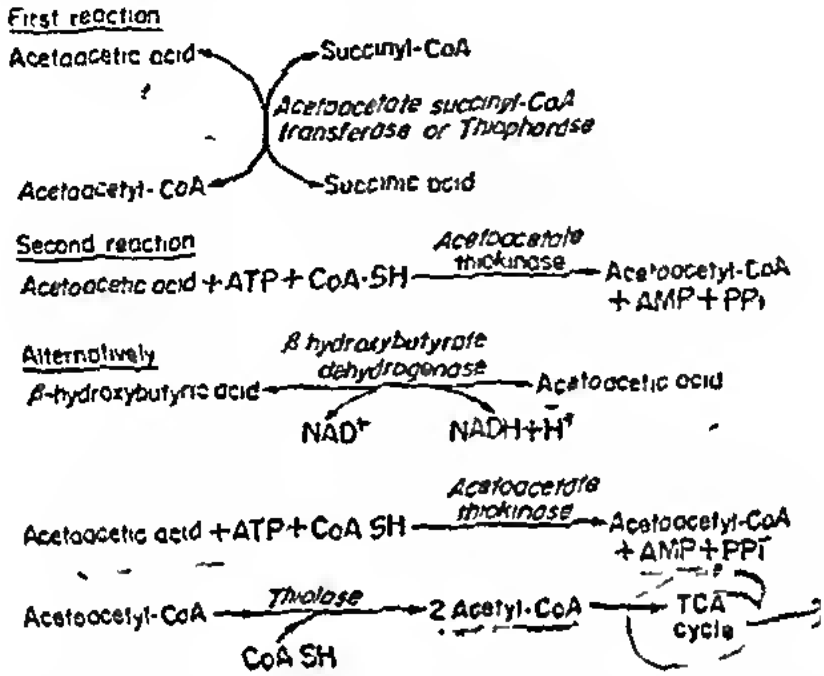


Fig. 17 18 Conversion of acetoacetate to acetoacetyl-CoA and further oxidation.

Most of the evidence suggests that ketonemia is due to increased production of ketone bodies by the liver rather than to a deficiency in their utilization by extra-hepatic tissues. In moderate ketonemia, the loss of ketone bodies through the

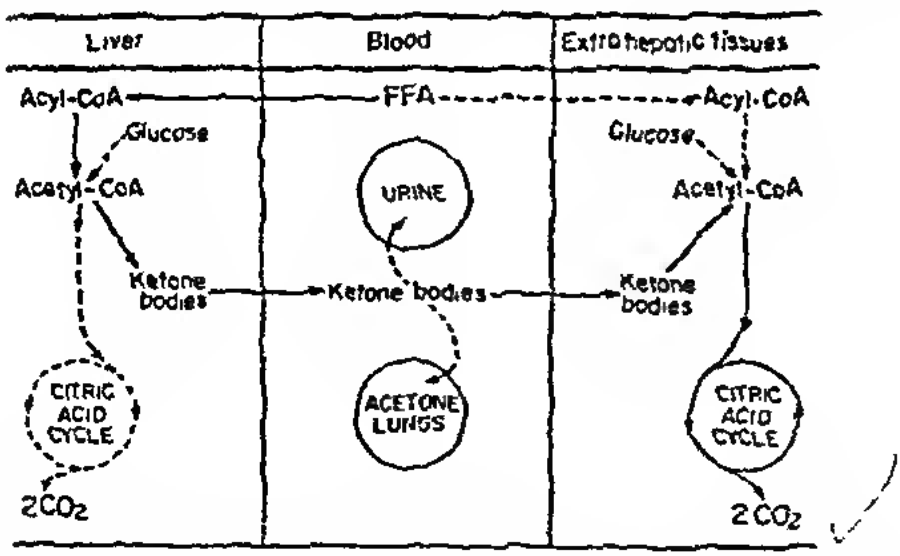


Fig 17 19 Formation, utilization and excretion of ketone bodies. (The main pathway is indicated by the heavier arrows)

urine is only a few per cent of the total ketone body production and utilization The formation, utilization and excretion of ketone bodies are mentioned in fig 16 19

Affects of ketosis

1 Both acetoacetate and β-hydroxybutyrate are moderately strong acids They neutralize bicarbonates resulting depletion of alkali of the body and produce metabolic acidosis In case of severe ketosis death may ensure from acidosis

2. The excretion of ketone body in the urine involves the loss of Na^+ in particular leading to total electrolyte and Na^+ deficiency

3 The severe diabetic patient excretes large quantities of both ketone bodies and glucose in the urine with a large quantity of water developing dehydration In diabetic acidosis, there is severe alteration in cation—anion balance in the plasma

Prevention of ketosis .

1. In case of diabetic ketosis carbohydrate diet, intramuscular injection of insulin and antiketogenic substance (aspartic acid) which may provide oxaloacetate by transamination should be administered

2 In case of prolonged starvation ketosis, carbohydrate diet and antiketogenic substance (aspartic acid) which may provide oxaloacetate by transamination should be given

3 The electrolytes and the fluids of the body must be restored by intravenous injection of isotonic solution of sodium salts such as NaCl, $NaHCO_3$ or sodium lactate Potassium salts are desired to be added

Test for ketone bodies in the urine

Rothera's test

1 5 ml of the urine is saturated with solid ammonium sulphate by shaking it vigorously

2 2 drops of freshly prepared 5 per cent solution of sodium nitroprusside and 1 ml of ammonium hydroxide are added

3 Allowed to stand for a while

A permanganate colour which appears just above the layer of the undissolved ammonium sulphate indicates the presence of ketone bodies

In normal individuals, the ketone bodies are excreted less in quantities in the urine This negligible amount does not respond to rothera's test Hence, the ketone bodies in the normal urine are not detected by this test

CHOLESTEROL METABOLISM

Occurrence :

Human body contains large quantities of cholesterol which are found in brain and nervous tissues Other tissues such as liver, kidney, spleen and skin also contain fairly good amounts of cholesterol The total amount of cholesterol is about 140 grams in the body of man weighing 70 kg The greatet part of the cholesterol of the body is synthesized (about 1 gram per day) whereas about 0 3 gram per day are provided by the average diet It is not synthesized in plants Dietary cholesterol is obtained only from animal sources like meat, liver, brain and egg yolk (a particularly rich source)

Chemistry :

1. Cholesterol is a white, waxy, solid found associated with fats but chemically different from them

2 It has a parent nucleus which is said to be cyclopentanoperhydrophenanthrene nucleus

3 It has a hydroxyl group at C_3, an unsaturated bond at C_5—C_6 two methyl groups at C_{10} and C_{13} and 8 carbon paraffin side-chain attached to C_{17}

4 It is an alcohol

5. It occurs free and combined with fatty acids by ester linkage at the hydroxyl group

6. Cholesterol in ester form is often referred to as "bound" cholesterol esters. These are normally rich in linoleic acid.

7 Steroids closely related to cholesterol include 7-dehydrocholesterol which occurs in skin and can be converted by ultraviolet radiation to vitamin D_3, dihydrocholesterol, bile acids, hormones of the adrenal cortex and of the sex glands

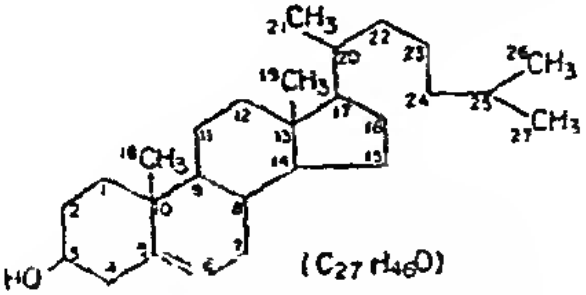


Fig 17 20 Structure of cholesterol
Structural relation of cholesterol to vitamin D and bile acids

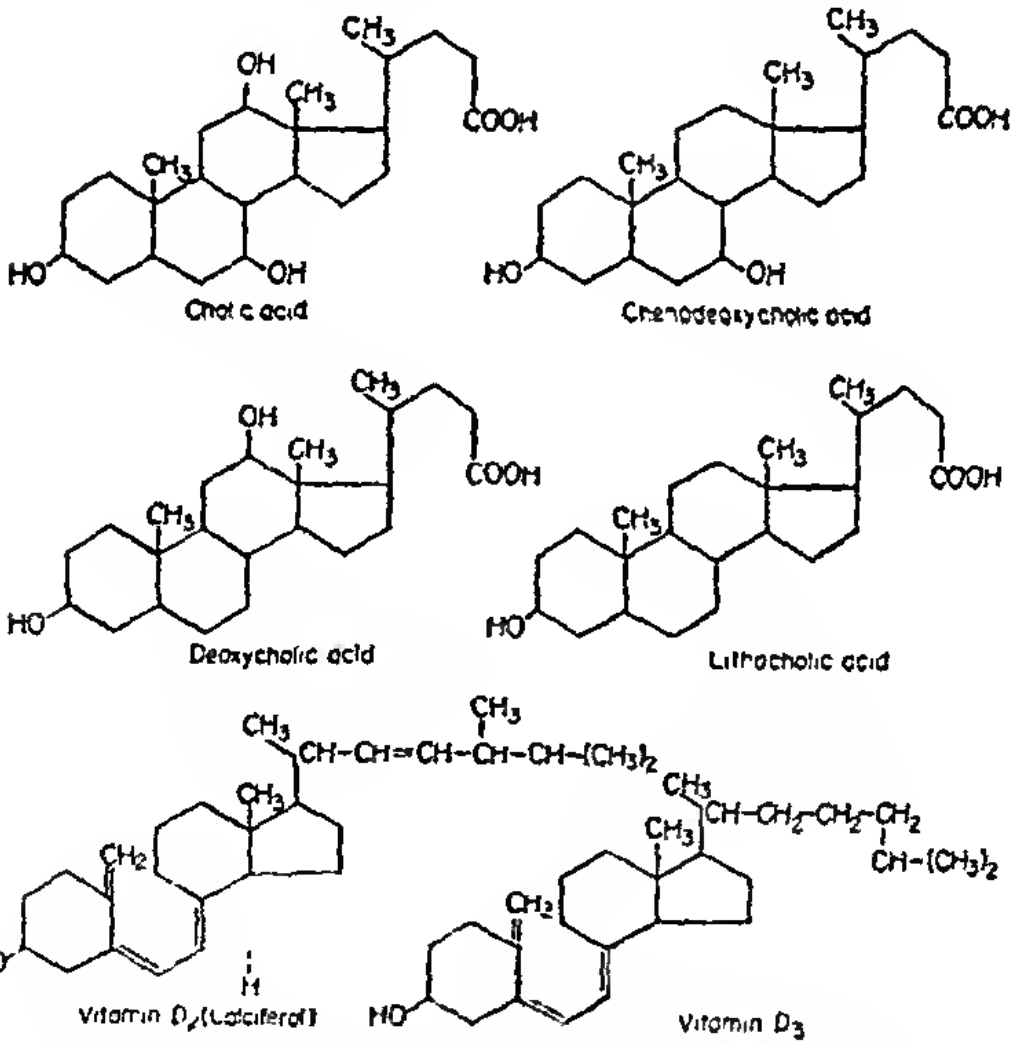


Fig 17 21 Structural relation of cholesterol to vitamin D and bile acids

Absorption :

1 A great part of the ingested cholesterol is absorbed Plant sterols are not absorbed

2 On a high cholesterol diet, man absorbs a maximum of only 15 mg of cholesterol per kg body weight per day

3 The cholesterol absorbed regulates the endogenous production of cholesterol which is about 14 mg per kg body weight per day

4 Bile is essential for its absorption

5 Pancreatic juice plays an important role because *cholesterol esterase* present in it hydrolyzes the esters of cholesterol present in the diet After absorption it is again esterified in the cell and then released for traosport via the lymphatic to the thoracic duct

Normal concentration of cholesterol in blood

140-250 mg per 100 ml of blood It increases with ages and also during pregnancy

Physiological Importance of cholesterol in the body

1 It is the essential constituent of cells

2 It aids in the permeability of the cells

3 It controls the red cells from being easily hemolyzed

4 It functions as the defensive action

5 It transports fat to liver in the form of cholesterol ester for oxidation

6 It assists the formation of bile acids and bile salts, 7 dehydrocholesterol and vitamin D_3, corticosteroid hormones androgens (*male sex hormones*) estrogens and progesterone (*female sex hormones*)

7 It helps the granulation of cell division

8 It acts as an antagonist to phospholipid

Factors affecting cholesterol level in blood

1 *Dietary fat* Fats (butter fat, hydrogenated fat) containing higher saturated fatty acids cause increased serum cholesterol But fats (corn oil, sun flower seed oil, safflower seed oil), fish oil cotton seed oil) rich in polyunsaturated fatty acids cause marked reduction in serum cholesterol level

2 *Dietary cholesterol* It tends to increase the serum cholesterol level

3 *Dietary carbohydrates* Consumption of excessive amounts of sucrose causes an increase in serum cholesterol level

4 *Heredity* Persons who are prone to become obese have a high level The level becomes slightly higher in persons belonging to blood group A and AB

5 *Caloric Intake* Intake of excess calories causes a significant increase in plasma cholesterol level

6 *Proteins* Increase in protein intake does not change the plasma cholesterol level but low protein intake causes reduction in plasma cholesterol level

7 *Vitamin B complex* Nicotinic acids in large amounts causes lowering of plasma cholesterol level whereas pyridoxine deficiency causes increased blood cholesterol level

8. *Minerals* Magnesium salts do not bring about any change. The conversion of acetate to cholesterol is depressed by iron salts and increased by manganese salt.

9 *Physical exercise* Physical exercise brings about a lowering in the serum cholesterol level.

10 *Fibre* Increasing the fibre content of the diet, causes the excretion of cholesterol and bile acids in the feces and brings about a significant reduction in serum cholesterol level on a high fat-high cholesterol diet.

Excretion of cholesterol

1 Half of the cholesterol eliminated from the body is excreted in the feces after conversion to bile salts

2 Coprostanol is the principal sterol in the feces which is formed from cholesterol in the lower intestine by the bacterial flora.

3 A large portion of the biliary excretion of bile salts undergoes enterohepatic circulation The bile salts not reabsorbed or their derivatives are excreted in the feces

Transport of cholesterol

1 Cholesterol in the diet after absorption from the intestine is incorporated into chylomicrons and VLDL being accompanied by other lipids

2. The greater part of cholesterol is found in the esterified form. It is transported as lipoprotein in the plasma The highest proportion is found in the LDL.

3 Some plasma cholesteryl ester may be formed in HDL as a result of the transesterification reaction in plasma by lecithin Cholesterol acyltransferase (LCAT)

4 Patients with parenchymal liver disease show a decrease of lecithin Cholesterol acyl transferase activity and abnormalities in the serum lipids and lipoproteins.

Biosynthesis of cholesterol

Liver is the principal organ for its synthesis. Other tissues such as adrenal cortex, intestine, skin, ovary kidney, testis also can synthesize cholesterol. The *microsomal* and *cytosol* fraction of the cell is responsible for cholesterol synthesis. It is interesting to note that the brain of the newborn can synthesize cholesterol while the adult brain cannot synthesize cholesterol.

Acetyl-CoA formed from fatty acid oxidation or oxidation of carbohydrate act as direct precursor of cholesterol. Synthesis of cholesterol takes place in several stages. The first is the synthesis of mevalonate The next major stage is the formation of isoprenoid units from mevalonate by loss of CO_2. The isoprenoid units are regarded as the building blocks of the steroid skeleton Six of these units condense to form squalene which gives rise to the parent steroid lanosterol Cholesterol is formed from lanosterol with the loss of 3 methyl groups.

The pathway through HMG CoA is more significant than the pathway through β-hydroxy β-methyl glutaryl-CoA S-enzyme complex. Cholesterol synthesis is extramitochondrial There are two HMG-CoA pools One in mitochondria is concerned with ketogenesis The other extramitochondrial pool is involved in the synthesis of isoprenoid units and cholesterol The pathway may

not involve formation of malonyl CoA because avidin does not inhibit the production of mevalonate from acetyl-CoA

HMG CoA is converted to mevalonate in a 2 stage reduction by NADPH catalyzed by *HMG-CoA reductase*

Mevalonate is phosphorylated by ATP to form several active phosphorylated intermediates By decarboxylation isopentenylpyrophosphate is formed This after condensation forms farnesyl pyrophosphate Two molecules of farnesyl pyrophosphate condense and follow reduction with NADPH to form squalene.

Squalene is converted to lanosterol by ring closures Before closures the methyl group of C_3 is hydroxylated This involves molecular oxygen and the reaction is catalyzed by a microsomal hydroxylase system

The formation of cholesterol from lanosterol involves changes to the steroid nucleus and side chain

The intermediates from squalene to cholesterol may be attached to a special carrier protein known as the *squalene and sterol carrier protein* This protein binds sterols and other insoluble lipids Cholesterol may affect the activity of HMG-CoA reductase as cholesterol-sterol carrier protein

The activity of HMG CoA reductase is decreased during fasting The activity is not reduced in diabetes mellitus But the activity of HMG-CoA reductase is inhibited by cholesterol feeding

Administration of insulin or thyroid hormone increases the activity of HMG-CoA reductase, whereas the administration of glucagon and glucocorticoids reduces the activity of the enzyme

More recent experiments have shown that cholesterol synthesis is inhibited by cAMP, indicating that one or more reactions in the synthetic pathway may be controlled by a cAMP-dependent protein kinase Plasma cholesterol in humans is made lower by reducing the amount of cholesterol in the diet An increase of 100 mg in dietary cholesterol causes a rise of 5 mg cholesterol per 100 ml of serum

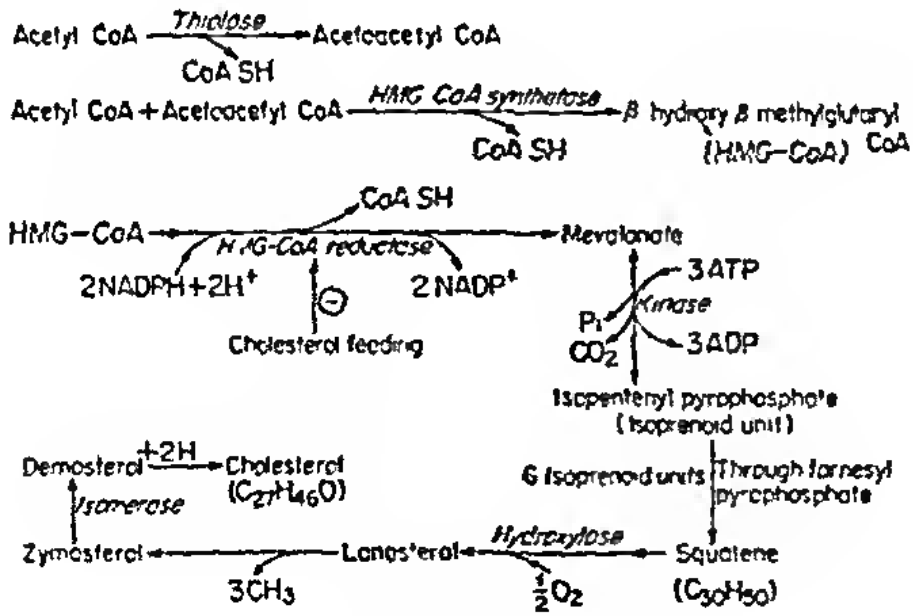


Fig 17 72 Biosynthesis of cholesterol

Hypercholesterolemia has been observed in uncontrolled diabetes mellitus, impairment of liver, obstructive jaundice, glomerulonephritis, hypothyroidism and nephrosis.

Hypocholesterolemia has been found in anemia, hyperthyroidism, hepatic diseases, infection, carcinoma, and acute pancreatitis.

Atherosclerosis is characterized by the deposition of cholesteryl ester and other lipids in the connective tissue of the arterial walls. Diseases in which prolonged elevated levels of LDL and VLDL occur in the blood are accompanied by severe atherosclerosis. Factors play a part in atherosclerosis include high blood pressure, obesity, lack of exercise. Rise in plasma free fatty acids also leads to increased VLDL secretion by the liver causing extra triacylglycerol and cholesterol output into the circulation. Factors leading to higher levels of free fatty acids include emotional stress, nicotine from cigarette smoking, coffee drinking and partaking of few large meals rather than more continuous feeding.

Polyunsaturated fatty acids can lower cholesterol level. Because the cholesteryl esters of polyunsaturated fatty acids are more rapidly metabolized by the liver and other tissues which may enhance their rate of turnover and excretion. Recently, it has been found that saturated fatty acids cause higher rates of secretion of VLDL by the perfused liver than do unsaturated free fatty acids. Cholesterol level is also decreased on the minimum intake of animal fat.

Affect of drugs :

1. Sitosterol is hypercholesterolemic agent which blocks the esterification of cholesterol in the gastrointestinal tract and thus reduces cholesterol absorption.
2. Drugs like choloxin and neomycin cause the increased fecal excretion of cholesterol and bile acids.
3. Clofibrate acts by inhibiting the secretion of VLDL by the liver or by inhibiting hepatic cholesterol synthesis.
4. The hypocholesterolemic drugs include nicotinic acid and estrogens.

Tests for cholesterol

1. *Salkowski's test* : A little cholesterol is dissolved in 2 ml of chloroform. An equal volume of conc. H_2SO_4 added to it. Shaked gently, upper layer of chloroform turns red and the sulphuric acid layer assumes a yellow colour with green fluorescence.
2. *Libermann-Burchard reaction* : A crystal of cholesterol is dissolved in 2 ml of chloroform in a dry test tube. 10 drops of acetic anhydride and 2 drops conc. H_2SO_4 are added. Mixed well. A red rose colour develops which quickly changes through blue to green.

ESSENTIAL FATTY ACIDS (EFA)

In 1928, Evans and Burr observed that rats fed on a purified nonlipid diet exhibited a reduced growth rate and a reproductive deficiency. The deficiency symptom was cured by the addition of essential fatty acids. Further symptoms due to EFA were scaly skin, necrosis of the tail and lesions in the urinary system. The essential fatty acids are linoleic acid, linolenic acid and arachidonic acid.

Chemistry :

Linoleic acid and linolenic acid contain 18 carbon atoms each and arachidonic acid contains 20 carbon atoms. Lenoleic acid, linolenic acid and arachidonic acid have 2, 3 and 4 double bonds respectively in their structure.

Their structures are given below

$CH_3(CH_2)_4$ $CH{=}CH\ CH_2\ CH{=}CH(CH_2)_7\ COOH$

Linoleic acid (18 2)

$CH_3\ CH_2\ CH{=}CH\ CH_2CH{=}CH\ CH_2CH{=}CH(CH_2)_7\ COOH$

Linolenic acid (18 · 3)

$CH_3\ (CH_2)_4(CH{=}CH\ CH_2)_4(CH_2)_2\ COOH$

Arachidonic acid (20 · 4)

Fig 17 23 Structure of Essential fatty acids

Chemical properties

1 The essential fatty acids of vegetable oils have low melting point and low iodine number

2 These essential fatty acids become saturated on hydrogenation and the oils are converted to solid fats

Sources :

1 Linoleic acid occurs in high concentrations in various edible vegetable oils e g corn, cotton seed, peanut, safflower

2 Arachidonic acid occurs in animal fats although in small amounts

Synthesis ·

Lenoleic acid cannot he synthesized by animals and therefore must he supplied preformed in the diet Arachidonic acid can be formed from linoleic acid in the animal hody

The pathway is first hy dehydrogenation of the CoA ester through γ-linolenate followed hy the addition of a 2 carhon unit (as acetyl-CoA in the mitochondrial system for chain elongation or as malonyl CoA in the microsomal system, which is the more active system) to give eicosatrienoate (homo γ-linolenate) which further forms arachidonate by dehydrogenation Therefore, the nutritional requirement of arachidonate is compensated by the linoleate in the diet The synthesis is given below

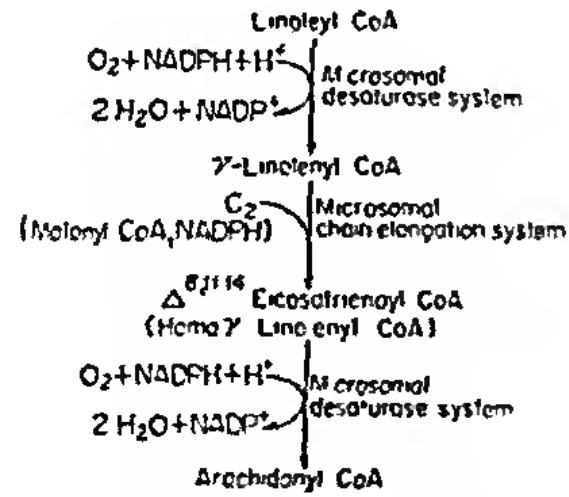


Fig 17.24 Synthesis of essential fatty acids

CHAPTER 18

REGULATION OF CARBOHYDRATE AND LIPID METABOLISM

Regulation of carbobydrate metabolism at the cellular and level:

1 The changes in the metabolism fully depend on the changes in bility of substrates The concentration of glucose, fatty acids and amino in blood influences their rate and pattern of metabolism in many tissues.

2 Alterations in the concentrations of glucose, fatty acids and in the blood owing to the changes in the dietary availability may ... secretion of hormones that influence the pattern of metabolism in ways,

3 Three types of mechanisms are responsible for regulating the enzymes concerned in carbohydrate metabolism

(i) Changes in the rate of enzyme synthesis

(ii) Conversion of an inactive to an active enzyme.

(iii) Allosteric effects

Regulation of Glycolysis, Gluconeogenesis and Hexose Shunt :

1 *Glucokinase* catalyzes the conversion of glucose to glucose-6-phosphate In the same extramitochondrial region *glucose-6-phosphatase* is also found which catalyses the same interconversion in the reverse direction on the supply of various carbohydrate, glucokinase activity is increased whereas glucose-6-phosphatase activity is decreased In starvation glucokinase activity falls as compared to glucose 6 phosphatase activity

2 Under the availability of glucose the enzymes utilizing glucose are activated but the enzymes producing glucose by gluconeogenesis are all depressed The secretion of insulin controls the activity of the enzymes responsible for glycolysis as well as gluconeogenesis

3 Both dehydrogenases of the HMP shunt are adaptive enzymes since their activity is very low in the well fed animal as well as when insulin is given to diabetic animal Their activity is low in diabetes or fasting Similar behaviour has been found in "Malic enzyme" and ATP-citrate lyase This indicates that these two enzymes are involved in lipogenesis rather than gluconeogenesis

4 The activity of pyruvate dehydrogenase is decreased since it is regulated by phosphorylation involving an ATP specific kinase and its activity is increased by dephosphorylation ... Thus, pyruvate dehydrogenase is ... is increased after administration

... is also

... of

Their structures are giveo below :

$CH_3(CH_2)_4 . CH{=}CH.CH_2.CH{=}CH(CH_2)_7.COOH$

Linoleic acid (18 : 2)

$CH_3.CH_2\ CH{=}CH.CH_2CH{=}CH.CH_2CH{=}CH(CH_2)_7.COOH$

Lioolenic acid (18 : 3).

$CH_3.(CH_2)_4(CH{=}CH.CH_2)_4(CH_2)_2.COOH$

Arachidonic acid (20 : 4).

Fig. 17.23 Structure of Essential fatty acids.

Chemical properties :

1. The esseotial fatty acids of vegetable oils have low melting point and low iodioe oumber.

2. These essential fatty acids become saturated on hydrogenatiou and the oils are converted to solid fats.

Sources :

1. Linoleic acid occurs io high conceotratioos io various edible vegetahle oils e.g. corn, cotton seed, peaout, safflower.

2. Arachidonic acid occurs in animal fats although in small amounts.

Synthesis :

Lenoleic acid cannot be synthesized by animals and therefore must be supplied preformed in the diet. Arachidonic acid can be formed from linoleic acid in the animal body.

The pathway is first by dehydrogenation of the CoA ester through γ-linolenate followed by the additioo of a 2 carhon uoit (as acetyl-CoA in the mitochondrial system for chain eloogatioo or as malonyl-CoA in the microsomal system, which is the more active system) to give eicosatrienoate (homo γ-linoleoate) whieh further forms arachidonate hy dehydrogenation. Therefore, the nutritiooal requirement of arachidonate is compensated by the linoleate in the diet. The synthesis is given below :

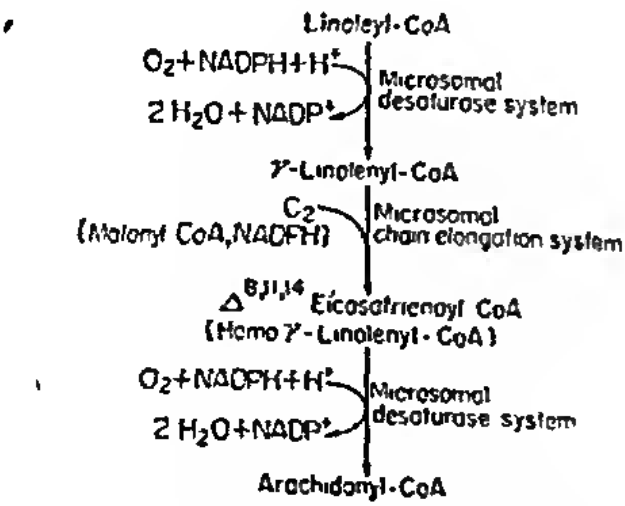


Fig. 17.24. Synthesis of essential fatty acids.

Functions :

1. Essential fatty acids found in the structural lipids of the cell are concerned with the structural integrity of the mitochondrial membrane.

2. These occur in high concentration in the reproductive organs.

3. These effect the prolongation of clotting time and increase the fibrinolytic activity.

4 These are esterified and emulsified with cholesterol and thus retard atherosclerosis.

5. These are incorporated into lipoproteins for transport to the liver for further oxidation

6. These are present in phospholipids mainly in the position 2.

7. The fatty livers due to deficiency of the essential fatty acids are cured only by the reintroduction of these substances into the diet.

8. The deficiency of these essential fatty acids causes skin lesion and impairment of lipid transfer.

9. The deficiency of these substances in the diet of babies also causes eczema.

PHOSPHORYLATION

1. All cells of the body, especially muscle cells, contain ATP which is composed of adenine-ribose-phosphoric acid -phosphoric acid-phosphoric acid.

2. ATP is the one of the intermediate phosphate donors of the body. It furnishes the phosphate for the phosphorylation of glucose to produce glucose-6-phosphate and ATP is converted to ADP

3. The liberation of phosphate from ATP is an energy-producing reaction. But the energy is utilized for chemical transfers, not as heat. Since, phosphorylation is going on continuously in the cell and the quantity of ATP is small, that ATP is reformed from ADP and inorganic phosphate.

4 In the cell, a number of organic phosphates are known as high-energy phosphate donors that serve for the reformation of ATP. Among these are 1, 3-diphosphoglyceric acid, phosphopyruvic acid and these are reformed during the breakdown of glycogen to lactic acid.

5 In muscle, another organic phosphate is phosphocreatine (phosphogens). This compound is the emergency store of phosphorylation energy which serves to regenerate ATP during muscle contraction During glycogenolysis or glycolysis there is accumulation of ATP, creatine is again built up to creatine phosphate.

LIPIDOSIS, XANTHOMATOSIS

According to Fredrickson, *lipidosis* means the abnormal concentrations of lipoproteins in blood or of specific lipids in tissues.

The specific lipids are the sphingolipids (Tay-Sachs disease, Gaucher's disease, Fabry's disease, metachromatic leukodystrophy, Niemann-Pick disease) or other lipids (Refsum's disease. Wolman's disease etc.). These disorders are discussed in the chapter on inborn error in metabolism.

Each of these disorders involves the nervous system. These are hereditary enzyme deficiency disorders.

Xanthomatosis means lipid accumulation in tissues in association with large "foam cells" The lipid is mostly cholesterol. Xanthomata develop in hyper-

lipoproteinemias, diabetes mellitus, biliary cirrhosis, hypothyroidism, glycogen storage disease etc

Exercise

1 Give an account of the metabolism of fat with particular reference to β-oxidation (Mith 64A)

2 What is lipogenesis ? Describe the cytoplasmic system of fatty acid synthesis (Bh U 75A,

3 Describe the oxidation of fatty acids in the body (P U 68A , Mith 60A)

4 *How are fatty acids synthesized and degraded in the body ?* (R U 74A)

5 Describe the fate of lipids in our body What are essential fatty acids ? (P U 66S)

6 Describe the complete oxidation of oleic acid in the body State how many molecules of ATP are formed during the process (P U 72A)

[Ans. Describe like unsaturated fatty acid oxidation (Linoleic acid)]

7 Describe briefly the chemistry of cholesterol and its physiological importance What is the normal blood cholesterol level ? (Muz 75S , R U 64A , Pun 70A)

8 Describe the occurrence, properties tests and physiological importance of cholesterol and show its structural relation to vitamin D or bile acids (Mith 61A)

9 Describe metabolism of cholesterol with reference to atherosclerosis Discuss the role of lipoprotein in it (P U 76A)

10 Name the ketone bodies Describe briefly the formation and metabolism of Ketone bodies Describe the test usually done in clinical laboratory to detect the presence of ketone bodies in urine (Mith 71A, 73A)

11 What are ketone bodies ? Describe how ketosis is produced in the body (Bh U 74S ; Mith 71A, 75S , P U 64S)

12 What is ketosis ? How are the ketone bodies produced in the body ? How does body try to combat ketosis ? (Muz 75S)

13 Short notes

(i) Essential fatty acids (Mith 75S , Bh U 76S , P U 74A)

(ii) Cholesterol (M U 73S 74S , R U 68A 70A)

(iii) Lipoprotein (R U 67A , Mith 65S)

(iv) Phospholipids (M U 73A , P U 68A 76A)

(v) Phosphorylation (Bh U 75S)

(vi) Ketosis (M U 73S 74S , R U 70A , Mith 65S)

(vii) Ketone bodies (Bh U 76A , P U 71A)

(viii) Ketogenesis (Mith 64A)

(ix) Rothera's test (P U 68S)

(x) Lipotropic factors (P U 71S , M U 73A)

(xi) Unsaturated fatty acids (Muz. 75A)

CHAPTER 18

REGULATION OF CARBOHYDRATE AND LIPID METABOLISM

Regulation of carbohydrate metabolism at the cellular and enzymatic level

1 The changes in the metabolism fully depend on the changes in the availability of substrates The concentration of glucose, fatty acids and amino acids in blood influences their rate and pattern of metabolism in many tissues

2 Alterations in the concentrations of glucose, fatty acids and amino acids in the blood owing to the changes in the dietary availability may alter the rate of secretion of hormones that influence the pattern of metabolism in metabolic pathways

3 Three types of mechanisms are responsible for regulating the activity of enzymes concerned in carbohydrate metabolism

(i) Changes in the rate of enzyme synthesis

(ii) Conversion of an inactive to an active enzyme

(iii) Allosteric effects

Regulation of Glycolysis, Gluconeogenesis and Hexose Monophosphate Shunt

1 *Glucokinase* catalyzes the conversion of glucose to glucose-6 phosphate In the same extramitochondrial region *glucose 6 phosphatase* is also found which catalyses the same interconversion in the reverse direction on the supply of sufficient carbohydrate, glucokinase activity is increased whereas glucose-6-phosphatase activity is decreased In starvation glucokinase activity falls as compared to glucose 6 phosphatase activity

2 Under the availability of glucose the enzymes utilizing glucose are all activated but the enzymes producing glucose by gluconeogenesis are all depressed The secretion of *insulin* controls the activity of the enzymes responsible for glycolysis as well as gluconeogenesis

3 Both dehydrogenases of the HMP shunt are adaptive enzymes since their activity is increased in the well fed animal as well as when insulin is given to a diabetic animal Their activity is low in diabetes or fasting Similar behaviour has been found in Malic enzyme and ATP-citrate lyase This indicates that these two enzymes are involved in lipogenesis rather than gluconeogenesis

4 The activity of pyruvate dehydrogenase is decreased since it is regulated by phosphorylation involving an ATP specific kinase and its activity is increased by dephosphorylation by a phosphatase Thus, pyruvate dehydrogenase is inhibited during fatty acid oxidation Its activity is increased after administration of insulin and decreased in starvation

5 The allosteric control of the activity of an enzyme is also available in carbohydrate metabolism In gluconeogenesis, the synthesis of oxaloacetate from pyruvate by the enzyme *pyruvate carboxylase* requires the presence of *acetyl CoA* as an allosteric activator The activation of pyruvate carboxylase and the

inhibition of pyruvate dehydrogenase by acetyl CoA formed from the oxidation of fatty acids helps to explain the sparing action of fatty acid oxidation On the oxidation of pyruvate and the stimulation of gluconeogenesis in the liver (figure 18 1) The main role of fatty acid oxidation in promoting gluconeogenesis is to supply ATP required in the pyruvate carboxylase and phosphoenolpyruvate carboxykinase reactions

6. Glucagon accelerates gluconeogenesis in the liver probably by increasing cAMP concentrations that stimulate the substrate concentration through the phosphoenolpyruvate carboxykinase reaction and inhibit pyruvate kinase Glucagon also stimulates triphosphatase in order to promote glycerol metabolism

7 **Phosphofructokinase,** the occupier of key position in regulating glycolysis, is inhibited by citrate and ATP and is activated by AMP The glycolysis is increased with the increase in the concentration AMP and with the decrease in the concentration of ATP during anoxia The inhibition of phosphofructokinase by citrate and ATP is the another explanation of the sparing action of fatty acid oxidation on glucose oxidation The consequence of the inhibition of phosphofructokinase is an accumulation of glucose 6 phosphate which, in turn, inhibits further uptake of glucose by allosteric inhibition of hexokinase

Regulation of Glycogen Metabolism:

1 Glycogen metabolism regulation is effected by the balance in activation between the enzymes of glycogen synthesis and those of glycogen breakdown as well as the hormonal control

2 Cyclic AMP dependent protein kinase activates phosphorylase b kinase and inactivates glycogen synthetase Thus, inhibition of glycogenolysis promotes glycogenesis and inhibition of glycogenesis enhances glycogenolysis

3 Glycogen metabolism in the liver is controlled by the concentration of phosphorylase a This enzyme not only controls the rate-limiting step in glycogenolysis but also inhibits to activity of synthetase, phosphatase and thereby controls glycogen synthesis

4 Inactivation of phospharylase is caused by glucose and activation is caused by 5 -AMP

5 It has been suggested that catecholamines, including epinephrine, stimulate glycogenolysis by an addition mechanism not involving cAMP These mechanisms probably involve direct stimulation of phosphorylase kinase by Ca^{++} Cyclic AMP independent glycogenolysis is also caused by vasopressin oxytocin and angiotensis 11

6 Phosphorylase is immediately activated followed by the activation of glycogen synthetase on the administration of insulin The presence of glucose is essential on the effects of insulin

Regulation of the Citric Acid Cycle:

1 The activity of the enzymes of citric acid cycle is immediately dependent on the supply of oxidized dehydrogenase cofactors (e g NAD) which, in turn, is dependent on the availability of ADP and ultimately on the rate of utilization of ATP

2 Control of the citric acid cycle occurs at the pyruvate dehydrogenase step Control may be experienced by allosteric inhibition of citrate synthase by ATP or long chain fatty acyl-CoA

3 Oxaloacetate inhibits succinate dehydrogenase and the availability of oxaloacetate, as controlled by malate dehydrogenase, depends on the ratios of the concentrations of NADH and NAD^+.

4 The increase in the ratio of the concentration of ATP and ADP is considered to raise the ratio of the concentration of GTP and GDP at the succinate thiokinase step, thereby increasing the concentration of succinyl-CoA.

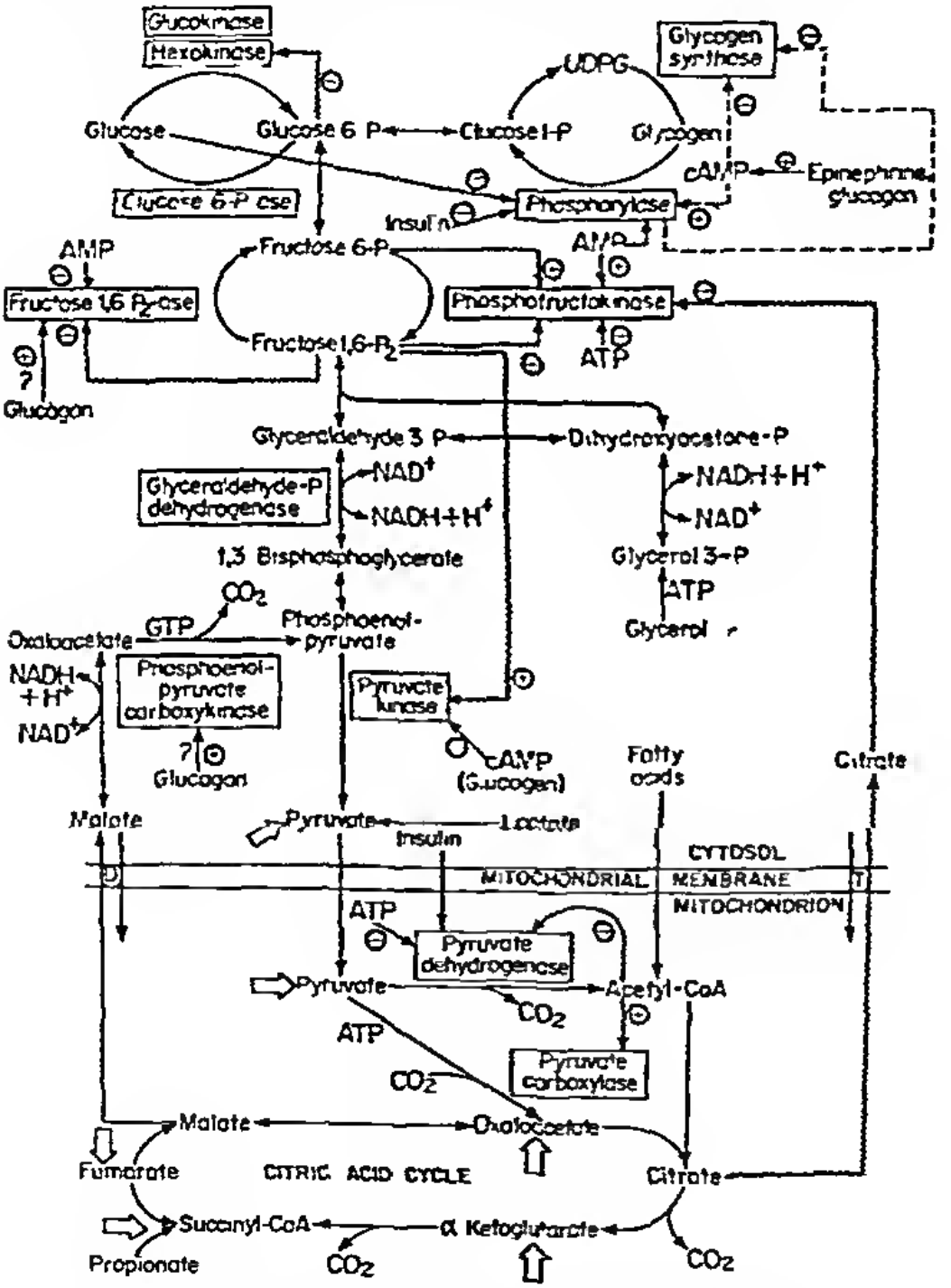


Fig 18 1. Key enzymes in the control of glycolysis, gluconeogenesis and glycogen metabolism in liver along with the necessary hormones

REGULATION OF LIPID METABOLISM

Regulation of Fatty Acid Synthesis:

(Lipogenesis):

1. Lipogenesis is concerned with the conversion of glucose and intermediate such as pyruvate, lactate and acetyl-CoA to fat.

2. The rate of lipogenesis is high in case of a diet containing a high proportion of carbohydrate. The rate is decreased on a high-fat diet or in the deficiency of insulin as in diabetes mellitus. All these conditions are related to increased concentration of plasma free fatty acids. There is an inverse relationship between hepatic lipogenesis and the serum free fatty acids concentration Lipogenesis is greatly inhibited over the high range of free fatty acids. Lipogenesis is depressed by the fat diet in the liver and carbohydrate is converted a little to fat when the fat diet is more than 10%.

3. Lipogenesis is higher when sucrose is fed instead of glucose. It is also blocked in fasting due to the lack of NADPH generation from the HMP shunt pathway.

4. Acetyl-CoA carboxylase in competitively inhibited with the activator citrate by the long chain acyl-CoA molecules. Therefore, if acyl-CoA is accumulated, it will automatically reduce the synthesis of new fatty acids.

5. Acyl-CoA may also inhibit the mitochondria transport of citrate into the cytosol. Free fatty acids also are inversely related to the proportion of active to inactive pyruvate dehydrogenase. Acyl-CoA may also inhibit pyruvate dehydrogenase by inhibiting the ATP-ADP exchange transporter of the inner mitochondrial membrane. As a result, the supply of acetyl-CoA from carbohydrate is blocked.

6. **Insulin** increases the transport of glucose into the cell making increased availability of pyruvate for fatty acid synthesis and glycerol-3-phosphate for esterification of the fatty acids. It also converts the inactive pyruvate dehydrogenase and acetyl-CoA carboxylase to the active form. It also depresses the level of intracellular cAMP, inhibits lipolysis and thereby reduces the concentration of long chain acyl-CoA which is an inhibitor of lipogenesis.

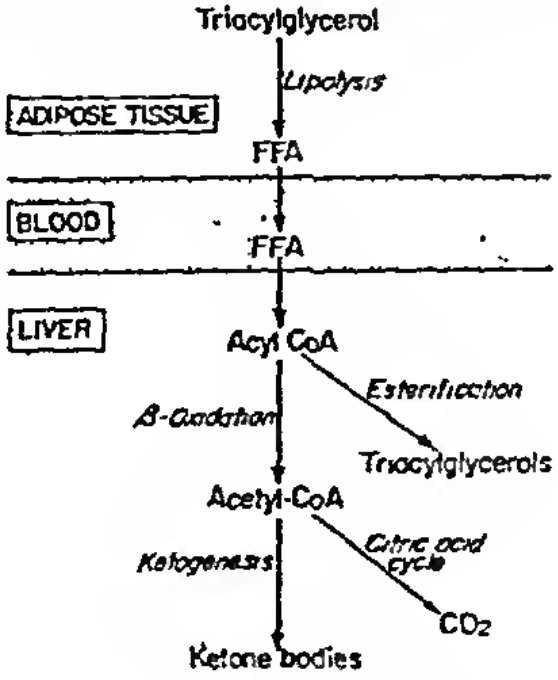


Fig. 18 2. Regulation of ketogenesis.

Regulation of Ketogenesis

1 In adipose tissue, very high concentrations of plasma free fatty acids (FFA) are available as a result of lipolysis of triacylglycerol In fed as well as in fasting conditions 30% or more of the free fatty acids pass to the liver After they are activated to acyl CoA they are either esterified mainly to triacylglycerol and phospholipid or they undergo β-oxidation to form acetyl CoA The acetyl CoA is either oxidized in the citric acid cycle or used to form ketone bodies (Fig 18 2)

2 The esterification which acts as an antiketogenic factor depends on the availability of precursors in the liver to supply sufficient glycerol 3 phosphate In the liver antiketogenic effects of glycerol and dihydroxyacetone are not correlated with the levels of glycerol 3 phosphate

3 Phosphatidate phosphohydrolase appears to increase in activity in livers in which extra triacylglycerol synthesis takes place Insulin increases the activity of glycerol phosphate acyltransferase which catalyses the first step in esterification

4 **Carnitine acyltransferase I** activity in the mitochondrial membrane regulates the entry of long chain acyl groups into the mitochondria before β-oxidation takes place In the fed state, fatty acid oxidation is depressed due to its lowered activity, but in fasting fatty acid oxidation increases owing to its increased activity This enzyme is inhibited in the fed state by the increased level of malonyl CoA the initial intermediate in fatty acid biosynthesis Therefore, in the fed state, the active lipogenesis and high malonyl CoA inhibit carnitine acyltransferase I

5 Low level of free fatty acids entering the liver cell are nearly all esterified to triacylglycerols and transported out of the liver in VLDL The concentration of free fatty acids increases with the onset of starvation and acetyl CoA carboxylase is inhibited and malonyl CoA decreases. Therefore, the inhibition of carnitine acyltransferase I is released with the permission of more acyl CoA to be oxidized The ratio of the concentrations of insulin and glucagon reinforces these events in starvation causing increased lipolysis in adipose tissue and inhibition of pyruvate kinase and acetyl CoA carboxylase in the liver

6 More free fatty acid is converted to ketone bodies and less is oxidized to CO_2 via TCA cycle with the increased concentration of serum free fatty acids The total free energy as ATP as a result of the oxidation of free fatty acids remains constant on the fact that the partition of acetyl CoA between the ketogenic pathway and the pathway of oxidation to CO_2 is regulated in such a particular manner On complete oxidation in the citric acid cycle, One mol of palmitate yields 129 mol of ATP, whereas this one mol of palmitate produces only 33 mol of ATP when acetoacetate is the end product Therefore ketogenesis is regarded as a mechanism in which the liver can oxidize large quantities of fatty acids without increasing its total energy expenditure

7 Reduced level of oxaloacetate within the mitochondria can cause impairment of the TCA cycle to metabolize acetyl CoA Krebs has suggested that a fall in the concentration of oxaloacetate owing to an enhanced gluconeogenesis may be the cause of the severe forms of ketosis found in diabetes and the ketosis of cattle It has been assumed that citrate synthase is inhibited either by long chain acyl CoA or by increased level of ATP It has also been shown that pyruvate carboxylase which converts pyruvate to oxaloacetate is activated by acetyl CoA Therefore, sufficient amounts of acetyl CoA signifies the presence of significant amounts of oxaloacetate to initiate the condensing reaction of TCA cycle

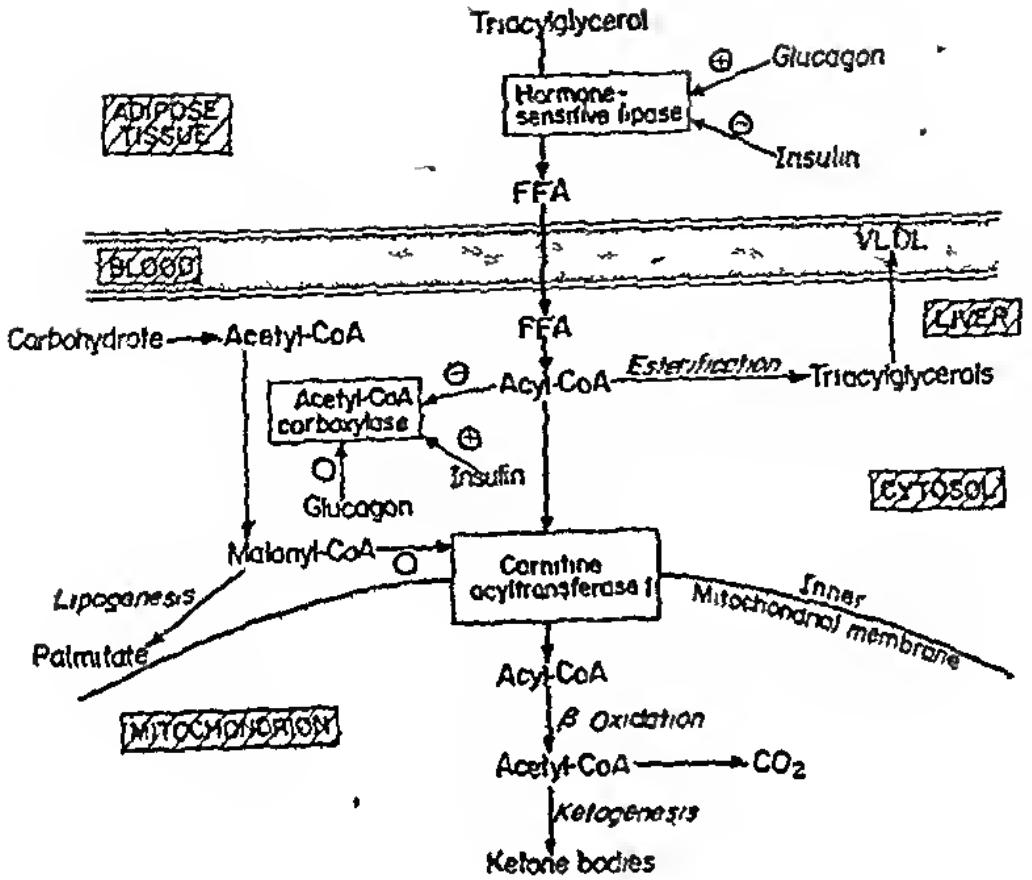


Fig 18.3 Regulation of long chain fatty acid oxidation in the liver

SUMMARY :

1 Free fatty acids (FEA) are the main substrates for ketone body formation in the liver Therefore, all factors, metabolic or endocrine, influence ketogenesis releasing free fatty acids from adipose tissue

2 Free fatty acids after entering the liver are established by the balance between their esterification and oxidation This balance is governed by carnitine acyltransferase I, whose activity is raised indirectly by the concentration of free fatty acids and the hormonal state of the liver

3 The regulation of more ketone bodies formation and less CO_2 formation with the increased oxidation of fatty acid is performed in such a manner that the total ATP production remains constant Ketone bodies without being oxidized significantly by the liver diffuse into the circulation and hence oxidized by extrahepatic tissues to other fuels

CHAPTER 19

PROTEIN METABOLISM

DYNAMIC STATE OF PROTEIN

1 All body proteins (plasma proteins, hemoglobin, and intracellular proteins) continually undergo degradation and synthesis More than half of the protein of the liver and intestinal mucosa are broken down and resynthesized in ten days, the rate is slower in muscle and erythrocytes.

2 Active resynthesis occurs even in starvation, and active breakdown in nitrogen equilibrium

3 Degradation of protein in one tissue is accompanied by synthesis in others

4 Antibody protein (γ-globulins) induced by immunization also undergo continual breakdown and synthesis The half life is about two weeks

5 The balance between the rates of synthesis and degradation of its constituents is maintained in adult organism. But in a growing organism, the rate of synthesis of its constituents exceeds the rate of breakdown and the rate of catabolism is greater than the rate of anabolism in wasting diseases and starvation

METABOLIC POOL

1 The mixture of endogenous and exogenous materials constitutes a so-called or "metabolic pool" of the compound.

2 This pool is contributed by substances derived from catabolism of protein [illegible] by the stimulation of the excessive amounts of thyroid hormones and [illegible] gonadal adrenocortical hormones and substances absorbed from the intestine Protein anabolism is stimulated by growth hormone, insulin and [illegible]

3 The metabolism of protein is integrated with that of carbohydrate and fat [illegible] pyruvate, oxaloacetate and α-ketoglutarate etc. and the members of this pool are continually equilibrated with other pools, e g. alanine is [illegible] pyruvic acid and hence the carbon skeleton of this amino acid is [illegible] which is directly connected to the carbohydrate pool

INTERMEDIARY METABOLISM

Metabolism is generally interpreted to mean intermediary metabolism It includes all chemical processes within cells and tissues which are concerned with [illegible] building up and breaking down and in their functional operation.

The building up and breaking down of protoplasm are concerned with protein metabolism Various substances of lipids, carbohydrates and inorganic metals are also involved

The [illegible] operation of tissues, such as muscular contraction, the [illegible] implies the secretory work of various glands, the selective [illegible] and the excretory processes of the kidney and other organs, [illegible] ons to necessary energy and specific [illegible]

2 Ketone bodies and free fatty acids spare the oxidation of glucose in muscle by impairing its entry into the cell, its phosphorylation of glucose-6-phosphate, the phosphofructokinase reaction, and the oxidative decarboxylation of pyruvate Free fatty acids and ketone bodies oxidation increases the concentration of intra cellular citrate which inhibits phosphofructokinase Olson has justified that under carbohydrate shortage available fuels are oxidized in the following order of preference (i) ketone bodies (and other short chain fatty acids, e g acetate), (ii) free fatty acids, (iii) glucose (shown in Fig 18 4)

3 Under certain conditions, fat mobilization can be reduced by the ad ministration of noncarbohydrate calorigenic substrates If glucose oxidation is spared by free fatty acids and ketone bodies, more glucose will be available causing a reduction in output of free fatty acids from adipose tissue and the free fatty acid level of plasma will fall The combination of the effects of free fatty acids in sparing glucose utilization in muscle and heart and the effect of the spared glucose in inhibiting free fatty acid mobilization in adipose tissue has been called the "glucose-fatty acid cycle'

Starvation

1 Under fasting condition, glucose concentration becomes less, glycogen is drawn upon to maintain the blood glucose level Blood insulin level decreases and glucagon level increases Since the glucose utilization in the adipose tissues decreases and the inhibitory effect of insulin on lipolysis becomes less, fat is mobi lized as free fatty acids and glycerol The free fatty acids are esterified in the liver and the remainder are oxidized In the liver and kidney, glycerol joins the carbohydrate pool after being converted to glycerol 3 P The endogenous glucose production cannot keep pace with its utilization and oxidation Therefore, the liver glycogen stores become depleted and blood glucose tends to fall Then fat is mobilized at the increased rate but in several hours the plasma free fatty acids and the blood glucose stabilize at the fasting level Hence increased fatty acid oxidation takes place resulting in the formation of ketone bodies

2 The adipose tissues provide carbohydrate in the form of glycerol together with that provided by *gluconeogenesis* from protein

3 In prolonged starvation in humans, *gluconeogenesis* from protein is diminished owing to the diminished release of amino acids, particularly alanine, from muscle The brain then utilizes ketone bodies in place of glucose

4 *Free fatty acids are under most conditions mobilized in excess of oxidative* requirements since a large amount is esterified even during fasting When the carbohydrate supplies are sufficient, most of the influx is esterified and ultimately retransported from the lives as VLDL to be utilized by other tissues In the case of increased influx of free fatty acids, ketogenesis is available which allows the liver to retransport much of the influx of free fatty acids in a form which is readily utilized by extrahepatic tissues under all nutritional conditions

5 The carbohydrate cycle involves the release of glycerol from adipose tissue and its conversion to glucose in the liver The lipid cycle involves the release of free fatty acids by adipose tissue, its transport to and esterification in the liver and retransport of VLDL back to adipose tissue These two interrelated cycles, are involved during the disturbances in carbohydrate or lipid metabolism where they interact in adipose tissue and in the liver

Exercise

1 *Discuss in* detail the regulation of carbohydrate metabolism in the body
2 How is the metabolism of l p d regulated in the body?
3 Discuss the economics of carbohydrate and l pid metabol sm in the whole body

THE ECONOMICS OF CARBOHYDRATE AND LIPID METABOLISM IN THE WHOLE BODY

1 Glucose is converted to fat under optimal nutritional intake Except glycerol, fat (as fatty acid) cannot produce glucose due to the irreversibility of the oxidative decarboxylation of pyruvate to acetyl-CoA. Certain tissues are more dependent on the continued supply of glucose than others A minimum supply of glucose is necessary in extrahepatic tissues to maintain the integrity of TCA cycle Glucose is the main source of glycerol-3-phosphate in tissues devoid of glycerokinase Large quantities of glucose are also required for fetal nutrition and the synthesis of milk. Certain mechanisms are also involved to safeguard essential supplies of glucose during shortage by allowing other substrates to spare its oxidation

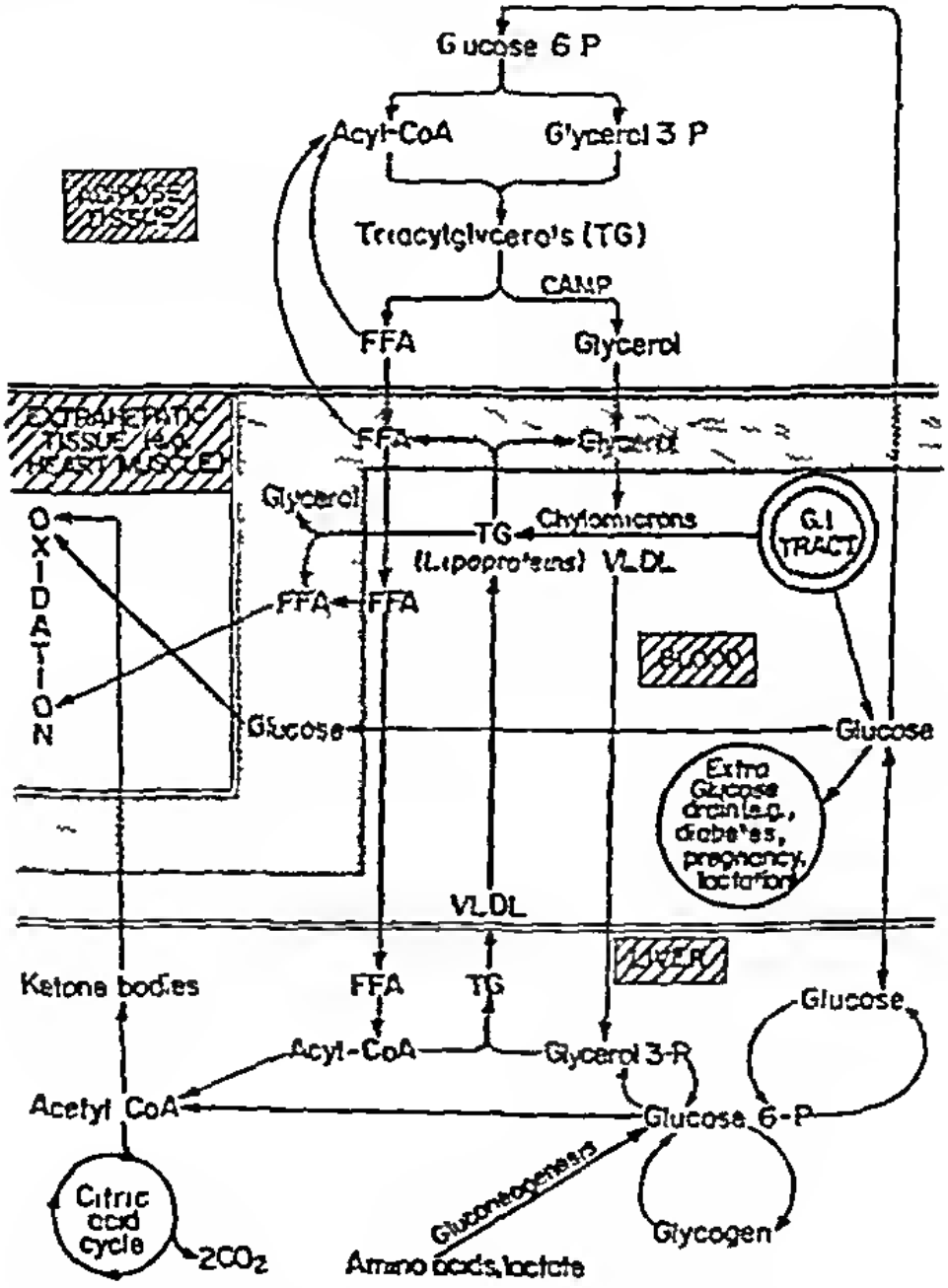


Fig 18 4 Metabolic interrelationship between adipose tissue, the liver and extrahepatic tissues

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2 How is the metabolism of lipid regulated in the body?
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1 The mixture of endogenous and exogenous materials constitutes a reservoir or "metabolic pool" of the compound

2 This pool is contributed by substances derived from catabolism of protein in the tissues by the stimulation of the excessive amounts of thyroid hormones and 11 oxygenated adrenocortical hormones and substances absorbed from the intestine Protein anabolism is stimulated by growth hormone, insulin and testosterone

3 The metabolism of protein is integrated with that of carbohydrate and fat through pyruvate, acetate, oxaloacetate and α ketoglutarate etc and the members of one pool are metabolically equilibrated with other pools, e g alanine is deaminated to pyruvic acid and hence the carbon skeleton of this amino acid is involved in the pyruvate pool which is directly connected to the carbohydrate pool

INTERMEDIARY METABOLISM

Metabolism is generally interpreted to mean intermediary metabolism It includes all chemical processes within cells and tissues which are concerned with their building up and breaking down and in their functional operation

The building up and breaking down of protoplasm are concerned with protein metabolism Various substances of lipids, carbohydrates and inorganic metals are also involved

The functional operation of tissues, such as muscular contraction, the propagation of nerve impulses, the secretory work of various glands, the selective absorption of intestine and the excretory processes of the kidney and other organs require a number of chemical reactions to provide necessary energy and specific chemical substances

Metabolism does not only mean the chemistry of tissue formation and breakdown but also the chemistry of the processes in tissues necessary for the formation of various specific compounds required for the operation and regulation of the metabolic machine

INTERMEDIARY METABOLISM OF PROTEINS

1 The amino acids abstracted from the *liver* are not utilized for *repair or* special synthesis but are broken down and the ammonia formed thus is converted into urea which is excreted

2 The amino acids abstracted from the tissues are used for the repair of the tissues The unwanted amino acids are either used up by the tissue, or converted into urea in the liver

3 Amino acids provide the nitrogen for the synthesis of many nitrogenous compounds of the body including bile acids, creatine, purine, pyrimidines, epinephrine, thyroxine, amino sugars, enzymes and the bases of the phospholipids

4 Fates of amino acids result in (i) Catabolism in the liver, (ii) Formation of tissue protein (iii) Formation of other nitrogenous substances

5 The essential and excess metabolism of proteins are usually differentiated by the term "endogenous" and "exogenous" metabolism Endogenous metabolism is involved in the formation of tissue and the other nitrogenous substances and also the normal breakdown of tissue proteins Exogenous metabolism is the metabolism of all proteins ingested over and above the essential requirements

6 In normal health, the nitrogen ingested equals to that excreted in urine, feces and sweats

7 Fates of sulphur containing amino acids result in (i) Catabolism in the liver producing sulphuric acid which is excreted (ii) Synthesis of tissue protein including that of hair (iii) Synthesis of other substances including taurine, glutathione, insulin, ethereal sulphate, sulphated mucopolysaccharide and lipids

In general it can be summarized as follows

Fig. 19.1 Intermediary metabolism of proteins

CATABOLISM OF AMINO ACID NITROGEN SYNTHESIS OF UREA

In mammalian tissues, the alpha amino groups of amino acids derived from the diet or from tissue protein breakdown are ultimately excreted in urine as urea Several enzymes are involved in the biosynthesis of urea which involves four processes

1 Transamination

2 Oxidative deamination

3 Ammonia formation and transport

4 Reactions of the urea cycle

In human and other ureotelic organisms, *urea* is the end product of amino acid nitrogen metabolism. But in uricotelic organisms (e.g. reptiles and birds), *uric acid* is the end product and in ammonotelic organisms (e.g. bony fish), *ammonia* is the end product of amino acid nitrogen metabolism.

1 Transamination

(i) Transamination involves interconversion of a pair of α amino acids and a pair of α-keto acids and is catalyzed by *transaminases* or *aminotransferases*.

(ii) Pyridoxal phosphate ($B_6—PO_4$) is the coenzyme essential for the transaminase activity.

(iii) Alanine pyruvate transaminase (*alanine transaminase*) and glutamate α ketoglutarate transaminase (*glutamate transaminase*) present in most mammalian tissues, catalyze the transfer of amino groups from most amino acids to form alanine (from pyruvate) or glutamate (from α keto glutarate) shown below.

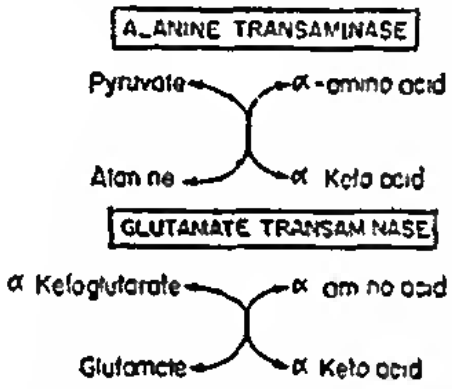


Fig. 19.2. Alanine and glutamate transaminases

(iv) Transamination is a reversible process. This reversibility allows transaminases to function in amino acid catabolism and biosynthesis.

(v) Each transaminase is specific for the specified pair of amino acid and keto acid as one pair of substrates.

(vi) Most amino acids are substrates for transamination except lysine, threonine, proline and hydroxyproline. Not only δ amino acid, δ-amino group of ornithine undergo transamination.

2 Oxidative deamination

(i) Oxidative conversion of many amino acids to their corresponding α keto acids occurs in mammalian liver and kidney tissues.

(ii) Both L and D aminoacid *oxidase* activities occur in mammalian liver and kidney tissue and are also widely distributed in other animals and microorganisms.

(iii) The amino acid oxidases are *auto-oxidizable flavoproteins* i.e. the reduced FMN or FAD is reoxidized directly by molecular oxygen forming H_2O_2 without the help of cytochromes or other electron carriers shown below. The toxic product (H_2O_2) is then split to O_2 and H_2O by *catalase* which occurs widely

in tissues, especially liver If catalase is absent, the α-keto acid is decarboxylated by H_2O_2 forming a carboxylic acid with one carbon atom less.

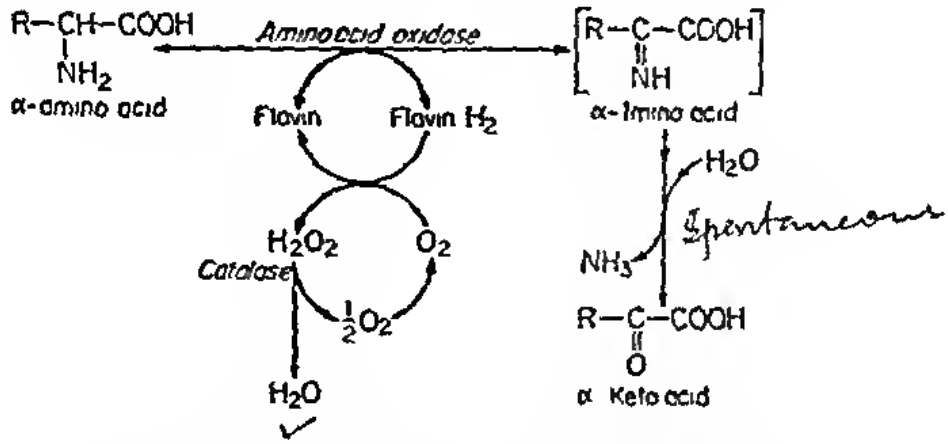


Fig 19 3 Oxidative deamination catalyzed by L amino acid oxidase

In the amino acid oxidase reactions in the above figure, the amino acid is first dehydrogenated by the flavoprotein of the oxidase forming an α-imino acid which spontaneously decomposes to the corresponding α-keto acids by the addition of water.

(iv) Mammalian L-amino acid oxidase, an FMN-flavoprotein is restricted to kidney and liver tissue and its activity is quite slow Mammalian D-amino acid oxidase, an FAD-flavoprotein, occurs in the liver and kidney tissue of most mammals

3 **Ammonia formation and transport**

(i) The amino groups of most amino acids are ultimately transferred to α-ketoglutarate by transamination Release of this nitrogen as ammonia is catalyzed by *L-glutamate dehydrogenase* which is widely distributed in mammalian tissues Certain hormones also influence glutamate dehydrogenase activity

(ii) Glutamate dehydrogenase uses NAD^+ or $NADP^+$ as cosubstrate The reaction is reversible

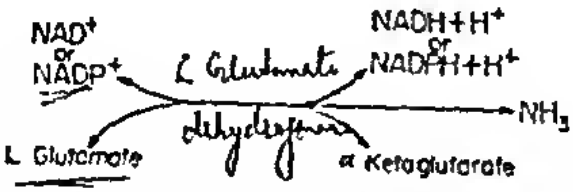


Fig 19 4 L-Glutamate dehydrogenation reaction

(iii) Intestinal bacteria produce ammonia from dietary protein as well as from the urea present in fluids secreted into the gastrointestinal tract. This ammonia is absorbed from the intestine into the portal venous blood Under normal conditions, the liver promptly removes the ammonia from the portal blood Minute quantities of ammonia are toxic to the central nervous system

The symptoms of ammonia intoxication include slurring of speech blurring of vision, a peculiar flapping tremor and in severe cases coma and death These symptoms occur when brain ammonia levels are increased

Ammonia is produced in the kidney from intracellular amino acid, glutamine, catalyzed by renal *glutaminase* Ammonia production by the kidney is highly increased in metabolic acidosis and depressed in alkalosis

Fig 19 5

(iv) Ammonia is excreted as ammonium salts during metabolic acidosis but the majority is excreted as urea Ammonia is present only in traces in blood (10-20 μg/100 ml) because it is rapidly removed from the circulation by the liver and converted to glutamine or urea

(v) Formation of glutamine is catalyzed by *glutamine synthetase*, a mitochondrial enzyme present in renal tissues in highest concentration Synthesis of glutamine is accompanied by the hydrolysis of ATP to ADP and P_i The reaction is irreversible Asparaginase and glutaminase are employed as antitumor agents because certain tumors require glutamine and asparagine

(vi) In *brain*, the major mechanism for removal of ammonia is glutamine formation and in the *liver*, the most important pathway is urea formation Brain tissue can form urea but this does not play a significant role in ammonia removal

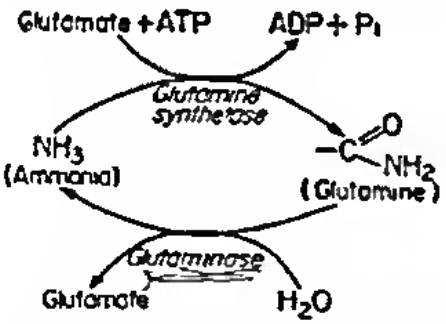


Fig 19 6 Formation and transport of ammonia

UREA CYCLE

4 Reactions of the urea cycle

(i) Carbamoyl phosphate is formed by the condensation of one mol of phosphate (derived from ATP) being catalyzed by the enzyme *carbamoyl phosphate synthetase* which is present in liver mitochondria of all ureotelic organisms including humans In addition to magnesium ion (Mg^{++}) N acetyl glutamate (a dicarboxylic acid) is required Probably, the presence of N acetyl glutamate brings about a marked change in the structure of carbamoyl phosphate synthetase which exposes certain sulfahydryl groups and affects the affinity of the enzyme for ATP

In hacteria, glutamine in place of ammonia serves as a substrate for carhamoyl phosphate synthesis.

(ii) Carhamoyl moiety is transferred to ornithine to form citrulline being catalyzed by *ornithine transcarbamaylase* of liver mitochondria.

(iii) Arginosuccinic acid is formed by the combination of citrulline and aspartic acid in presence of *argina succinic acid synthetase* and ATP.

(iv) Arginosuccinic acid is cleaved to arginine and fumaric acid by *arginosuccinase* which is present in mammalian liver and kidney. The fumarate formed is converted to oxaloacetate via the fumarase and malate debydrogenase reactions and then transaminated to regenerate aspartate.

(v) The hydrolytic cleavage of arginine is catalyzed by *arginase* wbich is present in the livers of all ureotelic organisms forming ornithine and urea.

Smaller quantities of arginase also occur in renal tissue, brain, mammary gland, testicular tissue and skin. Orinithine and lysine are the competitive inhibitors of arginine.

The cycle for the overall reactions is given below :

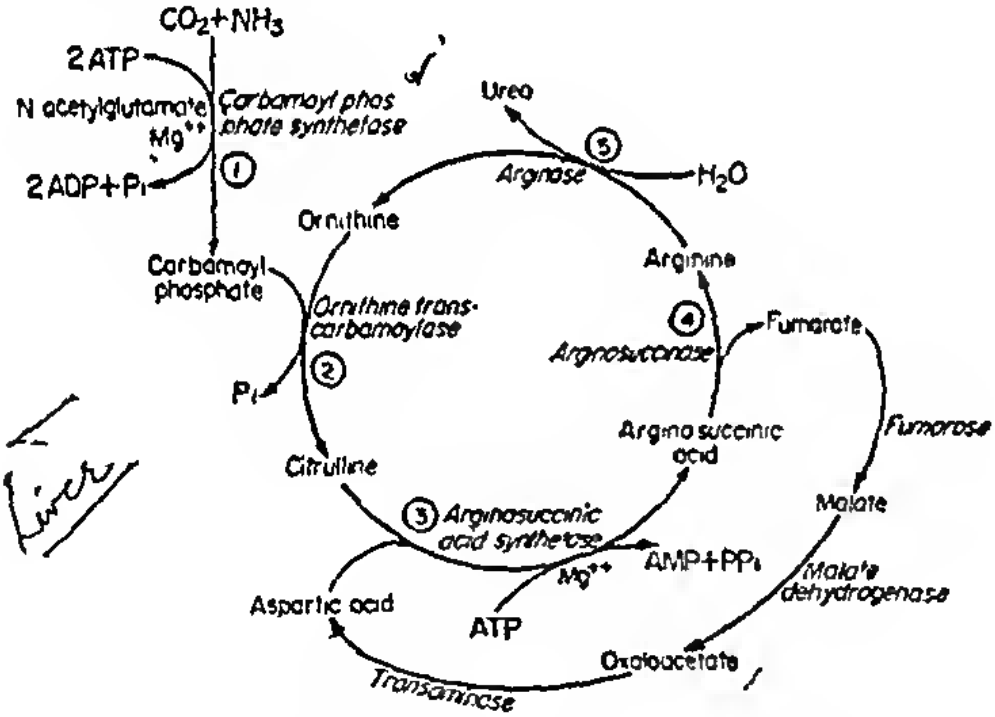


Fig 19.7. Biosynthesis of urea or ornithine-urea cycle.

The hiosynthesis of urea occurs mainly ia the liver. 1 mol. of urea is synthesized from 1 mol. of ammonia, 1 mol. of carbon dioxide, 3 mols. of ATP (2 of whicb are converted to ADP and Pi and 1 to AMP+PPi), 5 enzymes catalyzing the reactions and 6 amino acids iavolved in the reaction.

One amino acid, N-acetyl-glutamate, serves as an enzyme activator. The remaining 5 amino acids aspartate, arginine, ornithine, citrulline and arginosuccinic acid—all function as carriers of atoms wbich ultimately become urea. Aspartate and arginine occur in protein while ornithinine, citrulline, arginosuccinate do not.

Urea formation is partly a cyclical process Ornithine used in reaction (2) is regenerated in reaction (5)

An active man consuming about 300 g of carbohydrates, 100 g of fat, 100 g of protein daily excrete about 16 5 g of nitrogen daily 95 per cent is eliminated by the kidneys and 5 per cent in the stool The major pathway of nitrogen excretion in human is as urea which is synthesized in the liver

Blood level Normal concentration of urea in the blood is 20 to 40 mg/ 100 ml

Excretion The daily output of urea through urine is 20 to 30 grams A less quantity is excreted in the sweat. The quantity of urea excreted is proportional to the total protein metabolism The excretion of urea is decreased in certain liver diseases In severe acidosis the output of urea is decreased In nephritis when the ability of the kidneys to excrete urea is severely impaired, the concentration of urea in the blood is increased (uremia)

TEST OF UREA IN URINE

Specific urease test

Principle Urea is split into ammonia and CO_2 by the enzyme urease present in plants at the optimum temperature and PH Ammonium carbonate formed as a result of ammonia and carbon dioxide shows the alkali characteristic by the indicator

Method

1 2 ml of urine in one test tube and 2 ml of water in another test tube

2 A drop of phenol red is added to each of the test tubes

3 2 per cent Na_2CO_3 added drop by drop till the deep pink colour develops

4 1 or 2 drops of acetic acid added to each test tube till the colour is just yellow

5 A pinch of soyabean powder added to each and the test tubes are rotated between the palms or the temperature is raised to 60°C, the optimum temperature for the enzyme urease

6 The development of pink colour in the test tube containing urine indicates the presence of urea

METABOLIC DISORDERS IN THE UREA CYCLE

All disorders of urea synthesis cause ammonia intoxication Clinical symptoms in urea cycle disorders are vomiting in infancy, avoidance of high protein foods, irritability, lethargy and mental retardation Significant improvement has been observed on a low protein diet and much of the brain damage is prevented The daily food intake should be given in frequent small feedings to avoid sudden increases in blood ammonia levels Uses of antibiotics to decrease the absorption of ammonia formed by bacterial decomposition in the intestine

Hyperammonemia type II

1 Patients suffer from a deficiency of ornithine transcarbamoylase

2. The clinical finding is an elevation of glutamine in the blood, cerebrospinal fluid and urine

3 There is enhanced synthesis of glutamine by the glutamine synthetase reaction for which tissue level of ammonia is increased

Citrullinaemia

1. Complete absence of *arginosuccinate synthetase* activity produces this condition.

2 Large quantities of citrulline are excreted in the urine and plasma and CSF citrulline levels are elevated

Arginosuccinic aciduria

1 Arginosuccinic aciduria is caused due to the absence of arginosuccinase activity This enzyme is also absent from brain, liver, kidney and erythrocytes of patients with this disease

2 The disease is characterized by the increased level of arginosuccinic acid in blood, CSF, and urine

3 It is frequently associated with the occurrence of tufted hair

Hyperargininaemia

1 Low erythrocyte level of arginase causes this disease

2 The urinary amino acid pattern resembles that of lysine cystinuria

3 A low protein diet results in the lowering of plasma ammonia level

BIOSYNTHESIS OF AMINO ACIDS

NUTRITIONALLY ESSENTIAL AND NONESSENTIAL AMINO ACIDS

Plants and bacteria can form all 22 amino acids from amphibolic intermediates But humans and other animals cannot synthesize some of these Therefore, these are supplied by the diet and are termed nutritionally essential amino acids The remainders are synthesized in the body Therefore, these are termed nutritionally nonessential amino acids

According to the nutritional scientists, the nutritionally essential amino acids are termed "essential" or "indispensable" amino acids and the nutritionally nonessential amino acids are termed "nonessential" or "dispensable" amino acids

Nutritionally nonessential amino acids are more important to the cell than the nutritionally essential ones

The essential amino acids are methionine, tryptophan valine, leucine, isoleucine, phenylalanine threonine lysine, histidine

Essential amino acids

An "essential" or "indispensable" amino acid is defined as one which cannot be synthesized by the organism from substances ordinarily present in the diet at a rate commensurate with certain physiological requirements

Certain of these essential amino acids are replaced by the corresponding α-keto acids or α hydroxy acids Ten amino acids are required for the optimal growth of animals found in the experiments on white rats But in case of humans, eight essential amino acids are required for the optimal growth of the young and for the maintenance of nitrogen equilibrium in the adult These eight essential amino acids are methionine, tryptophan, valine, phenylalanine, leucine, isoleucine, threonine and lysine Two amino acids, arginine and histidine, which are required for animals, are "nutritionally semi-essential" for humans because they may be synthesized in tissues at rates inadequate to support growth of children

Certain nonessential amino acids in the diet serve as the sparing action of certain essential amino acids, e g tyrosine spares phenylalanine and cystine spares methionine In phenyl ketonuric individuals, who are unable to convert phenylalanine into tyrosine, the latter becomes an essential amino acid

If a single essential amino acid is omitted from the group and fed separately several hours later, the nutritional effectiveness of the entire group is impaired

The omission of an essential amino acid from the diet results in the negative nitrogen balance or decrease of growth

Nutritionally nonessential amino acids formed from amphibolic intermediates

Alanine Alanine is formed from pyruvate by transamination in presence of the coenzyme pyridoxal phosphate (B_6 Po_4) in all forms of life

Glutamic acid In all forms of life, glutamic acid is formed from α ketoglutarate by glutamate dehydrogenase

Bacteria contain only an NAD^+-dependent dehydrogenase whereas yeast and fungi contain two glutamate dehydrogenases specific for NAD^+ and for $NADP^+$

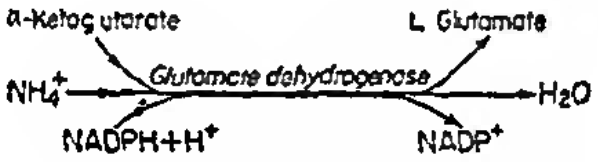


Fig 19 8 Synthesis of glutamic acid

It is also to be noted that NAD^+ functions in glutamate catabolism and $NADP^+$ in glutamate biosynthesis in animals

In many bacteria glutamate is formed by glutamate synthetase which is given below

Fig 19 9 Formation of glutamate

Aspartic acid Aspartic acid is formed by transamination of oxaloacetate

Glutamine In plants animals and bacteria, glutamine is synthesized from glutamate by glutamine synthetase NH_4^+ aminates glutamate requiring ATP and Mg^{++} which is shown below

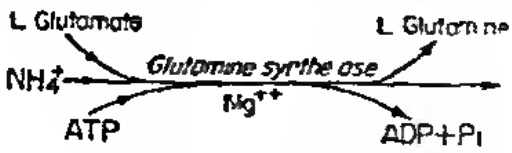


Fig 19 10 Synthesis of glutamine

Asparagine Asparagine is synthesized from aspartate by asparagine synthetase ATP and Mg^{++} are also required in this reaction ATP is hydrolyzed to AMP+PP_i R NH_3^+ aminates aspartate which is shown below

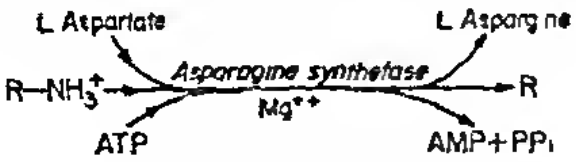


Fig 19 11 Synthesis of asparagine

Serine In mammalian tissues serine is synthesized from 3 phosphoglycerate, an intermediate of glycolysis, by two pathways One pathway uses phosphorylated intermediates and the other use nonphosphorylated intermediates Majority of the serine is synthesized by the pathway via phosphorylated intermediates Plants and micro organisms follow this pathway

Synthesis via phosphorylated intermediates

3 phosphoglycerate is oxidized to phosphohydroxypyruvate which by transamination is converted to phosphoserine Finally phosphoserine is converted to serine by phosphatase

Synthesis via *nonphosphorylated intermediates*

3 phosphoglycerate is dephosphorylated to glycerate by a phosphatase Glycerate is oxidized to hydroxypyruvate which is finally transaminated to form serine The reactions are shown below

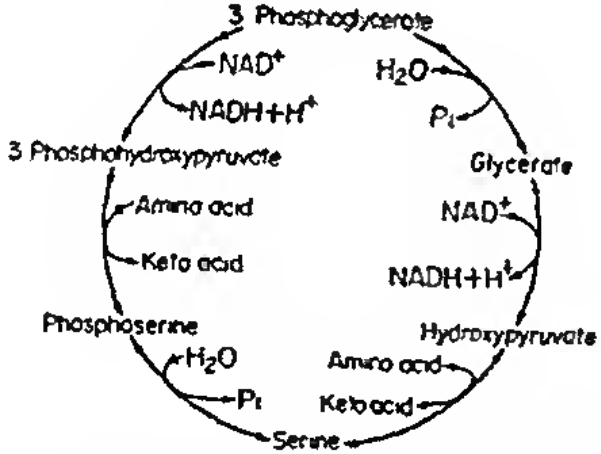


Fig 19 12 Synthesis of nonphosphorylated intermediates

Glycine Glycine is synthesized from serine as well as choline which are given below .

Fig 19 13 Synthesis of glycine

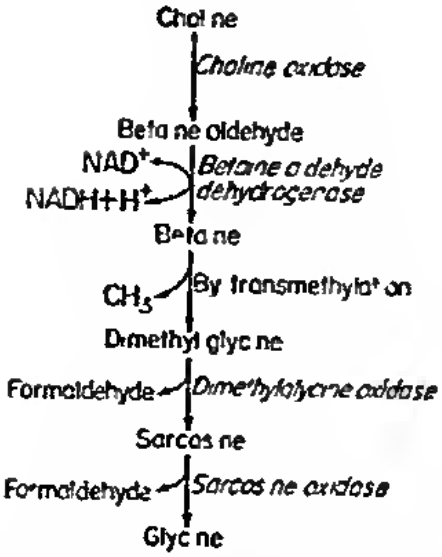


Fig 19 14, Synthesis from choline

Nutritionally nonessential amino acids formed from other nutritionally nonessential Amino acids

Proline Proline is synthesized from glutamate by reversal of reactions for proline catabolism

Hydroxyproline Since proline serves as a precursor of hydroxyproline, this is also synthesized from glutamate

Nutritionally nonessential amino acids formed from nutritionally essential amino acids

Cysteine Cysteine is formed from methionine (essential amino acid) Methionine is first converted to homocysteine which is converted to cysteine in conjugation with serine shown below

$$\text{Homocysteine} + \text{Serine} \xrightarrow{H_2O} \text{Cystathionine} \xrightarrow{H_2O} \text{Homoserine} + \text{Cysteine}$$

Tyrosine The conversion of phenylalanine (an essential amino acid) to tyrosine is catalyzed by phenylalanine hydroxylase complex, a mixed function oxygenase present in mammalian liver but absent from other tissues One atom of molecular oxygen is incorporated into the para position of phenylalanine and the other atom is reduced forming water (shown in the figure below) The reducing power supplied ultimately by NADPH, is immediately provided as tetra hydrobiopterine a pteridine resembling that in folic acid The reaction is not reversible

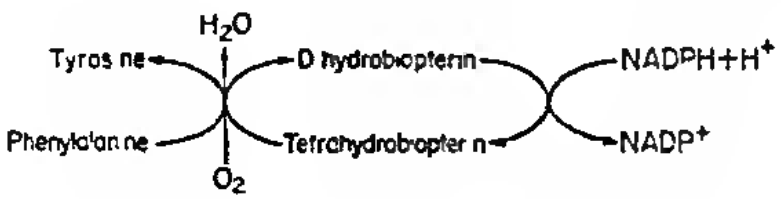


Fig 19 15 Conversion of phenylalanine to tyrosine

Nutritionally essential amino acids

Nutritionally essential amino acids are synthesized by bacteria but the synthesis does not take place in mammalian tissues, hence the synthesis is not discussed here

CATABOLISM OF AMINO ACIDS

List of amino acids converted to carbohydrate and fat or both

GLYCOGEN [GLYCOGENIC L-AMINO ACIDS]	FAT [KETOGENIC L-AMINO ACIDS]	BOTH GLYCOGEN AND FAT [GLYCOGENIC AND KETOGENIC L-AMINO ACIDS]
1 ALANINE	1 LEUCINE	1 ISOLEUCINE
2 ARGININE		2 LYSINE
3 ASPARTATE		3 PHENYLALANINE
4 CYSTINE		4 TYROSINE
5 GLUTAMATE		5 TRYPTOPHAN
6 GLYCINE		
7 HISTIDINE		
8 HYDROXYPROLINE		
9 PROLINE		
10 METHIONINE		
11 SERINE.		
12 THREONINE		
13 VALINE		

L-amino acids are catabolized to amphibolic intermediates

Amino acids forming oxaloacetate

Asparagine and aspartate are converted to oxaloacetate by the successive actions of asparaginase and a transaminase (shown below)

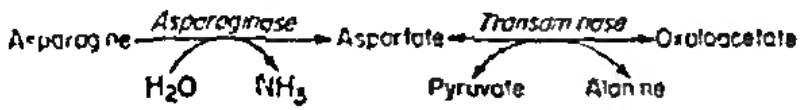


Fig 19 16 Conversion of asparagine to oxaloacetate

Amino acids forming α ketoglutarate

Glutamine and *glutamate* are catabolized like that of asparagine and aspartate but with the formation of α ketoglutarate (shown below)

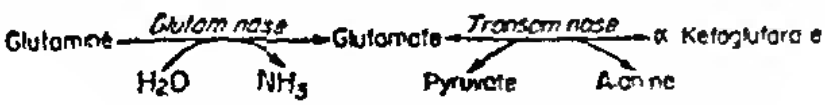


Fig 19 17 Conversion of glutamine to α ketoglutarate

Proline is oxidized to a form of dehydroproline which, on addition of water, forms glutamate γ-semialdehyde This is then oxidized to glutamate and trans aminated to α-ketoglutarate (shown below)

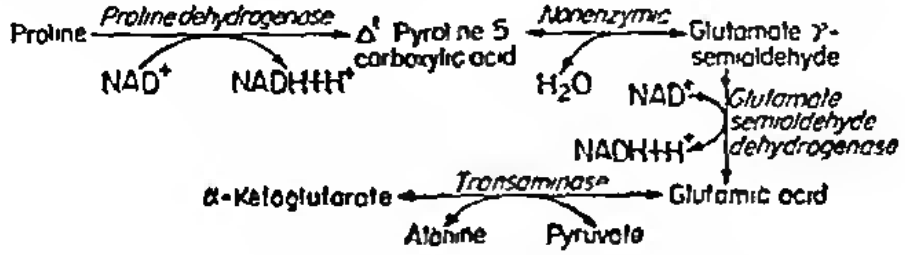


Fig 19 18 Conversion of proline to α ketoglutarate

Arginine and *histidine* are both converted to α-ketoglutarate Arginine is converted to ornithine by arginase with the removal of urea Ornithine by transamination forms glutamate γ-semialdehyde which is oxidized to glutamate and transaminated to α-ketoglutarate (shown below)

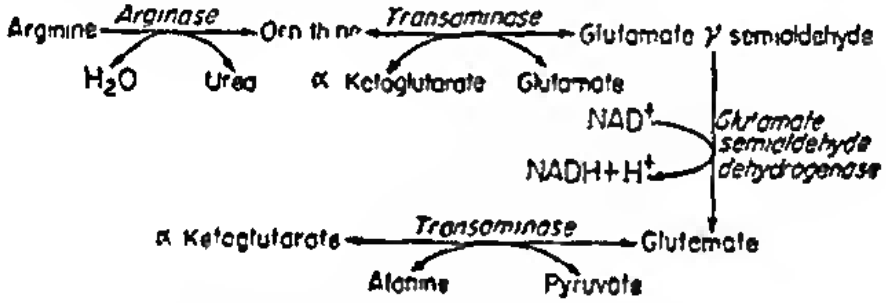


Fig 19 19 Conversion of arginine to α-ketoglutarate

Histidine on deamination produces urocanic acid which is converted to 4-imidazolone 5 propionate by urocanase This product on addition of water and internal oxidation reduction forms glutamate which is transaminated to α-keto-glutarate (shown below)

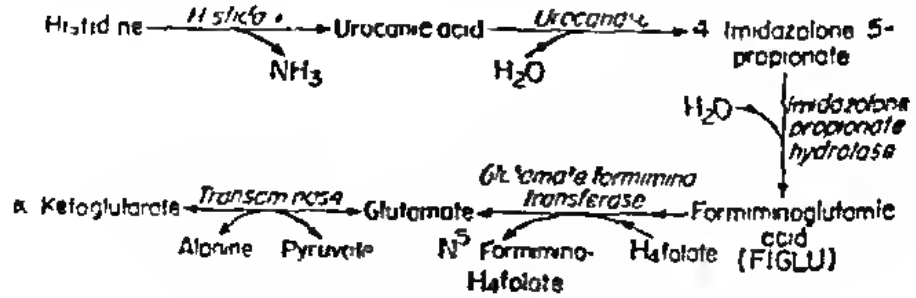


Fig 19 20 Conversion of histidine to α ketoglutarate

Amino acids forming pyruvate

Glycine is converted to serine by serine hydroxymethyltransferase Serine then forms pyruvate by serine dehydratase shown below).

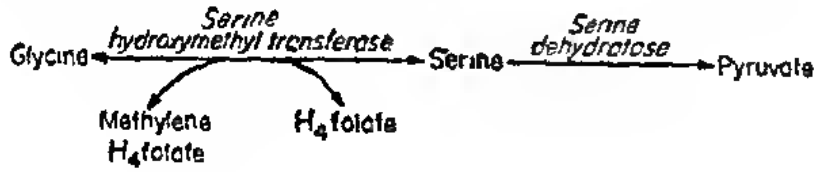


Fig 19 21 Conversion of glycine to pyruvate

Alanine forms pyruvate by transamination

Fig 19 22 Conversion of alanine to pyruvate

Serine is converted to pyruvate by serine dehydratase, a pyridoxal phosphate protein Addition and loss of water as well as loss of ammonia are involved in this reaction. The reaction is shown below

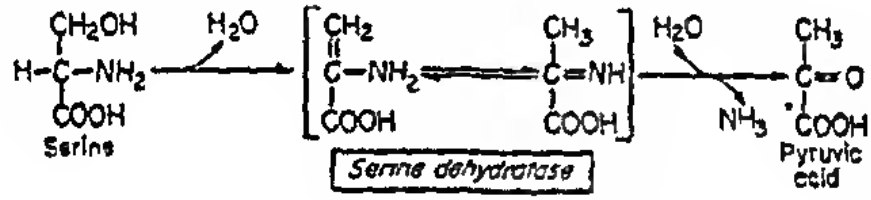


Fig 19 23 Conversion of serine to pyruvate

This conversion of serine to pyruvate is prominent in the liver tissue of rats and guineapigs because serine dehydratase is rich in this tissue of these animals

But in humans and many other vertebrates, serine is degraded to glycine by serine hydroxymethyltransferase The further catabolism follows the glycine cleavage system

Cystine is converted to cysteine by an NADH-dependent cystine reductase as follows :

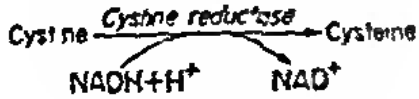


Fig 19 24.

Cysteine is converted to pyruvate by (1) transamination and loss of H_2S

(2) By oxidation of the sulfhydryl group forming cysteine sulfinic acid, transamination and by desulfination (shown below)

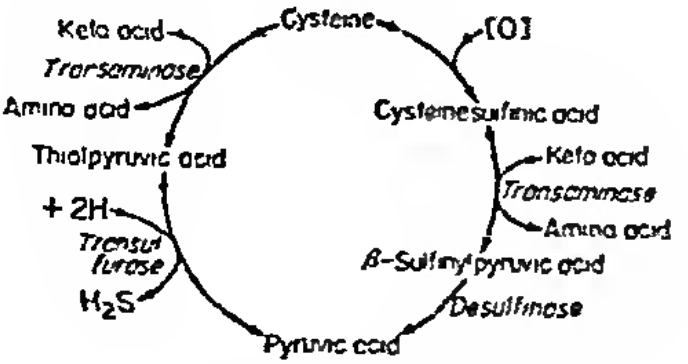


Fig 19.25 Conversion of cysteine to pyruvate

Threonine aldolase cleaves *threonine* to acetaldehyde and glycine Glycine is catabolized to pyruvate as discussed before Both pyruvate and acetaldehyde then form acetyl-CoA (shown below)

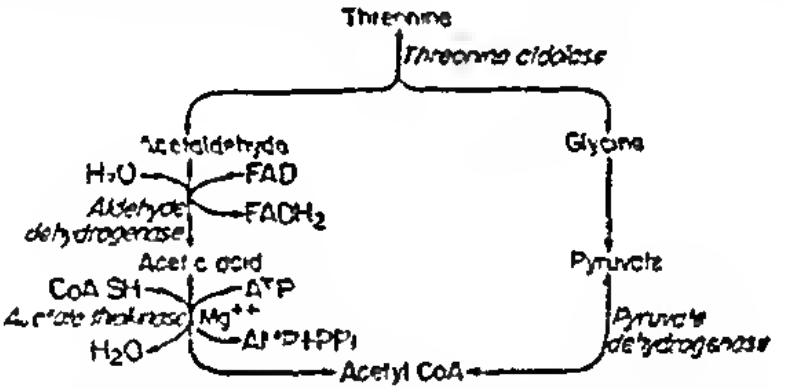


Fig 19.26 Conversion of threonine to acetyl-CoA

Hydroxyproline is converted to pyruvate and glyoxylate The conversion is indicated below

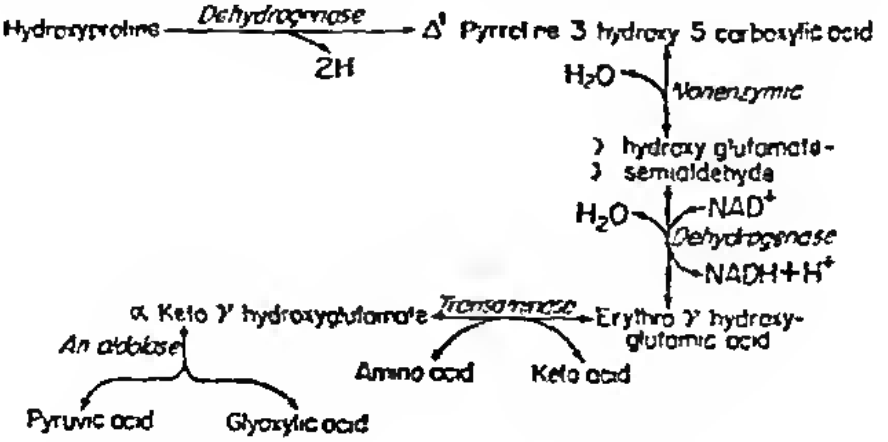


Fig 19.27 Conversion of hydroxyproline to pyruvate

AMINO-ACIDS FORMING ACETYL-CoA

Amino acids forming pyruvate are convertible to acetyl-CoA In addition to this, 5 amino acids form acetyl CoA directly without first forming pyruvate

Pheoylalanine

Phenylalanine (an essential amioo acid) is converted to tyrosioe by pheoylalaoioe bydroxylase , tetrahydropteridine, NADPH and O_2 are required The reaction is not reversible

Fig 19 28

The catabolism of phenylalanine and tyrosioe may be discussed under the following heads :

1 Formation of fumarate and acetoacetate
2 Formation of melanin
3 Conversion to epineplirine
4 Conversion to thyroxine

Major pathway

1. Formation of fumarate and acetoacetate

(a) Tyrosine is transaminated to P-hydroxyphenylpyruvate by *tyrosine-α-ketoglutarate transaminase*, an enzyme of mammalian liver tissue

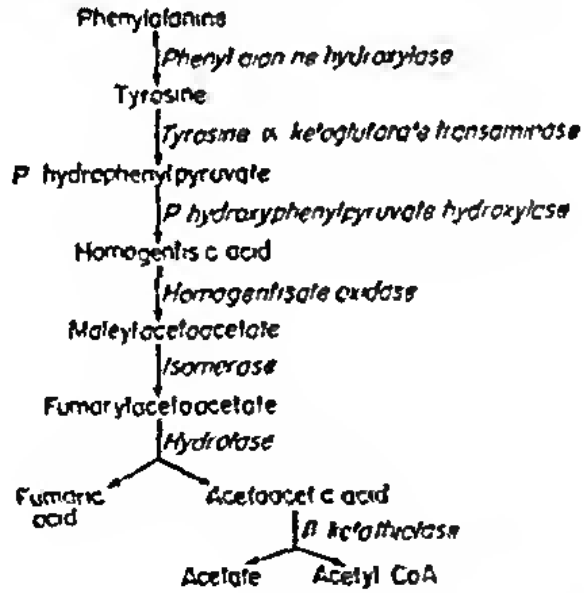


Fig 19 29 Formation of fumarate and acetoacetate

(b) P hydroxyphenylpyruvate hydroxylase, a copper metalloprotein with properties similar to those of tyrosinase, converts P-hydroxypheoylpyruvate to homogentisic acid Ascorbic acid acts as a cofactor in this reaction

(c) By the oxidative reaction catalyzed by *homogentisate oxidase*, an iron metalloprotein of mammalian liver, maleylacetoacetate is formed by the rupture of the benzene ring of homogentisate. The reaction is inhibited by a chelating agent that binds iron.

(d) Maleylacetoacetate is converted to fumarylacetoacetate by *maleylacetoacetate cis-trans isomerase*, present in mammalian liver.

(e) Fumarylacetoacetate on hydrolysis by *fumarylacetoocetate hydrolase* forms fumarate and acetoacetate. Acetoacetate can be converted to acetyl-CoA and acetate by β-ketothiolase

2. Formation of melanin :

(a) Melanin, the black pigment present in the skin, hair and retina of the eye, is formed from pheoylalanine and tyrosine in the specialised cells (melanoblasts) present in the skin. The pathway of melanin formation is given below.

(b) Tyrosine is oxidized to dihydroxyphenylalanine (DOPA) catalyzed by *tyrosinase* in presence of ascorbic acid as cofactor.

(c) DOPA is converted into dopaquinone which is further converted into 5 : 6 dihydroxyindole-2-carboxylic acid This is oxidized to dihydroxyindole which polymerises spontaneously to melanin

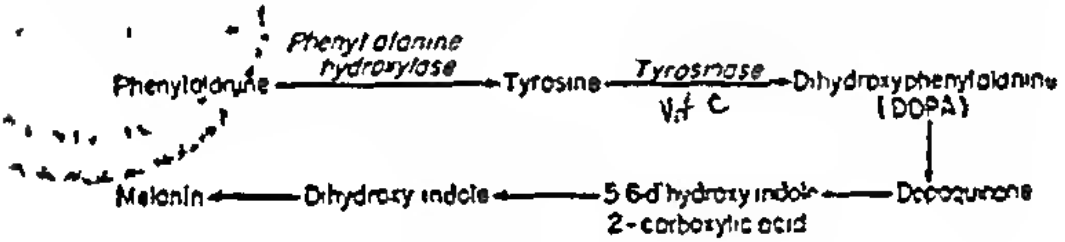


Fig 19 30 Formation of melanin

3. Conversion to Epinephrine :

(a) Pheoylalanine is converted into tyrosine which is further converted into 3 : 4-dihydroxypheoylalanine (DOPA) by *tyrosine hydroxylase* with tetrahydropteridine as cofactor.

(b) DOPA is decarboxylated to dopamine by a *decarboxylase* which is present in many tissues including adrenal medulla with pyridoxal phosphate as cofactor.

(c) The hydroxylation of dopamine is carried out by *dopamine β-hydroxylase* to form norepinephrine in the presence of ascorbic acid and molecular oxygen.

(d) The methylation from methionine converts norepinephrine to epinephrine.

The sequence of reactions are given below.

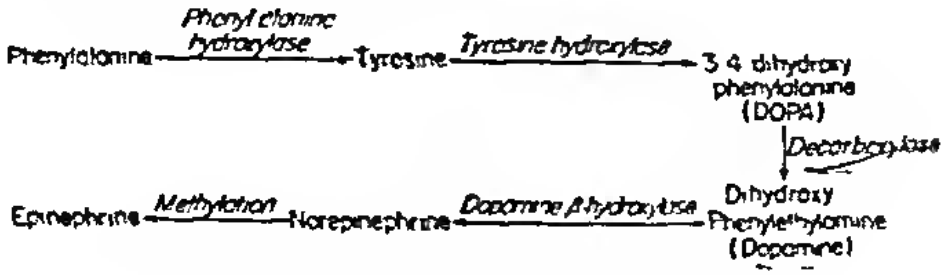


Fig 19 31. Formation of epinephrine

4 **Conversion to thyroxine**

The synthesis of thyroxine takes place in the thyroid gland

(a) Phenylalanine is converted to tyrosine which on iodination forms monoiodotyrosine This on iodination forms diiodotyrosine

(b) Coupling of 2 mols of diiodotyrosine yields thyroxine

(c) Coupling on 1 mol of monoiodotyrosine and 1 mol of diiodotyrosine produces triiodothyronine

The steps are shown below

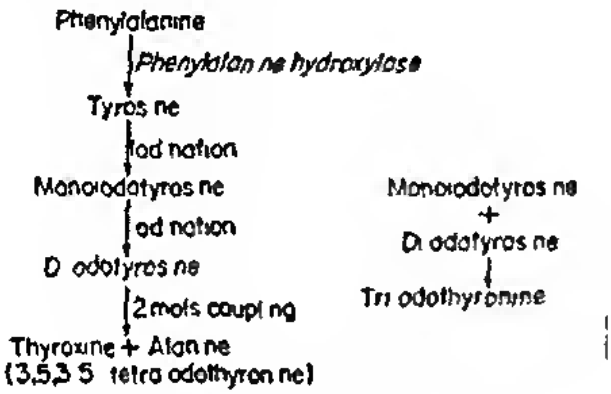


Fig 19 32 Formation of thyroxine

Minor pathway

1 Formation of tyramine
2 Formation of tyrosine-o sulphate

1 **Formation of tyramine** Tyramine is produced by the bacterial decomposition involving decarboxylation of tyrosine by decarboxylase present in bacteria

Fig 19 33 Formation of tyramine

2 **Formation of tyrosine o-sulphate** This is present in fibrinogen Two peptides are liberated during the conversion of fibrinogen into fibrin One of the peptides contain tyrosine-o-sulphate

Tryptophan

The carbon atoms of the side chain and of the aromatic ring of tryptophan may be completely degraded to amphibolic intermediates This proceeds via kynurenine anthranilate pathway This pathway is important for degradation of tryptophan as well as niacin formation from tryptophan

The catabolism of tryptophan may be discussed under the following pathways

1 Kynurenine-anthranilate pathway

2 Serotonin pathway

3 Minor pathways

1 **Kynurenine-anthranilate pathway**

(a) *Tryptophan oxygenase (Tryptophan pyrrolase)* cleaves the indole ring of tryptophan with the incorporation of 2 atoms of molecular oxygen forming N-formylkynurenine The oxygenase enzyme is an iron porpbyrin metalloprotein which is present in the liver of mammals, amphibians birds and insects Four forms of hepatic tryptophan pyrrolase have been described the active holo-enzyme, the apoenzyme, third form is combined with hematin and the fourth form requires prolonged incubation The chief inducing agents of tryptophan pyrrolase is adrenal corticosteroids induction is blocked by puromycin Tryptophan stabilizes the enzyme toward proteolytic degradation

(b) *Kynurenine formylase* of mammalian liver catalyzes the hydrolytic removal of the formyl group of N formyl kynurenine producing *kynurenine*

(c) Kynurenine on deamination produces 2 amino 3 hydroxybenzoyl pyruvate which loses water and then undergoes spontaneous ring closure forming *kynurenic acid* This is not formed in the main pathway of tryptophan breakdown

(d) Kynurenine is hydroxylated by kynurenine hydroxylase with molecular oxygen in presence of NADPH to *3 hydroxy-kynurenine*

(e) 3 hydroxyanthranilic acid is formed from 3 hydroxy kynurenin by the enzyme *kynureninase* which requires *vitamin* B_6 (pyridoxal phosphate) as coenzyme. In the deficiency of vitamin B_6 kynurenine derivatives reach the extrahepatic tissues where they are converted to xanthurenic acid This is found in the urine of human, monkeys and rats when there is dietary deficiency of vitamin B_6 Excess tryptophan feeding can induce excretion of xanthurenic acid if vitamin B_6 deficiency exists The kidney can produce xanthurenic acid derivatives from kynurenine

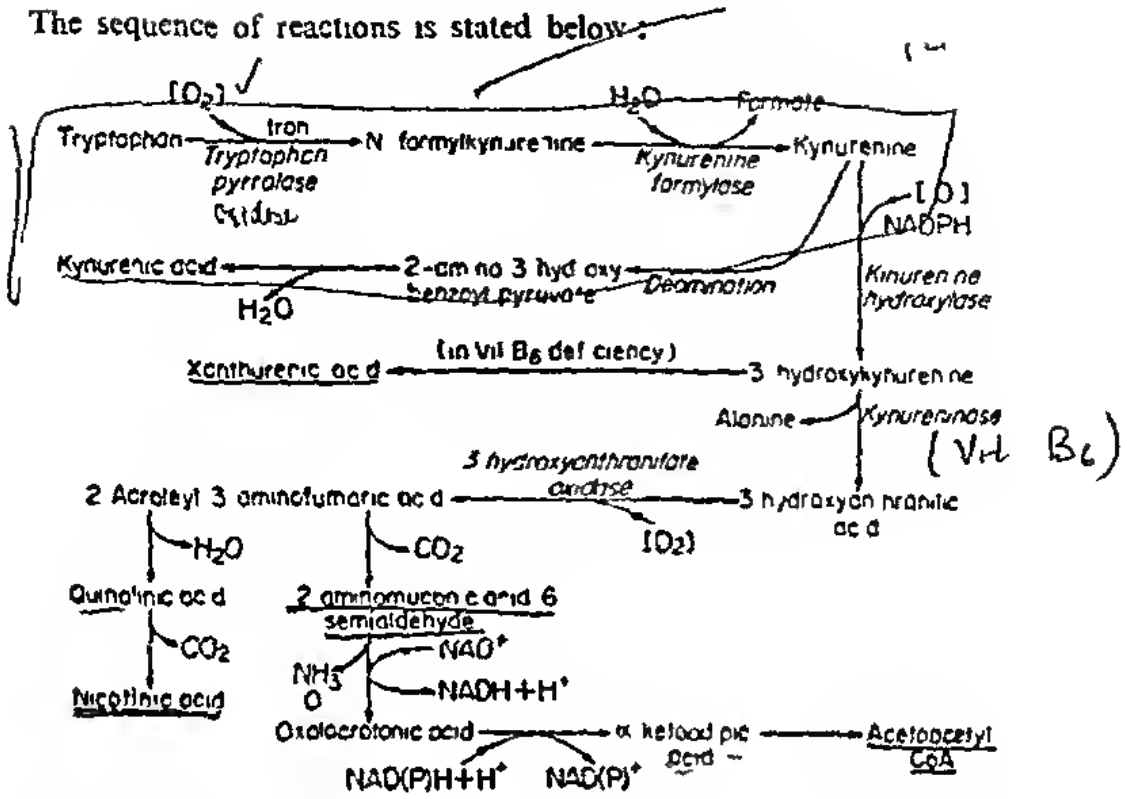
In the deficiency of vitamin B_6 the synthesis of pyridine nucleotides (NAD and NADP) in the tissues is impaired owing to the nonconversion of tryptophan to nicotinic acid 60 mg of tryptophan produce 1 mg of nicotinic acid

(f) 3 hydroxyanthranilic acid is then converted to 2-acroleyl 3 aminofumaric acid by the specific oxidase 2 acroleyl 3 aminofumaric acid is dehydrated to quinolinic acid which on decarboxylation produces nicotinic acid

(g) *Riboflavin* is also necessary for the formation of 3 hydroxy kinurenine In riboflavin deficiency, anthranilic acid and *5* hydroxyanthranilic acid are excreted In febrile state, man often excretes 3 hydroxykynurenine

(h) 2 acroleyl 3 aminofumaric acid is decarboxylated to form 2 amino-muconic acid 6-semialdehyde which on deamination and oxidation produces oxalocrotonic acid This on reduction forms α ketoadipic acid which ultimately forms acetoacetyl CoA

The sequence of reactions is stated below:



In the deficiency of riboflavin :

Kynurenine ⟶ Anthranilic acid ⟶ 5-hydroxy-anthranilic acid

Fig 19.34 Kynurenine—anthranilate pathway

2 Serotonin pathway

Tryptophan on hydroxylation in the liver forms 5-hydroxytryptophan which on decarboxylation produces 5-hydroxytryptamine (serotonin), a stimulant of the central nervous system and also a vasoconstrictor. Serotonin is stored in platelets. It also occurs in intestinal mucosa where it promotes peristalsis. Serotonin is broken down by oxidative deamination to 5-hydroxyindoleacetic acid which is excreted in the urine.

The sequence of reaction is given below

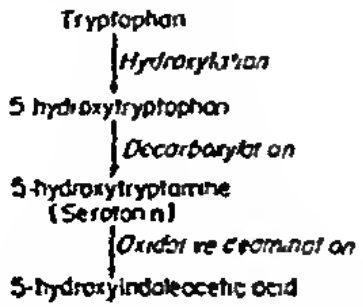


Fig 19.35 Serotonin pathway for tryptophan catabolism

Serotonin on acetylation and methylation produces N-acetyl-5-methoxytryptamine (the hormone *melatonin*)

3 Minor pathways

Minor pathways include .

(i) Formation of kynurenic acid

(ii) Formation of tryptamine

(iii) Formation of indoleacetic acid

(i) *Formation of kynurenic acid* Mentioned in the kynurenic anthranilate pathway (Fig 19 34)

(ii) *Formation of tryptamine* Small quantities of tryptophan are converted into tryptamine by tryptophan decarboxylase present in bacteria in large intestine

$$\text{Tryptophan} \xrightarrow{\text{Decarboxylase}} \text{Tryptamine}$$

Fig 19 36 Formation of tryptamine

(iii) *Formation of indoleacetic acid* • Oxidative deamination converts tryptophan to indolepyruvic acid which in turn, is oxidized to indoleacetic acid Indoleacetic acid is further converted to indole, skatole and indoxyl by intestinal bacteria The reactions are shown below

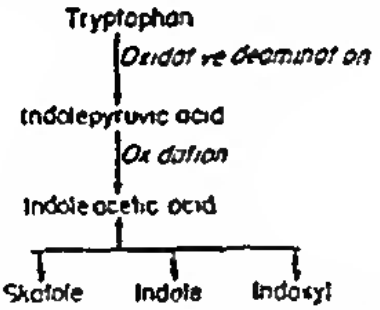


Fig 19 37 Conversion of tryptophan (minor pathways)

AMINO ACIDS FORMING SUCCINYL-CoA

Methionine

(a) Methionine is first activated by ATP forming S adenosylmethionine ("active methionine")

(b) After removal of the methyl group, the active methionine is converted into S-adenosylhomocysteine This is hydrolyzed to produce homocysteine and adenosine

(c) Homocysteine then condenses with a molecule of serine forming cystathionine which produces homoserine and cysteine on hydrolytic cleavage.

(d) Homoserine is then converted to α ketobutyric acid by the enzyme homoserine deaminase

(e) The oxidative decarboxylation converts α-ketobutyrate to propionyl CoA.

(f) Propionyl CoA is converted to D-methyl malonyl-CoA by propionyl-CoA carboxylase in presence of ATP and biotin as coenzyme

(g) Methylmalonyl-CoA racemase converts D-methyl malonyl-CoA to L-methylmalonyl-CoA which in turn, is converted into succinyl-CoA by the specific isomerase with B_{12} as coenzyme

The reactions are given below :

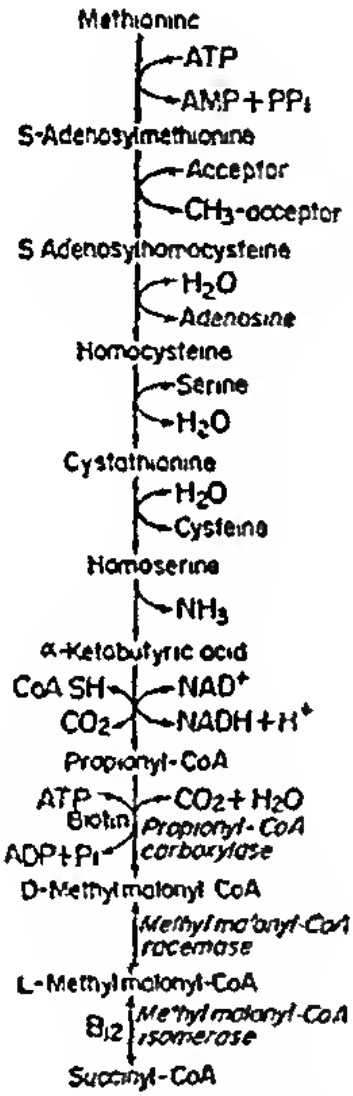


Fig 19.38 Catabolism of methionine

Leucine :

(a) Leucine is transaminated to produce α-ketoisocaproic acid which on decarboxylation yields isovaleryl-CoA. This on dehydrogenation produces β-methylcrotonyl-CoA.

(b) Carboxylation converts β-methylcrotonyl-CoA into β-methylglutaconyl-CoA which in turn, is converted to β-hydroxy-β-methylglutaryl-CoA on hydration. This product is cleaved into acetoacetate plus acetyl-CoA.

The reactions are given below

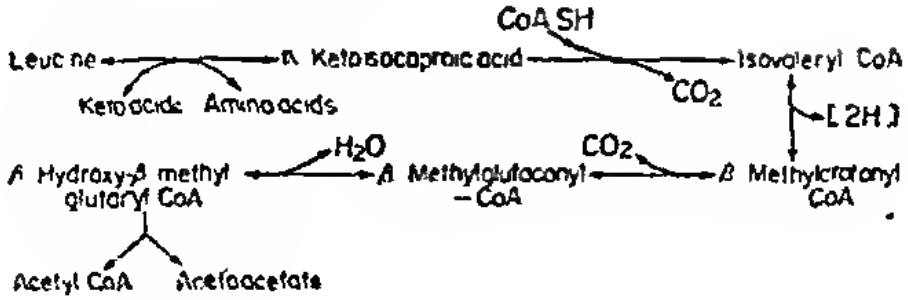


Fig 19 39 Catabolism of leucine

Valiae

(a) Valine on transamination produces α-keto-isovaleric acid which on decarboxylation yields isobutyryl-CoA This on dehydrogenation produces methylacrylyl-CoA

(b) Methylacrylyl-CoA is hydrated to produce β hydroxyisobutyryl-CoA which on hydrolysis gives β-hydroxyisobutyric acid This is converted into methylmalonic acid semialdehyde

(c) The semialdehyde is oxidized to yield methylmalonic acid which on acylation yields methylmalonyl-CoA This ultimately produces succinyl-CoA as shown in leucine catabolism

The reactions are given below

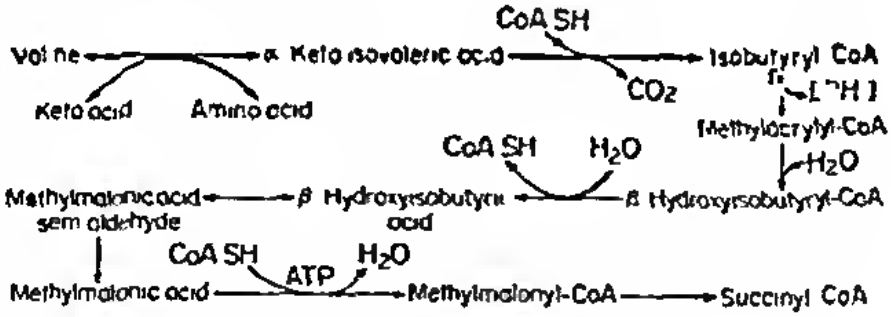


Fig 19 40 Catabolism of valine

Isoleucine

(a) Isoleucine on transamination produces α-keto β-methylvaleric acid which on decarboxylation yields α methylbutyryl-CoA This on dehydrogenation produces tiglyl CoA

(b) Tiglyl-CoA is hydrated to produce α methyl-β-hydroxybutyryl-CoA which on dehydrogenation forms α-methylacetoacetyl-CoA Thiolytic cleavage forms acetyl CoA plus propionyl-CoA Propionyl CoA is converted to succinyl-CoA as mentioned in leucine catabolism

Reactions are given below :

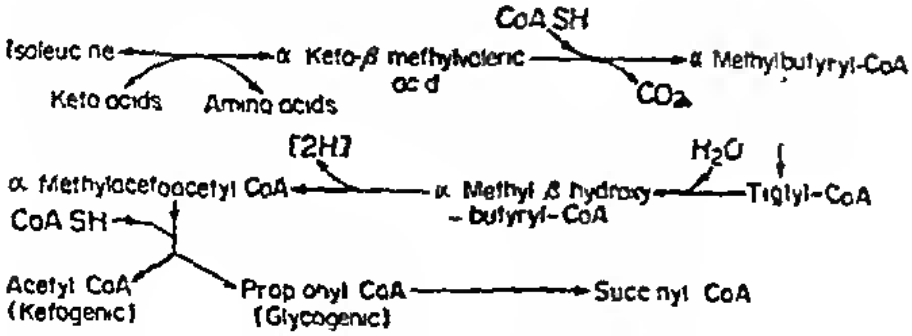


Fig 19.41 Catabolism of Isoleucine

Catabolism of Histidine

The catabolism of histidine proceeds under the following pathways

1 Urocanic acid pathway
2 Histamine pathway
3 Imidazole pyruvic acid pathway
4 Minor pathways

1 Urocanic acid pathway

This is the major pathway of histidine catabolism

(a) *Histidine* is first converted into urocanic acid by the removal of one mol of ammonia by the enzyme histidase

(b) The enzyme urocanase with pyridoxal phosphate (B_6-Po_4) as coenzyme converts urocanic acid to imidazolone-3 propionic acid which in turn is converted into α formiminoglutamic acid (FIGLU) by hydrolase

(c) α-Formimino glutamic acid is then converted into glutamic acid resulting in the transfer of C_1 carbon atom to tetrahydrofolic acid

In human beings and animals, deficiency of folic acid or vitamin B_{12} causes an excessive excretion of FIGLU. A small amount of imidazole-3 propionic acid may be oxidized to hydantin-3-Propionic acid which is excreted in the urine

The reactions are stated below

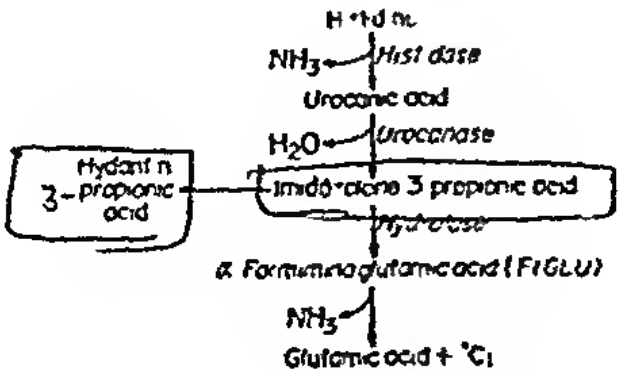


Fig 19.42 Urocanic acid pathway

2 Histamine Pathway

Histidine is decarboxylated by histidine decarboxylase to form histamine This is formed at a significant rate in fetal and regenerating liver as well as in healing skin wounds

Histamine is detoxified as its methyl or acetyl derivatives or to oxidize to imidazole acetate which are excreted in urine The reactions are given below

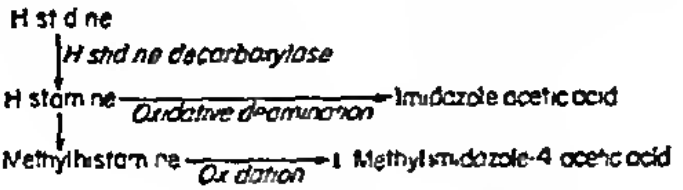


Fig 19 43 Histamine pathway

3 Imidazole Pyruvic acid pathway

Histidine by transamination is converted into imidazole pyruvic acid which is further oxidized to imidazole acetic acid as noted below

Histidine
↓
Imidazole pyruvic acid
↓
Imidazole acetic acid

4 Minor pathways

(a) Histidine is converted into carnosine and anserine by minor pathways. These compounds are found in muscle Carnosine is a peptide of histidine and β-alanine whereas anserine is a peptide of methylhistidine and β-alanine

(b) Ergothioneine the betaine of 2 mercaptohistidine, is present in high concentration in human erythrocytes It is also found in liver and brain

GLUTATHIONE

Formation

Glutathione is a tripeptide of glycine cysteine and glutamic acid. Its synthesis is as follows

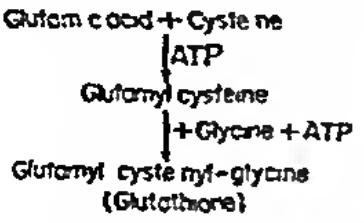


Fig 19 44 Synthesis of glutathione

Functions

1 It acts as a coenzyme for the enzyme glyoxalase which converts methyl glyoxal to lactic acid.

2 It acts as a coenzyme for formaldehyde dehydrogenase which converts formaldehyde to formic acid

3 It acts as coenzyme for maleyl acetoacetate isomerase

4 It is involved as a coenzyme in the γ glutamylacyl transferase for amino acid transport in some cells

5 As a coenzyme it participates in the formation of prostaglandin PGE_2 from arachidonic acid

6 It takes part as a coenzyme in the prevention of peroxidation of poly unsaturated fatty acids in tissues

CREATINE AND CREATININE

Chemistry

Creatinine is the anhydride of creatine and a product of endogenous metabolism Both creatine and creatinine are readily interconverted in solution Acid favours the formation of creatinine The structures are given below

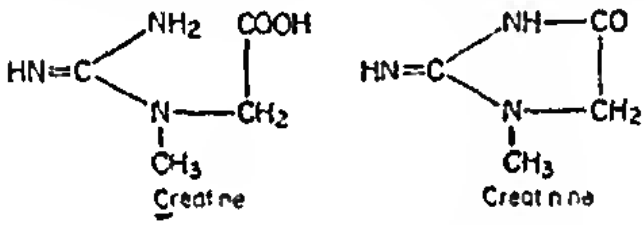


Fig 19 45 Structure of creatine and creatinine

Occurrence

Creatine is widely distributed in animal tissues It is present in muscle, brain and blood as phosphocreatine and also in the free state Skeletal muscle contains about 0 5 per cent creatine and heart muscle about half that amount 98 per cent of the total creatine in the body is in the muscles Creatinine is formed largely in the muscle by the irreversible and nonenzymic removal of water from creatine phosphate Traces of creatine are also normally present in urine Creatinine formation is a preliminary step required for the excretion of most of the creatine

Biosynthesis of creatine and creatinine

1 Three amino acids—Glycine, arginine, and methionine are directly involved

2 The first reaction is that of transamidination from arginine to glycine to form guanidoacetic acid (Glycocyamine) This reaction takes place in the *kidney* but not in the liver or in heart muscle Recently, evidence has shown that nephrectomized rats can still synthesize creatine The interpretation is that there is the existence of an extrarenal site or sites of transamidination in this animal

3 The synthesis of creatine is completed in the *liver* by the methylation of glycocyamine Active methionine is the methyl donor Other methyl donors-betaine or choline after oxidation to betaine—serve indirectly by producing methionine through the methylation of homocysteine The methylation of glycocyamine is not reversible Creatine or creatinine can not methylate homocysteine to methionine In the methylation of creatine, ATP and oxygen are required

The enzymatic mechanisms for the methylation of glycocyamine involves first the formation of active methionine (S-adenosylmethionine) which requires ATP, Mg^{++} and glutathione as well as a methionine activating enzyme. The methylation of glycocyamine by active methionine is catalyzed by *guaridoacetate methylferase* found in the liver of mammals. Glutathione or other reducing substances are required for the optimal activity of the enzyme. There is no evidence for the requirement of metal ions or other cofactors

It has also been found that *Pancreas* can synthesize glycocyamine. Therefore, *Pancreas* may play an important role in the synthesis of creatine within the body of mammals

Dietary creatine or high blood creatine has no effect on the rate of synthesis of creatine in the liver The rate of creatine biosynthesis is dependent on kidney transamidinase activity Hyperthyroidism is associated with reduced kidney transamidinase activity. The effect of hyperthyroidism on kidney transamidinase is mediated by the increased levels of blood creatine.

4 Creatinine is the anhydride of creatine and is formed by the non-enzymatic means in muscle.

The overall reaction is given below :

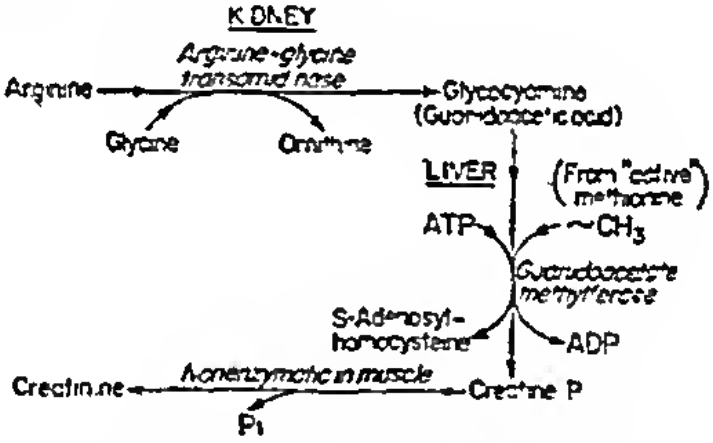


Fig 19 46 Biosynthesis of creatine and creatinine

Absorption

It is completely reabsorbed in the renal tubules at lower concentrations.

Normal level of creatine and creatinine in the blood.

Creatine .	2.6—7.6 mg/100 ml.
Creatinine	0 7—1 5 mg/100 ml.

Daily excretion in urine.

Creatine .	
Prepubertal children	4.2 mg/kg.
During pregnancy and occasionally in normal non-pregnant women	0—100 mg
Normal males on a balanced diet	Nil
Normal subject maintained on a very low carbohydrate intake and during fasting period (due to excessive catabolism of muscle tissue)	Upto 100 mg.

Creatinine :

Normal adult males 1 to 2 grams.
,, ,, females 0 8 to 1 8 grams.

Creatinine coefficient ·

This term is used to indicate the number of milligrams of creatinine (plus creatine) nitrogen excreted per kilogram of body weight in 24 hours.

The creatinine coefficient is an index of the amount of active muscle tissue in the body

Normal values of creatinine coefficient range from 20 to 26 mg creatinine for men and 14 to 22 mg creatinine for women

Clinical importance :

1. The amount of creatinine excreted is not proportional to the size of the subject but to his muscular development A man with well-developed muscles excrete more than a less muscular man of the same weight Ordinary muscular exertion does not affect the daily output of creatinine, which is approximately the same on a day of exercise as on a day of rest.

2 Excessive creatinuria may occur in starvation, febrile and wasting diseases, diabetes mellitus, in certain myopathies (myotonia congenita, various forms of myositis, congenital muscle dystrophies, congenital muscular hypertrophy and secondary muscle atrophy)

3 Excessive creatinuria has also been observed in hyperthyroidism, during the menstrual period and in pregnancy It has been observed after fractures (to 500 mg. daily) It disappears gradually during the period of healing of the fracture

4. Testosterone diminishes creatinuria in castrates by increasing the storage but not the synthesis of creatine The administration of methyltestosterone causes the increased excretion of creatine and creatinine due to increased synthesis of creatine.

5. In myasthenia gravis, the administration of large doses of glycine results in the increased excretion of creatine Such increase in creatinuria also occurs in the majority of normal subjects after the ingestion of glycine

Creatine tolerance :

1 When 1 or 2 gms of creatine are ingested by a normal adult, about 80 per cent is retained by normal men and about 70 per cent by normal nonpregnant women About 20 and 30 per cent respectively are excreted in the urine during the subsequent 24 hours

2. Diminished tolerance (i e. excessive excretion) is present in mild cases of above mentioned disorders

3 Creatine tolerance is decreased in the majority of patients with hyperthyroidism and increased in hypothyroidism This procedure carries much values in the diagnosis of hyperthyroidism or hypothyroidism in children

FATE OF AMINO ACIDS IN THE BODY :

1 Tho amino acids are required for the synthesis of protein

2 These are utilized for the synthesis of methionine which is methyl donor This methyl donor is required for the synthesis of choline, betaine, creatine and epinephrine. Methionine can also be converted into cystine when this amino acid is inadequately supplied in the diet.

3 Phenylalanine can be converted into tyrosine and tyrosine is the precursor of epinephrine, thyroxine and the pigment melanin

4 Glycine, arginine and methionine all participate in the synthesis of creatine

5 Glutamine, formed from glutamate, is the chief source of ammonia formation in the kidney Ammonia helps in the neutralization of acid to be excreted.

6 Glycine acts as a coupling agent to convert benzoic and salicylic acids into hippuric acid and salicyluric acid respectively which are readily excreted by the kidney

7 Histidine can be decarboxylated to produce histamine

8 Tryptophan can be converted into nicotinic acid in presence of vitamin B_6 and B_2 and into serotonin which on acetylation and methylation produces the hormone melatonin

9 Glutamate, aspartate and the ammonia formed from amino acids by deamination are utilized for purine and pyrimidine synthesis

10 Glutamate and aspartate are involved in transamination reaction to form keto acids which are converted into glucose by gluconeogenesis

11 Alanine on deamination forms pyruvic acid which is an important intermediate compound in the metabolism of glucose The fate of alanine is the same as that of glucose Hence, alanine can be regarded as a glucose former

12 In case, the diet contains more proteins than is required to replace the catabolized protein, the excess of amino acids is used for energy production The presence of an oxidative deaminating enzyme system in the liver (and kidney) can convert amino acids into keto acids liberating ammonia in the free state The ammonia is used for the synthesis of ammonia.

13 Some of the amino acids are metabolized to glucose derivatives while others form acetoacetate

NITROGEN BALANCE

Nitrogen balance is defined as the quantitative difference between the nitrogen intake and the nitrogen output, both expressed in gms N/day Intake means the nitrogen of the food and output means the excretion of nitrogen as urine, feces milk, sweat, vomiting, menstrual fluid and loss of hair

Positive nitrogen balance exists when intake exceeds output. This condition occurs whenever new tissue is synthesized such as during growth of the young and in pregnancy

In negative nitrogen balance, the output exceeds the intake This condition occurs in inadequate intake of protein (fasting, diseases of the gastro-intestinal tract), in accelerated catabolism of tissue protein (fevers, infections, wasting diseases and trauma) and in the absence of "essential" amino acid from the diet.

It is concluded from the above discussion that nitrogen equilibrium [intake= output] is maintained only in the adult organisms and only in the absence of the abnormal conditions

An "adequate diet" contains all requirements for minerals and vitamins the protein of the diet is of high "biological value" and is administered at a sufficiently high level, other caloric needs are met satisfactorily by the Carbohydrate and fat of the diet A minimum amount of dietary carbohydrate (5 grams/100 calories) is required for the maintenance of nitrogen equilibrium independently of the adequacy of the energy provision from fat and

protein This is referred to as the "protein sparing action' of carbohydrate Under these circumstances, the nitrogen output equals the intake An increase or decrease in the intake is followed by a corresponding adjustment in the output, so that nitrogen equilibrium is established at a new level There is no marked tendency for storage of surplus nitrogen

PROTEINURIA

The glomerular membrane permits only a very small amount of the normal plasma proteins. The glomerular filtrate normally contains 10 to 25 mg protein per 100 ml. This is almost completely reabsorbed by the tubular epithelial cells 100 mg is the upper limit of normal daily protein excretion in the urine Hence, it is difficult to detect albumin in the urine normally

In nephritic syndrome, urinary albumin Globulin ratios are very high In chronic glomerulonephritis, the ratio is lower In acute nephritis, the ratio is low Low values are found in amyloid disease of the kidneys

Certain foreign proteins entering the plasma are eliminated by the kidney, whereas normal plasma protein is retained in the blood Thus, hemoglobin when free in the plasma, egg albumin and other foreign proteins if introduced into the blood stream, appear in the urine

Two major mechanisms operate to produce abnormal proteinuria

1 *Humoral proteinuria* Plasma contains abnormal proteins of small molecular size which pass readily through normal glomeruli

2 *Renal proteinuria* The glomerular membranes are so injured that they become more permeable to the normal plasma proteins or tubular epithelial cell damage results in inadequate reabsorption of proteins from the glomerular filtrate

Abnormal proteinuria occurs when the quantities of proteins reaching the tubules exceed their reabsorptive capacity

The pathogenesis of proteinuria may be summarized as follows

1 In the majority of instances, glomerular abnormalities—structural or functional, primary or secondary—play a fundamental role

2 In many cases, associated tubular involvement is the primary factor

3 A few cases are due to abnormal proteins in the plasma

4 The urinary proteins originate in the lower urinary tract

Proteinuria is classified into three classes

1 Transitory proteinuria It never indicates permanent renal disease It may occur in fever, after intense physical exertion and after minor emotional stimuli

2 Continuous proteinuria : This is always pathological and is indicative of pyelonephritis

3 Orthostatic proteinuria : This is caused by erect posture in lumbar lordosis and is common in adolescence

METHIONINE

Methionine is an essential and a sulphur containing amino acid

Structure $CH_3\ S\ CH_2\ CH_2\ CH\ COOH$

NH_2

Concentration in blood	0 85 mg /100 ml	
Daily requirements	Infants	85 mg /kg body weight.
	Adult male	1 5 mg /kg ,, ,,
	Adult female	4 7 mg /kg , ,,

Physiological functions :

1 Methionine, as the sole source of sulphur containing amino acids, is capable of maintaining nitrogen equilibrium

2 Active methionine is the main methyl donor of the body

3 Choline can be formed from ethanolamine if enough methionine is available in the body

4 Methionine, after donating methyl group, is converted into homocysteine from which cysteine is formed

5 It donates methyl group for the synthesis of creatine from guanidoacetic acid

6 It is involved in the detoxification of nicotinic acid for the formation of the excretory product trigonelline

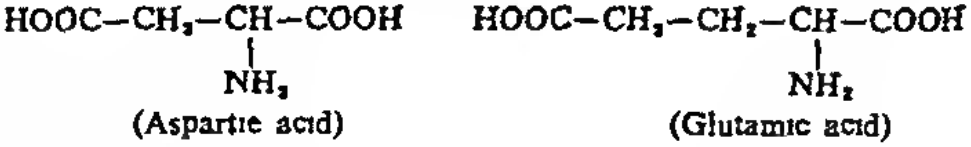
7 Methyl group is donated by methionine for the formation of epinephrine from norepinephrine

8 It acts as a lipotropic factor

ACIDIC AMINO ACIDS

The acidic amino acids are 1 Aspartic acid (α-amino succinic acid) 2 Glutamic acid (α amino glutaric acid)

Structure :



(Aspartic acid) (Glutamic acid)

Biological importance :

1 These amino acids are nonessential and readily synthesized in the body

2 These are metabolically the most reactive of the amino acids

3 These are the active agents in the processes of deamination and amination through the participation in transamination reactions

4 Glutamic acid (as acetyl glutamate) participates in the conversion of CO_2 to "active CO_2" and 'active CO_2' to carbamoyl phosphate which in—combination with ornithine forms citrulline

5 Aspartic acid participates in the conversion of citrulline to arginine

6 Decarboxylation of aspartic acid leads to the formation of β-alanine which is a constituent of pantothenic acid.

7 Both the amino acids are glucogenic only

8 Asparagine formed from aspartic acid plays an important role in the metabolism of plants

9 Glutamic acid is a component of glutathione and folic acid

10 Glutamic acid is converted into glutamine in the kidney which is the source of urinary ammonia Glutamine also serves as a reservoir of amino nitrogen in the body

Metabolism .

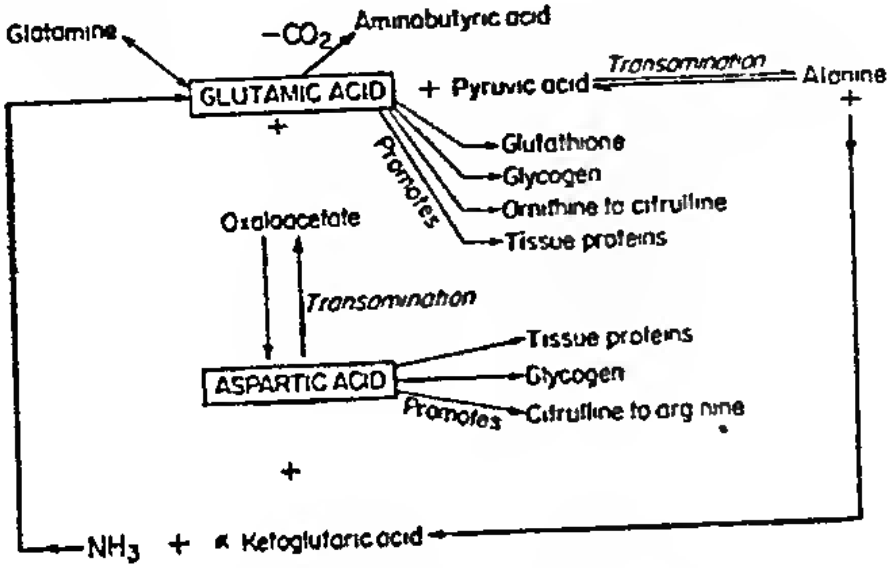


Fig 19 47 Metabolism of acidic amino acids

EDEMA

Edema is recognized by the increase in the volume of interstial fluid This is of three types Nephrotic type, nephritic type and cardiac edema

1. Nephrotic type : This type is produced by the lowering of serum protein concentration due to severe albuminuria or to low protein intake If the serum colloidal osmotic pressure drops below the normal, there is accumulation of interstitial fluid

2 Nephritic type : This type is formed by damage to the capillary endothelium allowing the passage of protein into the interstitial fluid The lowered osmotic pressure allows greater filtration and less reabsorption This mechanism is applied in acute glomerulonephritis Renal factors also play an important role in it

3 Cardiac edema : Increased venous pressure produces cardiac edema. Many believe that renal retention of sodium produced by forward failure of heart leads to retention of water, to increased venous pressure and then to edema

Clinical manifestations of edema :

1 Facial edema distinguishes nephritic or nephritic edema from cardiac edema The whole face becomes swollen Patients with uremia often have such facial edema

2 General dehydration of uremia usually minimizes the facial edema.

3 Edema of the brain has also been reported in acute glomerulonephritis

4 Edema produced by "forward failure" diminishes cardiac output and thereby decreases renal circulation The lowering of glomerular filtration produces a greater reabsorption of sodium which in turn causes a greater reabsorption of water

Management of edema :

Diuresis is essentially an increase in urine output sufficient to produce a significant negative balance of water and negative balance of water signifies negative balance of sodium In most edema, if glomerular filtration is not greatly diminished, diuresis can be established by sodium intake. The water intake need not be limited Normal or even higher than normal ingestion of water may enhance the urine output. Such management is beneficial in cardiac, nephrotic and cirrhotic edema.

Two new powerful diuretics—Ethacynic acid and furosemide—have recently been introduced Ethacynic acid inhibits the reabsorption of sodium in proximal and distal segments Furosemide produces a prompt excretion of water, sodium and chloride Potassium injection is moderate It inhibits reabsorption of sodium also

AMINOTRANSFERASES [TRANSAMINASES]

Aminotransferases catalyze the transfer of amino group from an amino acid to a keto acid , a new amino and keto acid are formed in this process

Clinically two most important aminotransferases are Glutamic oxalacetic transaminase (GOT), now designated asparate aminotransferase and glutamic pyruvic transaminase (GPT), now designated alanine aminotransferase These enzymes are widely distributed in tissues and are normally present also in the blood serum in concentrations upto 19 U/l

Aminotransferase activity is increased in certain diseases involving tissues like liver and myocardium owing to the liberation of abnormally large amounts of it from the damaged tissues

Serum amino transferase activity is increased in inflammatory, degenerative, and neoplastic lesions of the liver The extent of rise depends on the severity of the disease The highest values have been obtained in acute hepatic necrosis. Lower values (<250 U/l) are obtained in uncomplicated portal cirrhosis, biliary cirrhosis and extrahepatic biliary obstruction as well as in hepatic malignancy (Primary or metastatic)

Serum GOT activity is increased in acute heart failure , the values are usually below 100 U/l, but occasionally as high as 1500 U/l Slight or moderate increases may occur after intracardiac operations In acute myocardial infarction, GOT activity is significantly increased but no increase in GPT The determination of GOT is diagnostically important for the following circumstances

1 Differentiation between acute myocardial infarction and coronary insufficiency No increase occurs in coronary insufficiency

2. Diagnosis of acute myocardial infarction when the ECG changes are not definitive or are difficult to interprete because of previous infarction

3 Diagnosis of extension of the original infarction or of recurring acute infarction

Serum GOT may increase in muscular dystrophies, myositis and gangrene An increase may occur in patients with acute pancreatitis, leukemia and toxemia of pregnancy No increase in serum GOT occurs in muscular diseases of nervous origin Increases occur in patients with acute hemolytic anemia and in normal person after prolonged severe exercise Frythromycin can cause an increase in serum GOT

Isoenzymes · GOT exists in two isomeric forms which can be demonstrated by electrophoresis One is derived from cytoplasm and the other from mito-

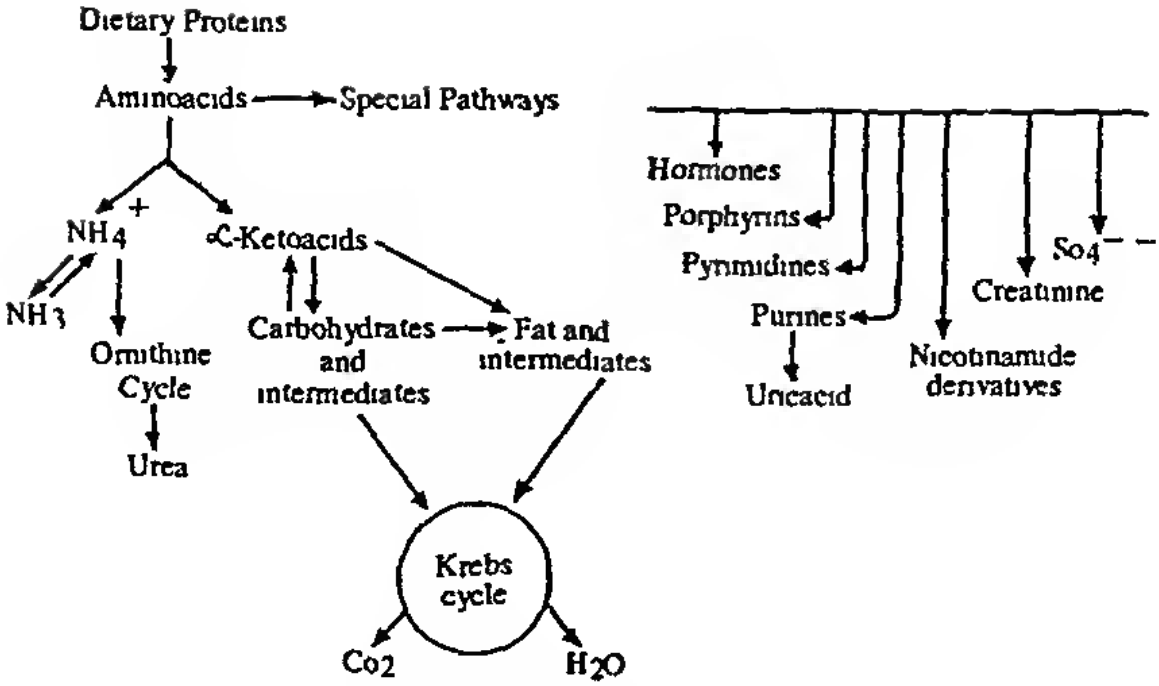


Fig 19 48 . General Pathways of Protein and amino acid metabolism

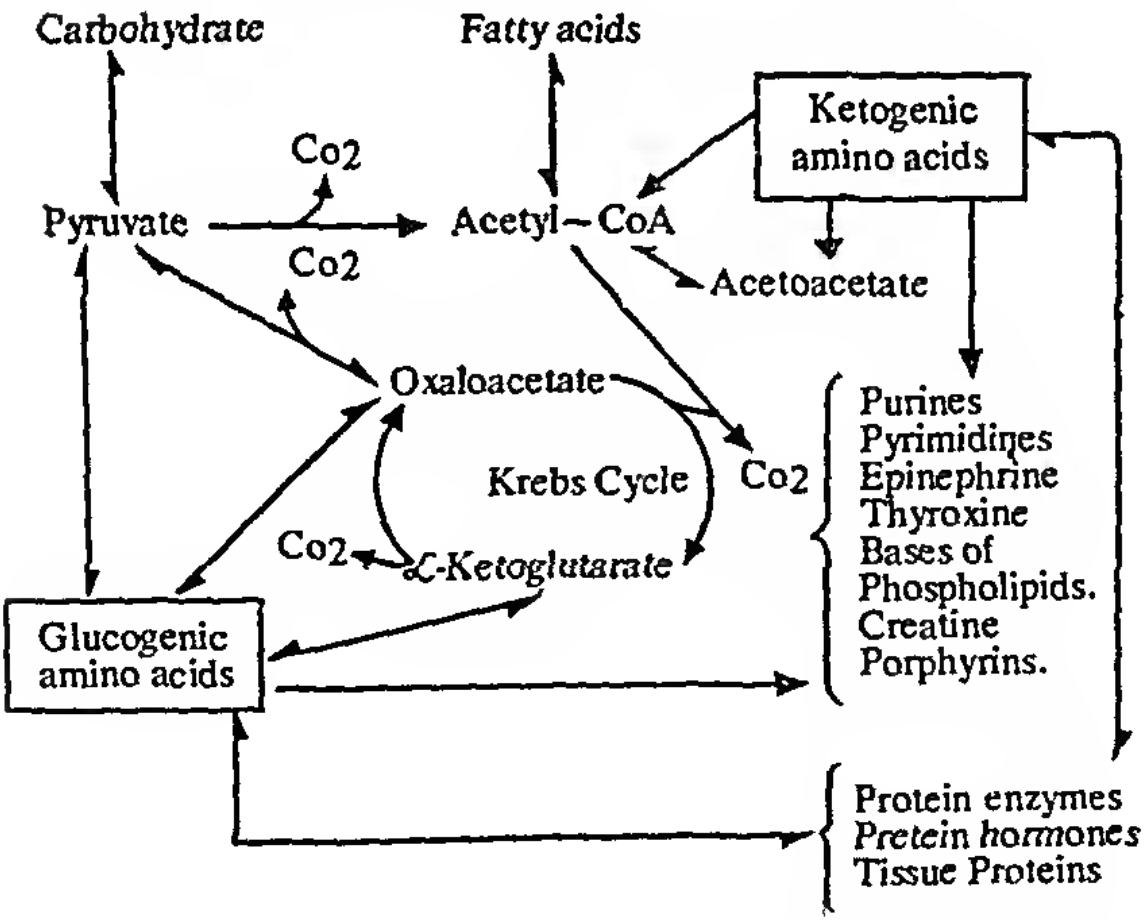


Fig. 19.49 : Metabolic interrelations of Proteins with other food stuffs

chondria. If the isoenzyme from mitochondria appears in serum, cell death occurs This indicates a severe lesion and affects the prognosis

Exercise

1 What do you mean by Intermediary metabolism ? Give an account of Intermediary metabolism of proteins (R U. 70A)

2 What is Urea ? Describe its formation and excretion in human body (Bh U 74A ; R U 70A)

3 Explain the chemical reactions involved in formation of Urea (R. U 73S)

4 What is Urea ? Describe the synthesis of urea in the body (Muz. 75S , P U 68A)

5 List the excretory products of nitrogen metabolism How are they formed in the body ? (R U 65A, 68S)

6 What are the sources and main functions of amino acids in our body ? Describe the process involved in the breakdown of amino acids (Mith 67A)

7 Name the essential amino acids and describe the specific metabolic role of any two of them (Pun 68S, 70A)

8 Describe the fate of amino acids in the body (Mith 74A ; P U 72S)

9 Describe the metabolic fate of phenylalanine and tyrosine in the body (Mith. 65A ; 75A)

10 Name one amino acid which takes part in synthesis of many hormones in the body Briefly outline the steps of synthesis of any one of these hormones (P U 72A)

11 Write notes on .

(a) Essential amino acids (P U. 75S ; Muz. 75A ; Bh U 74A)

(b) Acidic amino acids (P. U. 70A)

(c) Methionine (R. U. 64S, 70S)

(d) Tryptophan (Muz. 74A , Pun. 69A)

(e) Amino transferase (Mith. 75A)

(f) Edema (R. U 72A)

(g) Nitrogen balance (Muz 74A)

(h) Nitrogen equilibrium (M. U 73S)

CHAPTFR 20

OVERVIEW OF INTERMEDIARY METABOLISM

INTRODUCTION

1 The dietary constituents after digestion and absorption follows intermediary metabolism

2 The metabolic pathways fall into three categories

(i) *Anabolic Pathways* involving the synthesis of compounds constitute the body's structure and machinery Protein synthesis is one of them. The free energy required for these pathways are available from catabolic pathways

(ii) *Catabolic Pathways* involve oxidative processes and release free energy (usually in the form of high-energy phosphate) or reducing equivalents e g , biologic oxidation

(iii) *Amphibolic Pathways* are the links between the anabolic and catabolic pathways, e g , TCA cycle

BIOMEDICAL IMPORTANCE

1 The concept of metabolism in the normal animals provides a sound understanding of many diseases

2 The variations and adaptation in metabolism due to periods of starvation, exercise, pregnancy, and lactation are included in normal metabolism

3 Abnormal metabolism results from nutritional deficiency, enzyme deficiency, and excessive secretion of hormones

4 The example of a disease caused by abnormal metabolism is diabetes mellitus

THE BASIC METABOLIC PATHWAYS

1 The basic pattern of metabolism in the tissues is set by the diet

2 Humans require to process the absorbed products of digestion of dietary carbohydrate, lipid, and protein These are mainly glucose, triacylglycerol, and amino acids respectively

3 In ruminants, cellulose in the diet is digested by symbiotic micro-organisms to lower fatty acids (acetic, propionic, butyric) which are utilized as major substrates in the tissue metabolism of these animals.

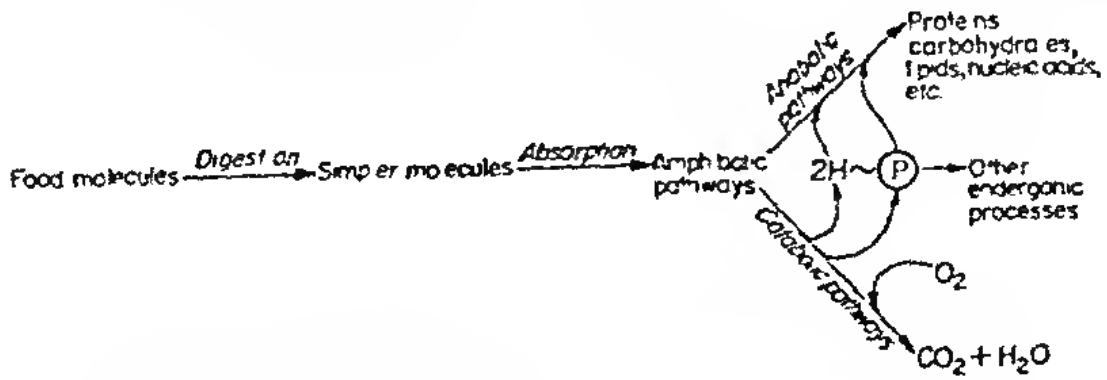


Fig 20 1 Three major categories of metabolic pathways. Catabolic pathways release free energy in the form of reducing equivalents (2H) or high-energy phosphate (~P) to power the anabolic pathways Amphibolic pathways act as links between the other two categories of pathways.

Carbohydrate metabolism

1 Pyruvate and lactate are formed in the mammalian cells as a result of the oxidation of glucose by glycolysis

2 Glycolysis occurs in the cytoplasm of cells in absence of oxygen producing lactate only

3 Under aerobic condition pyruvate is metabolized to acetyl—CoA which enters the citric acid cycle for complete oxidation to CO_2 and H_2O

4 Glucose also takes part in other metabolic processes as follows

(i) It is converted to glycogen as a storage particularly in liver and skeletal muscle.

(ii) The HMP shunt or the pentose phosphate pathways arising from intermediates of glycolysis is a source of reducing equivalents (2H) for biosynthesis of fatty acids, cholesterol etc., and it is a source of ribose which is important for nucleic acid formation

(iii) Triose phosphate of glycolysis is a source of glycerol of fat

(iv) Pyruvate and the intermediates of citric acid cycle form amino acids and acetyl—CoA is the building block for long-chain fatty acids and cholesterol, the precursor of all steroid hormones in the body

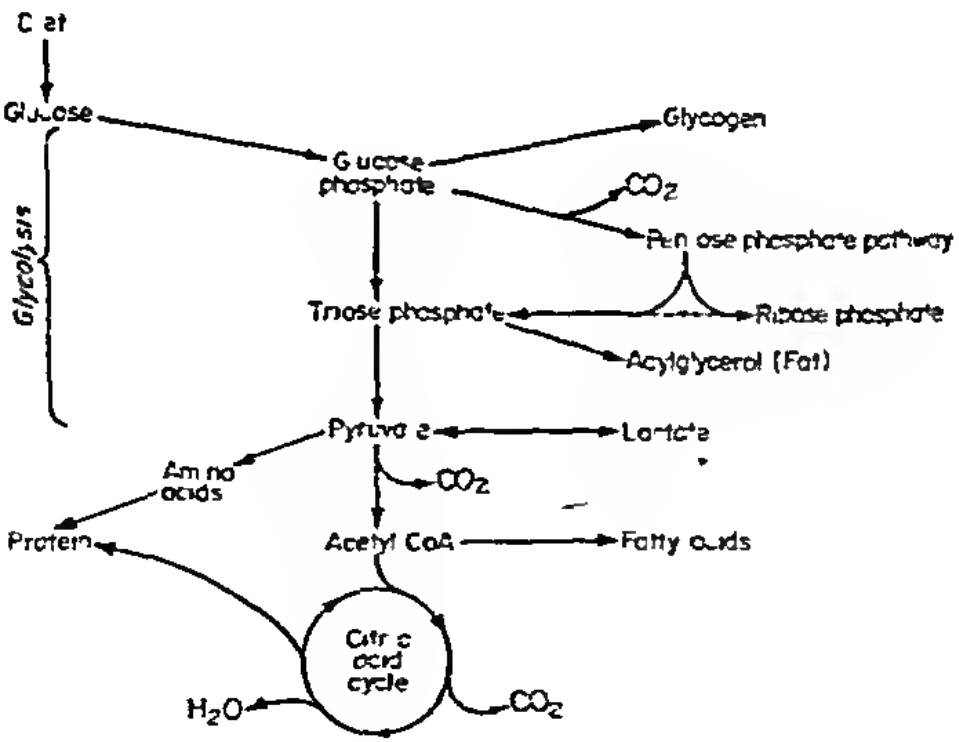


Fig 20.2 Schematic representation of carbohydrate metabolism showing the major end products.

Lipid metabolism

1 The long-chain fatty acids are synthesized from acetyl—CoA derived from carbohydrate or from dietary lipid

2 In the tissues, fatty acids are oxidized to acetyl—CoA or esterified to acylglycerol to form fat which is the main calorie reserve of the body

3 Acetyl CoA formed by β oxidation has the following significant roles in the body

(i) It liberates CO_2 and H_2O and also yields high energy Therefore, during the oxidation of fatty acids by β oxidation for their complete oxidation more energy is formed

(ii) It is a source of cholesterol biosynthesis

(iii) In the liver, it forms ketone bodies which are alternative water-soluble tissue fuels These fuels become important sources of energy under certain conditions (*e g starvation*)

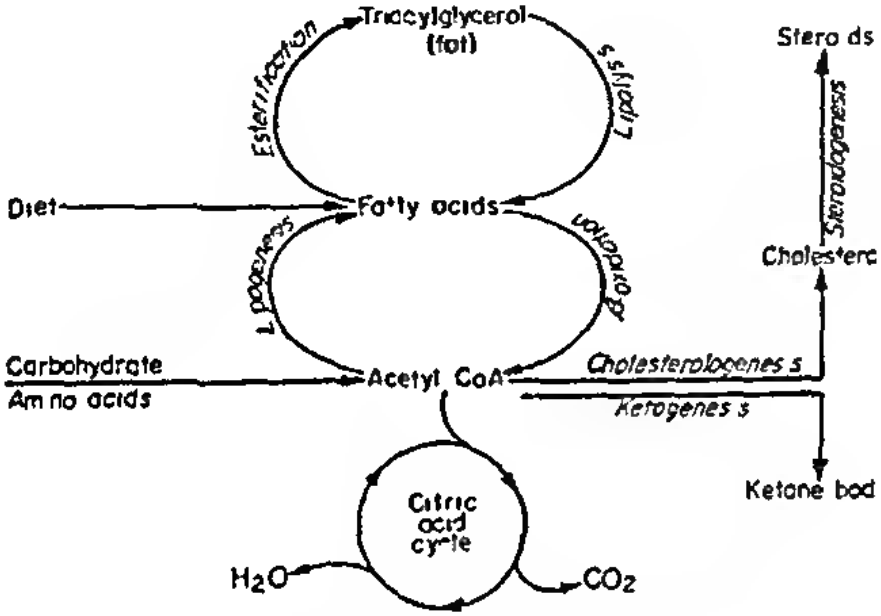


Fig 20.3 Schematic representation of lipid metabolism showing the major end products

Amino acid metabolism

1 Amino acids are required for protein synthesis

2 The essential amino acids must be supplied in the diet since these are not synthesized by the tissues

3 Diet can supply the non-essential amino acids which are also formed from the intermediates of citric acid cycle by *transamination*

4 Excess amino nitrogen as a result of *deamination* of amino acids is removed as *urea* and the carbon skeletons that remain after transamination give the following products

(i) Carbondioxide and water via the citric acid cycle

(ii) Glucose (by gluconeogenesis)

(iii) Ketone bodies

5 The amino acids are also the precursors of many other important compounds, e g , purines, pyrimidines, and hormones such as epinephrine and thyroxine

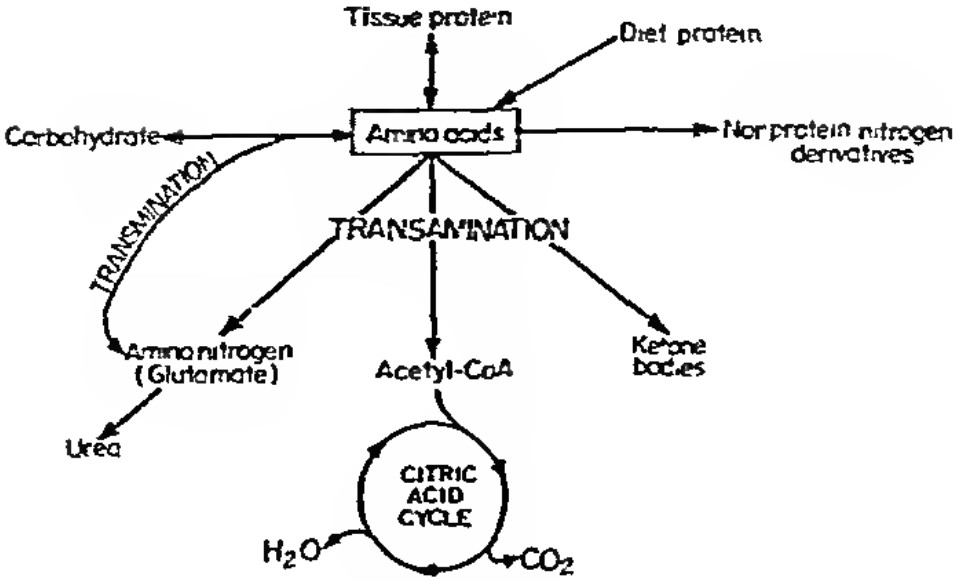


Fig 20.4. Schematic representation of amino acid metabolism showing the major end products.

THE LOCATION OF METABOLIC PATHWAYS

The metabolic pathways can be studied at many levels of organization. These levels are divided into two major groups:

1. *At the tissue and organ level*—the nature of the substrates entering and metabolites leaving tissues and organs is defined, and their overall fate is discussed.

2. *At the subcellular level*—each cell organelle (e.g., the mitochondrion) or compartment (e.g., the cytosol) carries out specific biochemical roles showing metabolic pathways.

Intermediary Metabolism at the Tissue and Organ Level

1. Amino acids formed from the digestion of dietary protein and glucose formed from the digestion of carbohydrate follow the common path of absorption via the *hepatic portal vein*. Both these metabolites and other-soluble products of digestion are initially directed to the liver (Fig. 20.5).

2. The *liver* primarily regulates the blood concentration of particularly glucose and amino acids.

3. The liver takes up excess glucose and converts it to glycogen (*glycogenesis*) or to fat (*lipogenesis*). During meals, it causes *glycogenolysis* to supply glucose in the blood or in company with the *kidney*, it converts non-carbohydrate metabolites such as lactate, glycerol, and amino acids to glucose (*gluconeogenesis*).

4. Sufficient concentration of blood glucose is maintained by certain tissues in which it is fuel, e.g., brain and erythrocytes.

5. The liver also *synthesizes the major plasma proteins* (e.g., albumin) and *deaminates amino acids* with the formation of *urea* which is transported via the blood to the kidney and excreted.

6. *Skeletal muscle* uses glucose as a fuel forming lactate and carbon dioxide. It stores glycogen for its use in muscular contraction and synthesizes muscle protein from plasma amino acids. During dietary shortage of protein, it can supply plasma amino acids from its stores of protein.

7. Lipids being digested form monoacylglycerols and fatty acids These are recombined in the intestinal cells with protein and secreted into the lymphatic system and then into the circulation as a *lipoprotein* known as *chylomicron*.

8. Chylomicron triacylglycerol is not taken up by the liver and it is metabolized by extrahepatic tissues having the enzyme *lipoprotein lipase* which hydrolyzes the triacylglycerol releasing fatty acids that are incorporated into tissue lipids or oxidized as fuel.

9. The long-chain fatty acid is synthesized (*lipogenesis*) from carbohydrate mainly in *adipose tissue* and *the liver*.

10. *Adipose tissue* triacylglycerol is hydrolyzed (lipolysis) to fatty acids which are released in the circulation.

11. Free fatty acids are taken up by most tissues (but not brain or erythrocytes) and esterified to acylglycerols or oxidized to carbondioxide.

12. The *liver* performs two additional important functions:

(i) Surplus triacylglycerol from both lipogenesis and free fatty acids is secreted into the circulation as very low density lipoprotein (*VLDL*). This triacylglycerol faces the fate similar to that of chylomicrons.

(ii) Partial oxidation of free fatty acids leads to ketone body formation (*ketogenesis*). These ketone bodies are transported to extrahepatic tissues where they become the major fuel source.

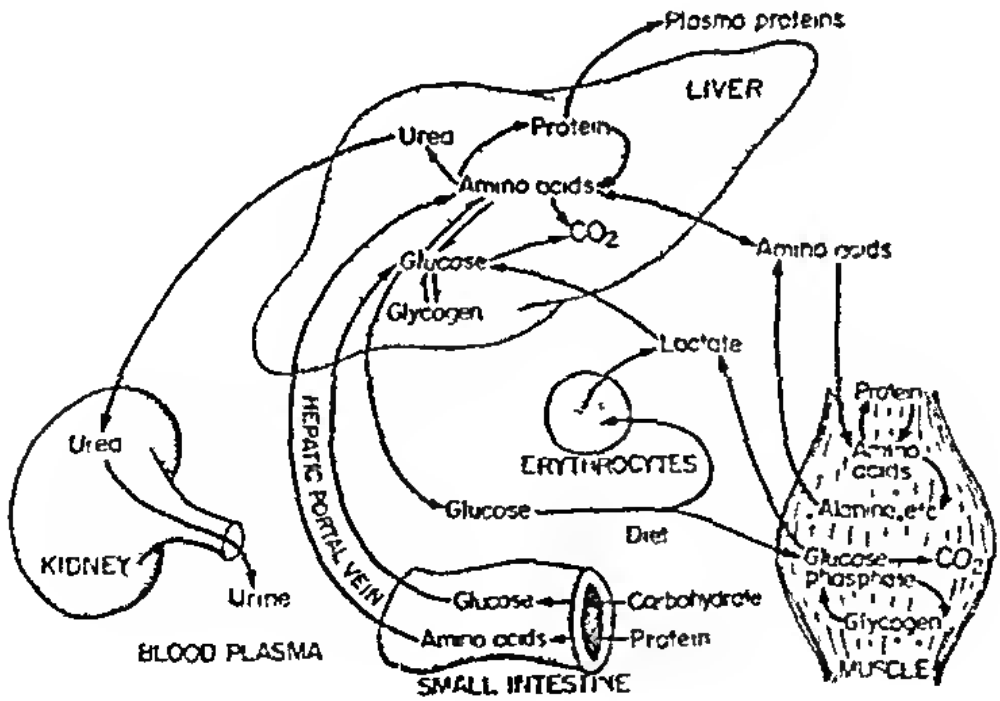


Fig. 20.5. Transport and fate of major carbohydrate and amino acid substrates and metabolites. Little free glucose in muscle is rapidly phosphorylated upon entry.

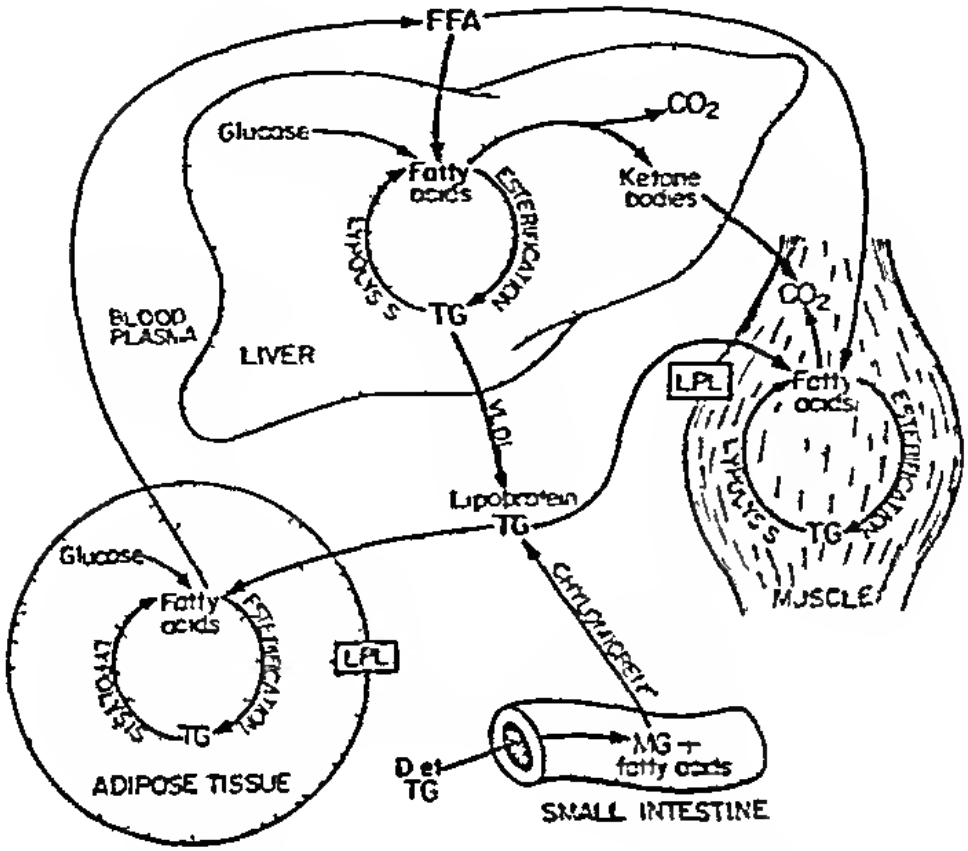


Fig 20.6 Transport and fate of major lipid substrates and metabolites.
FFA=Free fatty acids, LPL=Lipoprotein lipase MG=Monoacyl glycerol, TG=Triacyl glycerol. VLDL=Very low density lipoprotein.

Intermediary Metabolism at the Subcellular Level

1 *Mitochondrion* acts as the focus and crossroad of carbohydrate, lipid, and amino acid metabolism. Particularly, it is the house of the enzymes of citric acid cycle, of the respiratory chain and ATP synthase, of β-oxidation of fatty acids, and of ketone body production It is also the collecting chamber for the carbon skeletons of amino acids after transamination and for utilizing these skeletons for the synthesis of nonessential amino acids.

2 In the *cytosol*, glycolysis HMP shunt or the pentose phosphate pathway and fatty acid synthesis all take place Substances such as lactate and pyruvate that are formed in cytosol must enter the *mitochondrion* and form oxaloacetate before conversion to glucose in the process of *gluconeogenesis*

3 The membranes of the *endoplasmic reticulum* contain the enzyme system for acylglycerol synthesis and the ribosomes are responsible for protein synthesis.

4 The transport of metabolites of varying size, charge, and solubility through the membranes separating organelles involves complex mechanisms.

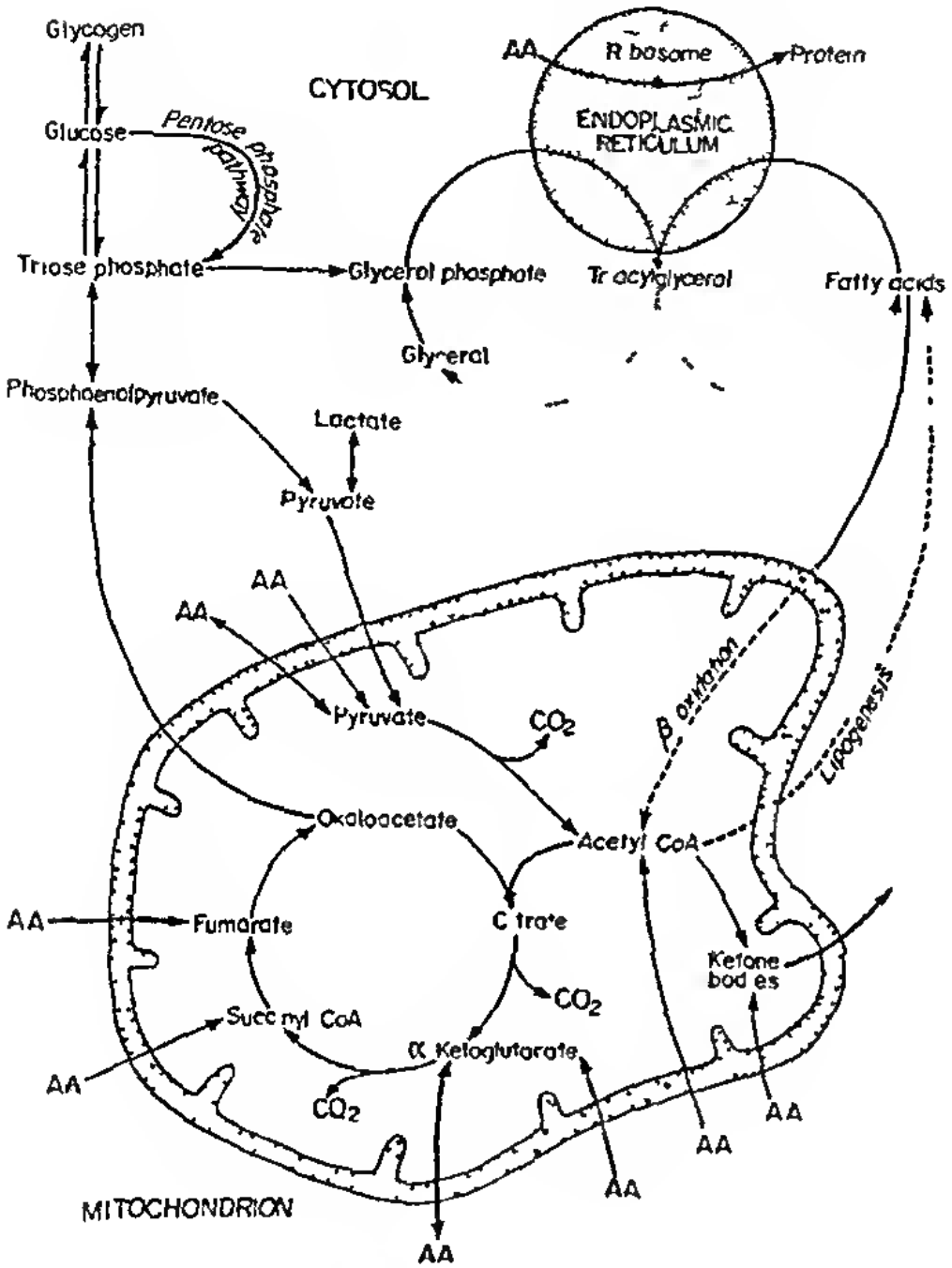


Fig 20 7 Intracellular location and integration of major metabolic pathways in a liver parenchymal cell

AA⟶metabolism of one or more essential amino acids

AA⟷metabolism of one or more nonessential amino acids

CHAPTER 21

INTERRELATIONSHIP IN THE METABOLISM OF PROTEIN, FAT AND CARBOHYDRATE

Glucose is converted into glycerol through triosephosphate and acetyl COA through pyruvate Acetyl COA helps the formation of fatty acids with malonyl-COA Glycerol and fatty acids combine to form triacylglycerol (neutral fat) The keto acids in TCA cycle are converted into amino acids by transamination

Fat is oxidized to form acetyl COA which enters citric acid cycle Malate of the citric acid cycle is permeable to pass through the mitochondrial membrane into the cytosol where it is converted ultimately to glucose by gluconeogenesis Glycerol is also converted into glucose Fatty acids with odd number of carbon atoms also enter citric acid cycle being converted to propionate

Protein is hydrolyzed to amino acids Certain amino acids are ketogenic forming acetoacetate which is further converted into acetyl-COA Acetyl COA enters the citric acid cycle which is converted into glucose via gluconeogenesis Some acetyl-COA can also be utilized for the formation of fat Some amino acids are glucogenic These are converted into pyruvate which can be converted into fat and glucose via acetyl COA and oxaloacetate formation respectively The amino acids—aspartic acid, alanine and glutamic acid—by transamination are converted into oxaloacetate, pyruvate and α-ketoglutarate respectively These products can be converted into glucose and fat

This interrelationship is represented by a schematic manner shown below :

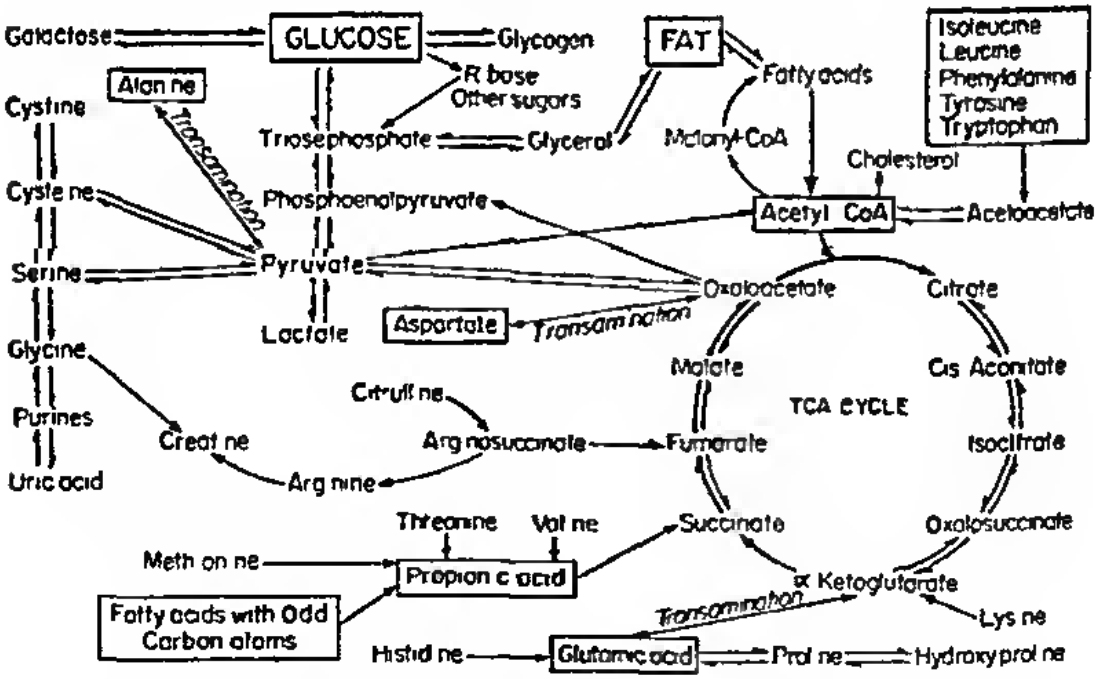


Fig 21 1 Interrelationship in the metabolism of protein, fat and carbohydrate

CHAPTER 22

METABOLISM OF PURINE AND PYRIMIDINE NUCLEOTIDES

Sources of the various atoms of the purine base :

1. Glycine is utilized to form the carbon positions 4 and 5 and its α-nitrogen forms the nitrogen in position 7.
2. The amino nitrogen of aspartic acid provides the nitrogen at position 1.
3. The N atoms at positions 3 and 9 are derived from the amide nitrogen of glutamine.
4. The carbon atom at position 6 is derived from CO_2 of Respiration
5. The carbons in positions 2 and 8 are supplied from a one carbon (C—I) compound given by the tetrahydrofolate carrier.

This is represented below :

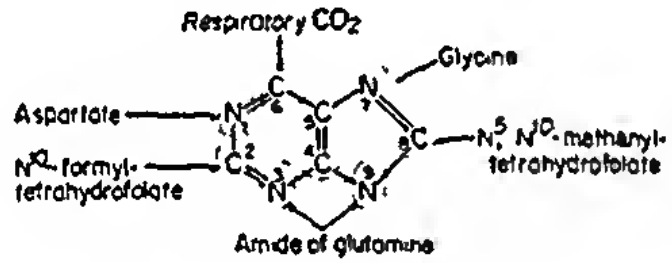


Fig 22.1. Sources of nitrogen and carbon atoms of the purine ring.

Biosynthesis of purine nucleotides [DE NOVO] :

1. Ribose-5-phosphate is converted into 1-pyrophosphoribosyl-5-phosphate (PP ribose P) by PP ribose P synthetase with ATP and Mg^{++}.
2. PP ribose P then reacts with glutamine by the enzyme *phosphoribosyl pyrophosphate glutamyl amidotransferase* to form 5-phospboribosylamine by the displacement of pyrophosphate and the formation of glutamate.
3. 5-phosphoribosylamine reacts with glycine to produce glycinamide ribosylphosphate by *glycinamide kinosynthetase* in presence of ATP.
4. The N_7 of glycinamide ribosylphosphate is formylated by the enzyme *glycinamide ribosylphosphate formyltransferase* to transfer the C_1 moiety.
5. Amidation from glutamine occurs at the C_4 of the formylglycioamide ribosylphosphate by *formylglycinamide ribosylphosphate synthetase* requiring ATP The amide N becomes position 3 in the purine.

6. Imidazole ring is closed by aminoimidazole ribosylphosphate synthetase requiring ATP.

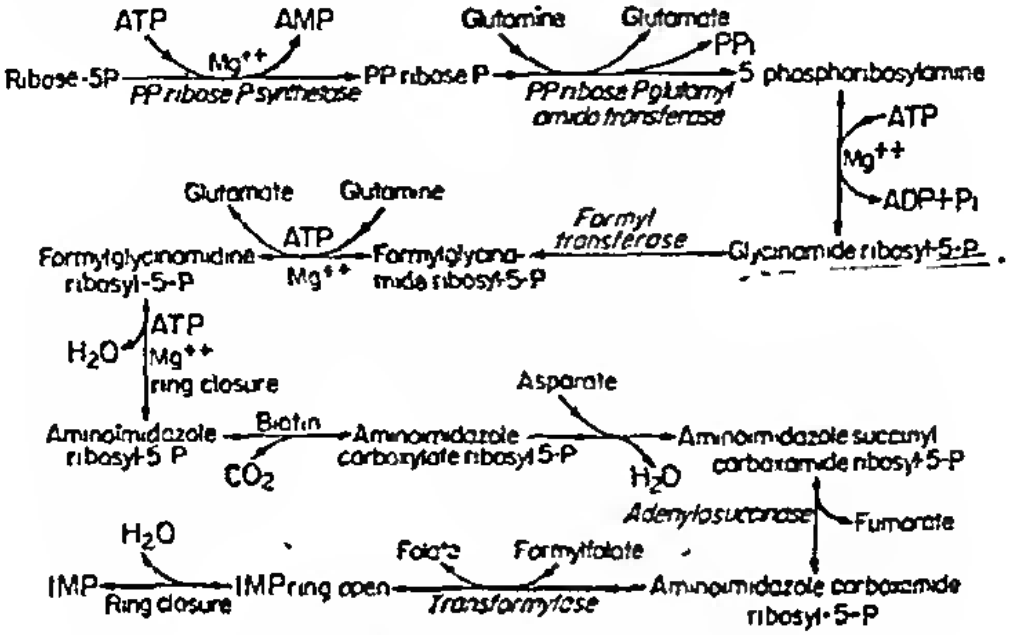


Fig. 22.2. The pathway of de novo purine biosynthesis.

7. Respiratory CO_2 is utilized requiring biotin to form aminoimidazole carboxylate ribosyl phosphate.

8. Addition of Aspartate forms aminoimidazole succinyl carboxamide ribosylphosphate which is converted into aminoimidazole carboxamide ribosyl-phosphate by *adenylosuccinase*.

9. Transformylase converts the above product into nucleoside phosphate and then the ring is closed.

Catabolism of purines :

Uric acid is the chief end-product of purine catabolism in man and the higher apes. Other mammals degrade uric acid to allantoin by means of the enzyme, uricase, which is lacking in primates.

Almost all tissues contain enzymes capable of breaking nucleoprotein down to nucleoside which can be oxidized to uric acid. Uric acid is always excreted even on a purine free diet or in starvation. Urinary uric acid is both endogenous and exogenous in origin.

Organisms that form uric acid as the major nitrogenous waste product are said to be *Uricotelic*. Birds, amphibius and reptiles do not possess uricase activity. These animals excrete uric acid and guanine as the end-products of purine metabolism and nitrogen (protein) metabolism. In man and most of the mammals, urea is the main product of nitrogen metabolism Hence, they are ureotelic.

In animals otber than mammals, uric is further degraded to urea and glyoxilic acid.

Uric acid —Uricase→ Allantoin —Allantoinase→ Allantoic acid —Allantoicase→ Urea + Glyoxylate

Formation of *uric acid* :

1. Adenine (6-aminopurine) is deaminated by-adenylate deaminase to form inosinic acid. Adenylate deaminase is quite abundant in skeletal muscle. Adenosine can also be deaminated to form inosine.

2. Both inosinic acid and inosine give rise to free bypoxanthine, which may be reutilized for nucleic acid synthesis but is most frequently oxidized to Xanthine by tbe enzyme xanthine oxidase, present in greatest amount in liver, small intestine and kidney.

3. Xanthine oxidase further nxidizes xanthine to urie acid (2, 6, 8-trioxypurine).

4. Free guanine (2-amino-6-oxypurine) is deaminated to form xantbine directly by the enzyme guanase, wbich is very active in most tissues. The liberated xantbine is then converted to uric acid by xanthine oxidase.

The patbway for the formation of uric acid is as follows :

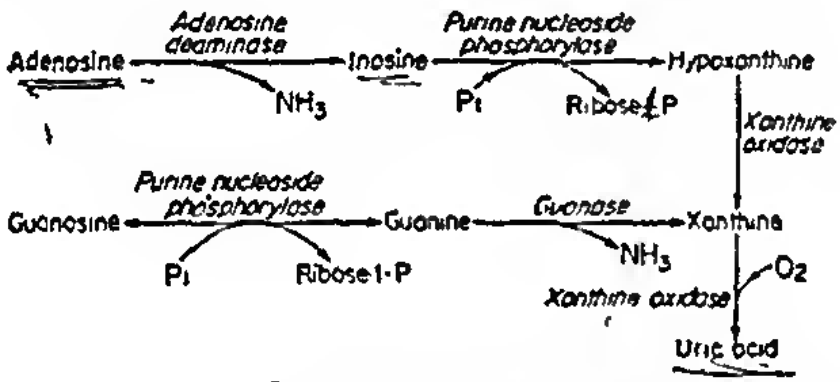


Fig. 22.3. Formation of uric acid.

Some uric acid may be produced from nucleic acid by the bacterial flora of *the intestinal tract, when it is absorbed and directly excreted. This pathway is a* minor contributor to the urinary uric acid of persons on a normal diet.

From recent studies it appears that sodium nrate is freely filtered by the mammalian glomerulus. It is reabsorbed and secreted in the proximal tubule and tbe loop of Henle and partially reabsnrbed in the distal convoluted tubules. The net excretinn of total uric acid in normal men is 400-600 mg. in 24 hours. Aspirin in bigh doses competitively inhibits urate excretinn as well as reabsnrptinn. Allnpurinnl competitively inhibits Xanthine oxidase for which uric acid cannnt be formed.

Uric acid is mainly excreted in urine, to a lesser extent in digestive fluid, and in small amounts in sweat and saliva. A portion of the uric acid is destroyed by bacterial actinn in the intestine. This intestinal uricolysis gives rise to urea and ammonia, which are absnrbed and excreted by the kidneys. Under enndtinns nf normal production and removal, the body contains n "readily miscible uric acid pool."

The normal uric acid content of serum is 2 5 to 7 0 mg /100 ml for adult males and 1.5 to 6 0 mg /100 ml for premenopausal females One third of it is loosely bound to plasma proteins—mostly albumin—but some is bound to an α_1—α_2 globulins Supersaturation of uric acid causes the disease gout which is much more common in males Only about 5 per cent of gouty patients are females and most of them are menopausal

Normal adults excrete less than 450 mg. uric acid daily on a low containing nucleoprotein diet This indicates that uric acid is formed from the catabolism of endogenous nucleic acids and nucleotides A high protein and caloric intake causes increased uric acid The output of uric acid may rise to 1 gram daily on a high purine diet (meat, liver, kidney, sweet breads) some uricosuric agents such as salicylates, cinchophen and carbamide increase urinary elimination of uric acid by inhibiting its reabsorption in the renal tubules by blocking the enzymatic transport mechanism. ACTH and adrenocortical oxysteroids also increase the urinary excretion of uric acid by inhibiting renal tubular reabsorption.

Hyperuricemia is due to overproduction, decreased destruction and decreased renal excretion

Increased values are observed in all forms of nephritis with nitrogen retention Values as high as 10 mg /100 ml are frequently observed Serum uric acid is also increased in eclampsia. In chronic leukemia, blood uric acid level is increased Uric acid level in blood may also shoot up in sickle cell anemia, thalassemia hemolytic anemia and macroglobulinemia

GOUT

Sodium urate crystals are precipitated out of solution and deposited in soft tissues, particularly in or about joints These urate deposits are referred to as *tophi* Acute inflammatory reactions called *acute gouty arthritis* is caused by the accumulation of sodium urate crystals in the tissues . The deposition of sodium urate tophi can cause *chronic gouty arthritis* which results in joint destruction

Primary gout is an arthritis characterized by a derangement of purine metabolism, occurring mostly in males, with the elevation of serum uric acid concentration The hyperuricemia of primary gout is due to excessive production of purines and to renal retention of uric acid Excessive purine synthesis has been found to be due to deficiency of hypoxanthine—guanine phosphoribosyl transferase

Decreased excretion of uric acid is due to a defect in the tubular secretory mechanism or the production of a metabolic inhibitor of this mechanism The renal circulatory disturbances also cause defective excretion of uric acid Hyperuricemia is a factor associated with an increased tendency to myocardial infarction

In acute attacks of gout, serum uric acid concentration is as high as 10 mg and occasionally 15 mg /100 ml The increased concentration of urate in the body fluids is the direct cause of the local tissue deposits in chronic gout Urates precipitate from the tissue fluids because of their low solubility at the usual p^H of the tissues and are deposited particularly in the kidneys, skin, subcutaneous tissues, cartilage, tendons, ligaments and synovial membranes. Acting as foreign bodies, these deposits incite an inflammatory reaction

The "urate pool", i e the amount of urate capable of mixing promptly with intravenously injected urate, is increased in subjects with gout ' Secondary gout" is applied to a condition with similar clinical signs with various disorders accompanied occasionally by elevation of serum uric acids The secondary gout is accompanied by an increased rate of metabolic turnover of nucleic acids

Treatment of Gout :

1. Bed rest and colchicine for the acute attack.
2. Low purine diet.
3. Salicylates and/or small doses of colchicine for the intercritical period.
4. Recently, the drug allopurinol has been discovered. This is an effective drug which can reduce the synthesis of uric acid.

In early stage of the disease, the patient should avoid foods of high purine content (Meat, fish, beans, peas, sweet breads, liver and kidney) but he should take diets of low purine content (Fruits, milk, cheese, eggs, sugar, sweets, vegetables).

Sources of various atoms of pyrimidines.

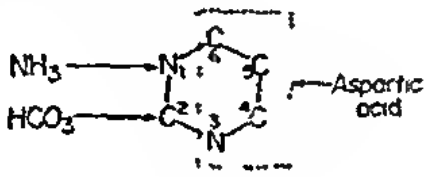


Fig. 22.4. Sources of nitrogen and carbon atoms of pyrimidine ring.

BIOSYNTHESIS OF PYRIMIDINES :

1. The synthesis of pyrimidine ring starts with the formation of carbamoyl phosphate from glutamine, ATP and CO_2 being catalyzed by *carbamoyl phosphate synthetase*, present in the cytosol of the cell.

2. Carbamoyl phosphate then reacts with aspartate by *aspartate transcarbamoylase* to form carbamoyl aspartate which, with the loss of water, is converted into dihydroorotic acid (DHOA) by the enzyme *dihydroorotase*.

3. Dihydroorotic acid on dehydrogenation by *dihydroorotate dehydrogenase* utilizing NAD as coenzyme is converted into *orotic acid* (OA) which is, by the action of *orotate phosphoribosyl transferase*, converted into orotidine monophosphate (OMP). This on subsequent decarboxylation by *orotidylic acid decarboxylase* forms uridine monophosphate (UMP).

4. By further phosphorylation UMP is converted into UDP and then to UTP.

5. UTP is converted into CTP in the reaction catalyzed by CTP synthetase utilizing ATP and glutamine.

6. The enzyme *Ribonucleotide reductase* converts UDP into deoxyuridine diphosphate (dUDP) which is converted into dUMP. This is, by the action of *thymidylate synthetase* with N^5, N^{10}-methylene H_4 folate, converted into thymidine monophosphate (TMP).

CATABOLISM OF PYRIMIDINES :

1. Liver is the main site for the catabolism of pyrimidines.

2. CO_2 is released from the pyrimidine nucleus representing a major pathway for the catabolism of uracil, cytosine, and thymine.

3. The major end products of cytosine, uracil and thymine are β-alanine and β-aminoisobutyric acid respectively.

The overall reaction is given below :

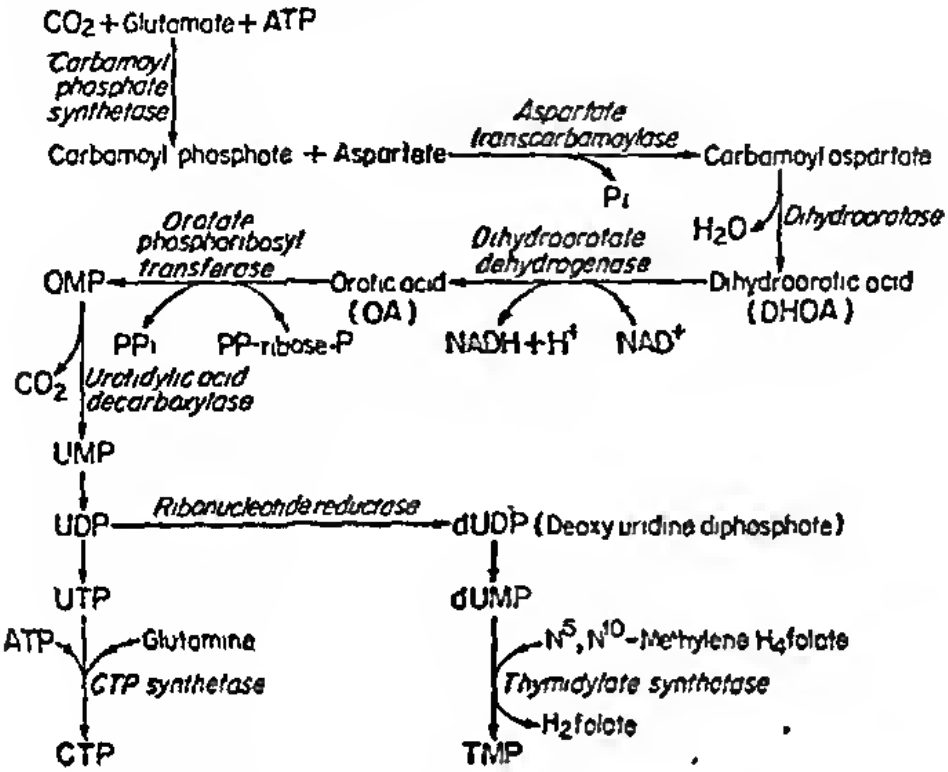


Fig 22.5. The biosynthetic pathway for pyrimidine nucleotides.

4. Thymine is the precursor of β-aminoisobutyric acid in humans and in animals. β-aminoisobutyric acid is excreted more in leukemia. This is due to increased destruction of cells and their DNA.

5. The β-aminoisobutyric acid is converted into methylmalonic semialdehyde and then to propionate which turns to succinate.

The overall reactions for degradation is noted below :

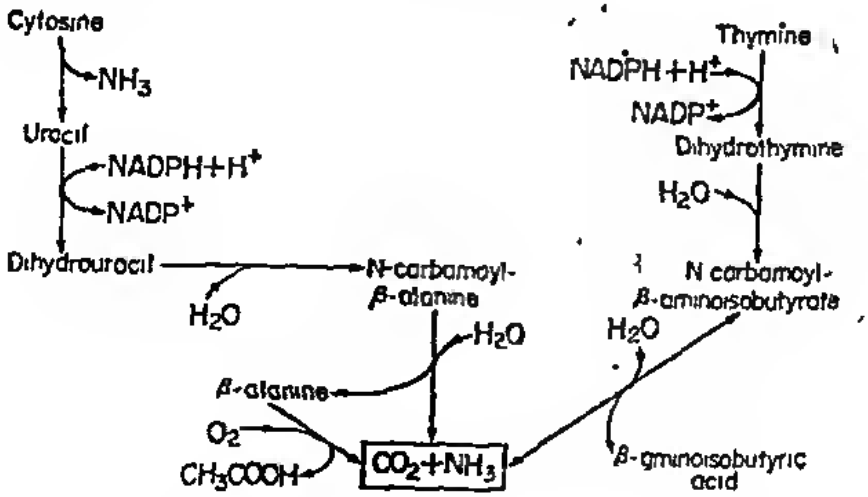


Fig. 22.6. Catabolism of pyrimidines.

Exercise

1. Discuss the catabolism of purines and pyrimidines. (R. U 68A)
2. Write notes on :
   (a) Uric acid (P U 64A ; 73S)
   (b) Gout. (M U. 72A ; R. U. 69S)

CHAPTER 23

RECOMBINANT DNA TECHNOLOGY

INTRODUCTION

1 Recombinant DNA technology is better referred to as *genetic engineering*

2 Much has been learned about the diseases from the study of affected proteins, but this mechanism cannot be applied where the specific genetic defect is unknown This new technology overcoming these limitations will approach directly to the DNA molecule for information

3 This chapter presents the basic concepts of recombinant DNA technology, its application to clinical medicine, and a glossary

BIOMEDICAL IMPORTANCE

1 It is helpful to give clear idea regarding the molecular basis of a number of diseases (e g , familial hypercholesterolemia, sickle cell disease, the thalassemia, cystic fibrosis, Huntington's chorea)

2 Using this technology, a large quantity of human proteins can be produced for therapy

3 By its aid proteins for vaccines (e g , hepatitis B) and for diagnostic tests (e.g , AIDS test) can be obtained.

4 This technology is utilized to diagnose existing diseases and predict the risk of developing a given disease

5 Gene therapy for sickle cell disease, the thalassemias, adenosine deaminase deficiency, and other diseases may be devised

CONCEPTS USED IN RECOMBINANT DNA TECHNOLOGY

Isolation and manipulation of DNA is the object of recombinant DNA research This requires several techniques and reagents

Restriction Enzymes

1 Some *endonucleases* that cut DNA at specific DNA sequences within the molecule are a key tool in recombinant DNA research These enzymes were originally said to be restriction enzymes More than 200 defensive enzymes protect the host bacterial DNA from foreign organisms (primarily infective phages) They are only present in cells that also have a companion enzyme that methylates the host DNA giving it an unsuitable substrate for digestion by the restriction enzyme

2 The restriction enzymes are named vide the bacterium from which they are isolated (e g , Eco RI from Escherichia Coli, Bam HI from Bacillus amyloliquefaciens)

3 Each enzyme recognizes and cleaves a specific double-stranded DNA sequence These DNA cuts result in *blunt ends* or overlapping (*sticky*) *ends* (Bam HI) (figure below), depending on the mechanism used by the enzyme Sticky ends are particularly useful in constructing hybrid or chimeric DNA molecules

4 In case, the nucleotides are distributed randomly in a given DNA molecule, one can easily calculate how frequently a given enzyme could cut a length of DNA

5 For each position in the DNA molecule there are 4 possibilities (A, C, G or T), therefore, a restriction enzyme that recognizes a 4-bp sequence will cut, on average, once every (4^4), whereas another enzyme that recognizes a 6-bp sequences will cut once every (4^6)

6 When DNA is digested with a given enzyme, the ends of all the fragments will have the same DNA sequence The fragments produced can be isolated by electrophoresis

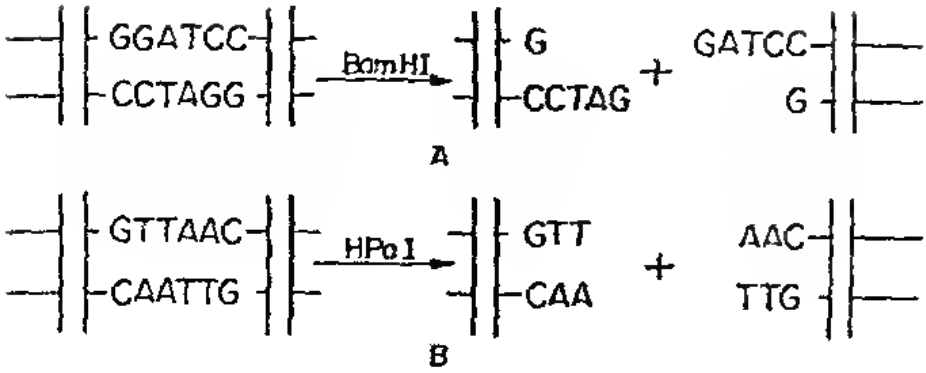


Fig 23 1 Results of restriction endonuclease digestion

Preparation of chimeric DNA molecules

1 Sticky ends of a vector may reconnect with themselves with no gain of DNA These ends of fragments can also anneal so that tandem heterogeneous inserts form These end sites may not be available or in a convenient position

2 To overcome the above problems, an enzyme that generates blunt ends is used and new ends are added using the enzyme terminal transferase

3 If poly d(G) is added to the 3' ends of the vector and poly d(C) is added to the 3' ends of the foreign DNA, the two molecules can only anneal to each other and thus overcome the above problem This procedure called *homopolymer tailing* also generates an Sma I restriction site

4 Sometimes, synthetic oligonucleotide linkers with a convenient restriction enzyme sequence are ligated to the blunt-ended DNA This technique has the advantage of joining together any pairs of ends The disadvantages are that there is no control over the orientation of insertion or the number of molecules annealed together

Cloning

1 A *clone* is a large population of identical molecules bacteria, or cells that arise from a common ancestor

2 Cloning allows for the production of a large number of identical DNA molecules which can then be used for other purposes

3 This technique is based on the fact that hybrid DNA molecules can be constructed in *cloning vectors*, typically bacterial plasmids, phages, or cosmids which then continue to replicate in host cell under their own control system Thus, the chimeric DNA is amplified

4 Bacterial *plasmids* are small, circular duplex DNA molecules whose natural function is to confer antibiotic resistance to the host cell

5 Plasmids have several properties that make them useful as cloning vectors They exist as single or multiple copies within the bacterium and replicate independently from the bacterial DNA

6 Plasmids are smaller than the host chromosome and are therefore easily separated from the latter, and the desired DNA is readily removed by cutting the plasmid with the enzyme specific for the restriction site into which the original piece of DNA was inserted

7. *Phages* usually have linear DNA molecules into which foreign DNA can be inserted at several restriction enzyme sites

8. The chimeric DNA is collected after the phage proceeds through the lytic cycle and produces mature, infective phage particles

9 Larger fragments of DNA can be cloned in *cosmids* which combine the best features of plasmids and phages

10 Cosmids are plasmids that contain the DNA sequences, so-called *cos sites*, required for packaging Lambda DNA into the phage particle

11 Insertion of DNA into a functional region of the vector will interfere with the action of this region,

12 The common plasmid vector *pBR* 322 has both *tetracycline* (tet) and *ampicillin* (amp) *resistance genes*

Libraries and library construction

1. Restriction enzymes and various cloning vectors in combination allow the entire genome to be packaged into a vector. The collection of these different recombinant clones is called a library

2 A *genome library* is formed by the total DNA of a cell line or tissue

3 A *cDNA library* is the population of mRNAs in a tissue

4 Genomic libraries are prepared by the partial digestion of total DNA with a restriction enzyme that cuts DNA frequently.

5. A human library that contains 10^6 recombinant fragments of large size has a 99 percent probability of being complete.

6. cDNA libraries are prepared by isolating all the mRNAs in a tissue and then copying these molecules into double-stranded DNA, using the reverse transcriptase and DNA polymerase

The protein coded by the gene introduced by recombinant DNA technology is actually synthesized in a vector is known as an *expression vector*. Such vectors are now used to detect specific cDNA molecules in libraries and to produce proteins by genetic engineering techniques. These vectors are specially constructed to contain very active inducible promoters

Probes

1 Probes are generally pieces of DNA or RNA labelled with a p^{32}—containing nucleotide

2 The probe must know a complementary sequence to be effective

3 A cDNA synthesized from a specific mRNA can be used to screen either a cDNA library for a longer cDNA or a genomic library for a complementary sequence in the coding region of a gene.

4. cDNA probes are used to detect DNA fragments on southern blot transfers and to detect and quantitate RNA on Northern blot transfers

Blotting and Hybridization Techniques

1 Observation of a specific DNA or RNA fragment in many "contaminating" molecules requires a number of techniques which are collectively termed *blot transfer*

2 Sometimes, if a specific base is altered and a restriction site is changed, these procedures can detect a point mutation.

3 The Northern and Western blot transfer techniques are used to size and quantitate specific RNA and protein molecules respectively

4. Colony or plaque hybridization is the method by which specific clones are identified and purified. Bacteria are grown on colonies on an agar plate and overlaid with a nitrocellulose filter paper.

5. Perfect matches hybridize readily and withstand high temperatures in the hybridization and washing reactions These complexes also form in the presence of low salt concentrations.

6. Less than perfect matches do not tolerate these *stringent conditions.*

7. Gene families can be detected by varying the stringency of the hybridization and washing steps

DNA Sequencing

1. The segments of specific DNA molecules can be analyzed for their nucleotide sequence. This method depends upon having a large number of identical DNA molecules.

2. This requirement can be satisfied by cloning the fragment of interest by the above techniques.

3. Enzymatic method uses specific deoxynucleotide analogs that terminate DNA strand synthesis at specific nucleotides as the strand is synthesized on purified template nucleic acid.

SOME PRACTICAL APPLICATIONS ON RECOMBINANT DNA TECHNOLOGY

Gene Mapping

1. Specific genes to distinct chromosomes are localized by this technique and thus to define a map of the human genome. This is already producing useful information in the definition of human disease.

2. Somatic cell hybridization and in situ hybridization are two techniques used to accomplish this.

3. In *situ hybridization,* the simpler and more direct procedure, a radioactive probe is added to a metaphase spread of chromosomes on a glass slide. The exact area of hybridization is localized by layering photographic emulsion over the slide and after exposure lining up the grains with some histologic identification of the chromosome. Some of the human genes are localized by this technique.

4. Genes that code for proteins with similar functions can be located on separate chromosomes.

5. Genes that form part of a family can also be on separate chromosomes (Growth hormone and prolactin).

6. The genes involved in many hereditary disorders known to be due to specific protein deficiencies, including X chromosome-linked conditions, are really located at specific sites.

Protein Prodnction

1. This technology has two prominent merits:

(i) It can supply large amounts of materials that could not be obtained by conventional purification methods.

(ii) It can provide human material (e.g., insulin, growth hormone).

2. Although the primary aim is to supply products, generally proteins, for treatment (Insulin) and diagnosis (AIDS test) of human and other animal diseases and for disease prevention (hepatitis B vaccine), there are other real and potential commercial applications, especially in agriculture.

RECOMBINANT DNA TECHNOLOGY IN MOLECULAR ANALYSIS OF DISEASE

Normal gene variations

1. *Polymorphisms* occur once in every 500 nucleotides, or about 10^7 times per genome.

2. There deletions and insertions of DNA as well as single base substitutions.

3. In healthy people, these alterations occur in noncoding regions of DNA or at sites that cause no change in function of the encoded protein.

4. The polymorphism of DNA structure can be associated with certain diseases.

Gene variations causing Disease

1. Most genetic diseases were due to point mutations that resulted in an impaired protein.

2. β-globin gene is located in a cluster on chromosome 11. Defective production of β-globin results in a variety of diseases and is due to many different lesions in and around the β-globin gene.

Point Mutations

1. *Sickle cell disease* is caused by mutation of a single base out of the 3×10^9 in the genome.

2. The altered codon specifies a different amino acid (valine rather than glutamic acid) and this causes a structural abnormality of the β-globin molecule. β-thalassemia is the result of these mutations.

Deletions, Insertions, and Rearrangements of DNA

1. Pieces of DNA can move from one place to another within a genome on the study of bacteria, viruses, yeasts, and fruit flies.

2. Disease is caused by the deletion of a critical piece of DNA, the rearraogement of DNA within a gene, or the insertion of a piece of DNA within a coding or regulatory region can all cause changes in gene expression.

3. Deletions in the alpha-globin cluster, located on chromosome 16, cause alpha thalassemia.

4. Deletions or insertions of DNA larger than 50 bp can often be detected by the southern blotting procedure.

Pedigree Analysis

1. Incubation of DNA from normal (AA), heterozygous (AS), and homozygous (SS) individuals results in three different patterns on southern blot transfer.

2. Pedigree analysis has been applied to a number of genetic diseases and is most useful in those caused by deletions and insertions or the rarer instances in which a restriction endonuclease cleavage site is affeteed.

Prenatal Diagnosis

1. Prenatal diagnosis is possible in case the genetic lesion is understood and a specific probe is available.

2. A fetus with the restriction pattern AA does not have sickle cell disease, nor is it a carrier.

3. A fetus with the SS pattern will develop the disease.

4. Probes are now available for this type of analysis of many genetie diseases.

Restriction Fragment Length Polymorphism (RFLP)

1 Inherited differences in the pattern of restriction is known as RFLP

2 This results from single base changes (e g , sickle cell disease) or from deletions or insertions of DNA into a restriction fragment (e g , the thalassemia) and are proving to be a useful diagnostic measure

3 RFLPs may disrupt the function of the gene or may have no biologic significance

4 RFLPs are inherited and they segregate in a mendelian fashion

5 These can be used to establish linkage groups, which in turn, by the process of *chromosome walking* will define the disease locus

6 In chromosome walking, a fragment representing one end of a long piece of DNA is used to isolate another that overlaps but extends the first.

7 20 p c of the defined RFLPs are on the X chromosome

8 X linked disorders such as Duchenne type muscular dystrophy will be defined using RFLPs

9 The defect that causes polycystic kidney disease is linked to the aloha globin locus on chromosome 16

Gene Therapy

1 Bone marrow precursor cells are being investigated because they presumably will resettle in the marrow and replicate there The introduced gene would begin to direct the expression of its protein product and this would correct the deficiency in the host cell

2 Some percentage of genes injected into a fertilized mouse ovum will be incorporated into the genome and found in both somatic and germ cells These *trangenic animals* are useful for analysis of tissue – specific effect on gene expression

3 The trangenic approach has recently been used to correct a genetic deficiency in mice

4 Fertilized ova obtained from mice with genetic hypogonadism were injected with DNA containing the coding sequence for the gonadotropin—releasing hormone (GnRH) precursor protein This gene was expressed and regulated normally in the hypothalamus of a certain number of the resultant mice, and these animals were in all respects normal Their offspring also showed no evidence of GnRH deficiency

GLOSSARY

cDNA: A single stranded DNA molecule which is complementary to an mRNA molecule and is synthesized from it by the action of reverse transcriptase

Chimeric molecule: A molecule (e g DNA, RNA Protein) containing sequences derived from two different species

Clone: A large number of cells or molecules which are identical with a single parental cell or molecule

Exon: The sequence of a gene that is represented as mRNA

Endonuclease: An enzyme which cleaves internal bonds in DNA or RNA.

Cosmid: A plasmid into which the DNA sequences from bacteriophage lambda that are necessary for the packaging of DNA have been inserted

Exonuclease: An enzyme which cleaves nucleotides from either the 3 or 5 ends of DNA or RNA

Hybridization: The specific reassociation of complementary strands of nucleic acids (DNA with DNA, DNA with RNA, or RNA with RNA)

Insert An additional length of base pairs in DNA

Library: A collection of cloned fragments that represents the entire genome. Libraries may be either genomic DNA or cDNA.

Ligation: The enzyme-catalyzed joining in phosphodiester linkage of two stretches of DNA or RNA into one. The respective enzymes are DNA and RNA ligases

Plasmid: A small, extrachromosal, circular molecule of DNA that replicates independently of the host DNA

Probe: A molecule used to detect the presence of a specific fragment of DNA or RNA in Common probes are cDNA molecules

Recombinant DNA: The altered DNA that results from the insertion of a sequence of deoxynucleotides not previously present into an existing molecule of DNA by enzymatic or by chemical means

Restriction enzyme: An endodeoxynuclease that causes cleavage of both strands of DNA at highly specific sites dictated by the base sequence

Reverse transcription: RNA –directed synthesis of DNA catalyzed by reverse transcriptase

Signal: The end product observed when a specific sequence of DNA or RNA is detected by autoradiography or some other method.

Sticky-ended DNA: Complementary single strands of DNA that protrude from opposite ends of a DNA duplex.

Tandem: It is described as the multiple copies of the same sequence (e.g, DNA) that lie adjacent to one another

Transcription: DNA—directed synthesis of RNA.

Transgenic: It describes the introduction of new DNA into germ cells by its injection into the nucleus of the ovum.

Translation: Synthesis of protein using mRNA as template.

Vector: A plasmid or bacteriophage into which foreign DNA can be introduced for the purposes of cloning

Northern blot: A method for transferring RNA from an agarose gel to a nitrocellulose filter on which the RNA can be detected by a suitable probe

Southern blot: A method for transferring DNA from an agarose gel to a nitrocellulose filter on which the DNA can be detected by a suitable probe.

Western blot: A method for transferring protein to a nitrocellulose filter on which the protein can be detected by a suitable probe (e.g , an antibody)

CHAPTER 24

METABOLISM OF NUCLEIC ACIDS

THE NATURE OF DNA :

1 DNA is a very long polymer of purine and pyrimidine mononucleotide monomers bound one to the other by phosphodiester bridges

2. It exists as a double-stranded molecule in nature The strands are held together by Vander Waals forces and the purine and pyrimidine bases of the two strands are held together by hydrogen bonding

3 The two strands extend in opposite directions, i e each is antiparallel

4 Each purine or pyrimidine base in one strand is related to pyrimidine or purine base of the other strand

i e, Adenine (A) is paired with Thymine (T) and
Guanine (G) is paired with Cytosine (C)

5 Genetic information is contained in the sequence of mononucleotide of the DNA molecule For each gene in the DNA molecule, there is a "sense" strand and its complementary "antisense" strand

6 Watson and Crick double stranded model of DNA strongly suggests the replication of DNA molecule in a semiconservative manner When each strand of the double stranded DNA molecule separates during replication, each can then serve as a template on which a new complementary strand can be synthesized

7 Each of the 2 newly formed double stranded DNA molecule contains one strand (but complementary) from the parent double stranded DNA molecule These two newly formed double stranded DNA molecule can then be sorted between 2 daughter cells Each daughter cell will contain DNA molecules with information identical to that which the parent possessed.

The double stranded structure of DNA and the template function of each old strand on which a new complementary strand synthesized and also the semiconservative and conservative replication are given below

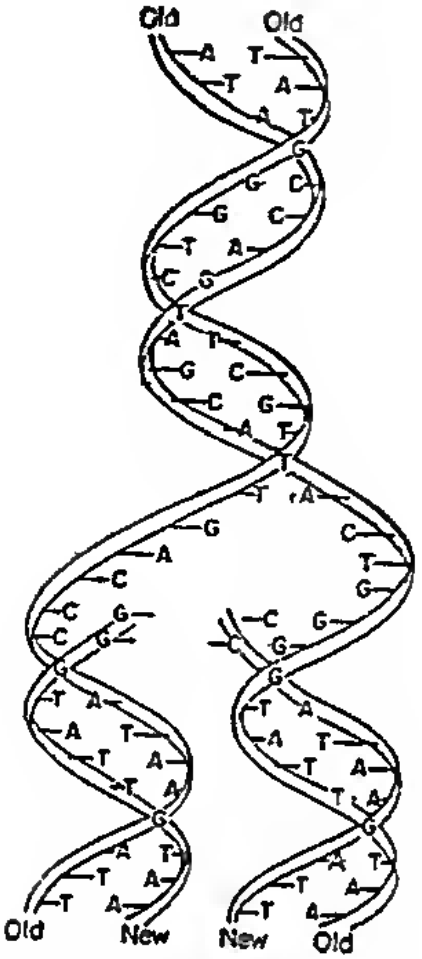


Fig 24 1 The double-stranded structure of DNA

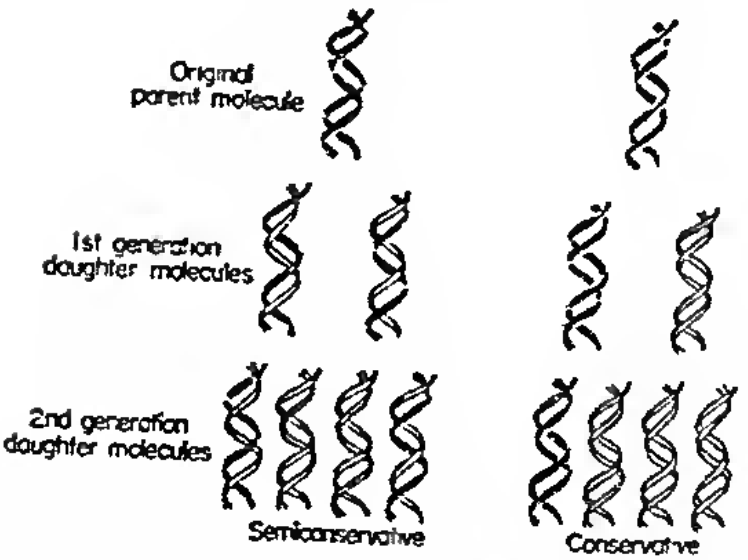


Fig 24.2 The distributions of parental DNA strands during semiconservative a conservative replication The parental strands are solid and the newly synthesized strands are on open

DNA METABOLISM

DNA Synthesis and Replication :

1. The first deoxyribonucleotide is attached at the 3'-hydroxyl end of a *short link of RNA* containing about 10 nucleotides in length 3'-hydroxyl group of the RNA initiator is attached to the alpha phosphate of deoxynucleotide triphosphate with the splitting off of pyrophosphate The 3'-hydroxyl group of the recently attached deoxyribonucleotide *monophosphate* is free to attack the next entering deoxyribonucleotide triphosphate again at its alpha phosphate moiety with the splitting off of pyrophosphate The selection of proper deoxyribonucleotide depends upon proper pairing with the other strand (template) of DNA molecule

2 The polymerization of deoxyribonucleotides takes place by such a process in a discontinuous phases of about 100 nucleotides in length These fragments of DNA attached to an RNA initiator component were discovered by Okazaki and are therefore referred to as *okazaki pieces* (shown in the figure 24 5)

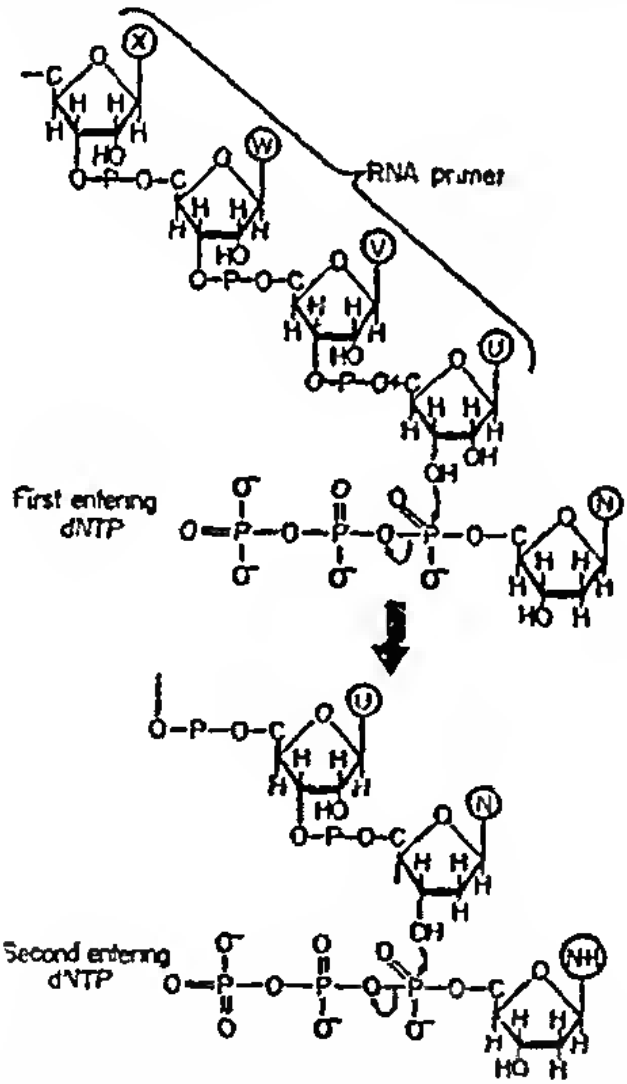


Fig. 24.3. The initiation of of DNA synthesis upon a primer of RNA.

3 In mammals, when many okazaki pieces are generated, the replication complex begins to remove the RNA primers and the gaps left by their removal are filled up by the proper base paired deoxynucleotide The enzymes *DNA ligases* seal the fragments of newly synthesized DNA

4 The replication of both the strands of DNA molecule cannot grow in the same direction The single enzyme replicates one strand in a continuous manner in the 5′ to 3′ direction and it replicates the other strand discontinuously by "turning its back" after the polymerization of 150-250 nucleotides again in the 5′ to 3′ direction This process is shown in the figure below

5 On the different chomosomes of mammalian cells there are multiple origin of DNA replication that occur in clusters of upto 100 of these replicatioa units

6 Replication occurs in both directions up and down the chromosomes and on both strands simultaneously This replication process generates "replication bubbles"

7 Proteins perform specific functions of promoting the unwinding of the DNA molecules during replication

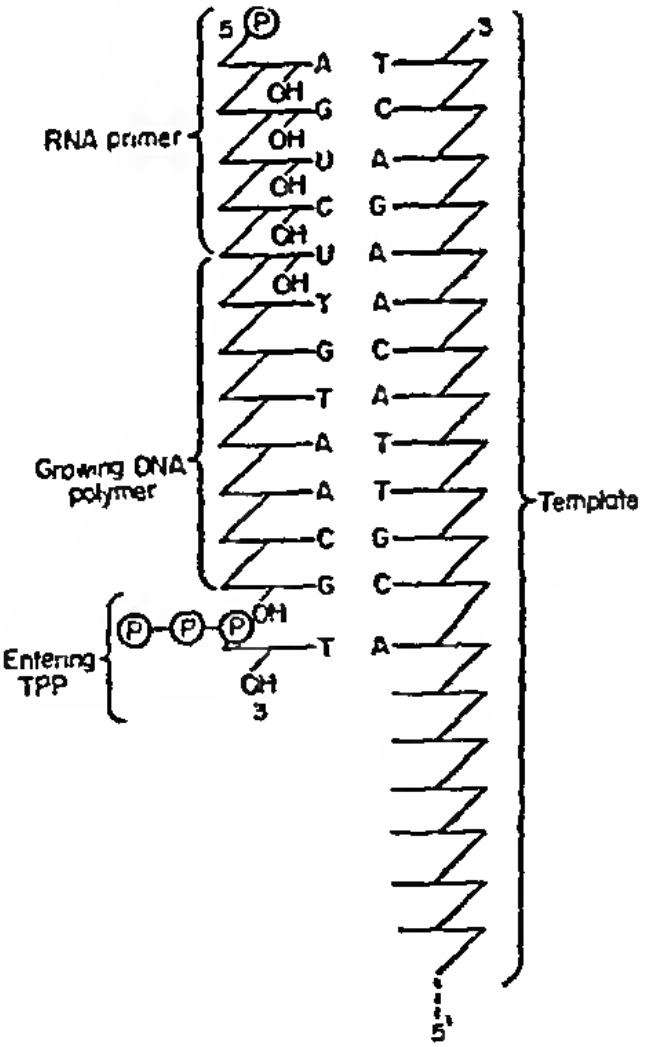


Fig 24 4 The synthesis of DNA on an RNA primer

8. DNA polymerase in mammalian cells are responsible for chromosome replication.

9. In recent years, a class of enzymes capable of syntbesizing a single-stranded and then a double-stranded DNA molecule from a single-stranded RNA template bas been discovered in many animal virus particles. This polymerase RNA-dependent DNA polymerase or "*reverse transcriptase*" synthesizes a double-stranded DNA molecule containing tbe information originally present in tbe RNA genome of tbe animal virus.

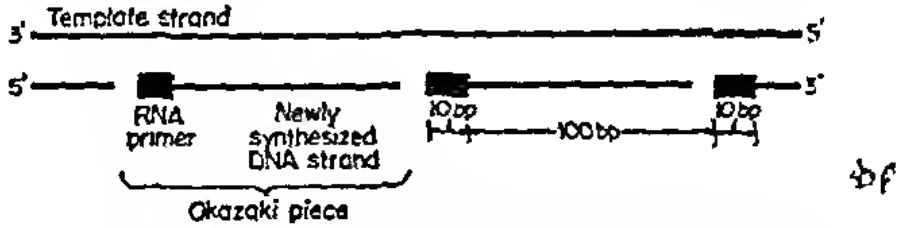


Fig. 24.5. The discontinuous polymerization of deoxyribonucleotions in formation of okazaki pieces.

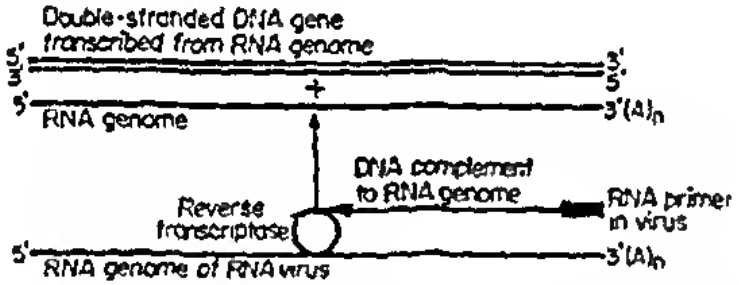


Fig. 24.6. The generation of a double-stranded DNA molecule from an RNA template by the action of reverse transcriptase.

Degradation of DNA :

DNA damage may be classified into 4 types :

1. Single base alteration. 2. Two base alteration. 3. Chain breaks. 4. Cross-linkage.

1. *Single base alteration :* The one base damage includes the bydration of the cytosine residue by ultraviolet irradiation.

2. *Two base alteration :* The two base damage includes Thymine—thymine dimer formation via a cyclobutane moiety.

3. *Chain breaks :* Chain breaks may be created by irradiation such as X-ray exposure

4. *Cross-linkage :* Cross-linkage agents whicb link bases of opposite strands also induce 2 base alterations. Cross-links can also occur between the DNA molecule and histones.

RNA METABOLISM

RNA Synthesis :

The strand which is transcribed into an RNA molecule is referred to as the *sense strand* of the DNA. The other DNA strand is referred to as the antisense strand of that gene. A double stranded DNA molecule contains many genes. The sense strand for each gene is not necessarily be the same strand of the DNA double helix. DNA-dependent RNA polymerase is responsible for the polymerization of ribonucleotides into a sequence complementary to the sense strand of the gene to be transcribed.

1. RNA synthesis is first involved in the binding of holo RNA polymerase molecule to the template of DNA at the promoter site. RNA molecule formation is initiated at 5′ end and then follows with the release of factor when the elongation of the RNA molecule continues from the 5′ to its 3′ end antiparallel to its template.

2. Synthesis of RNA molecule is terminated by a signal which is recognized by a termination protein, the Rho(ρ) factor.

3. After termination of the synthesis of RNA molecule, the core enzyme is separated from the DNA template. The core enzyme then recognizes a promoter site at which the synthesis of new RNA molecule begins with the help of another σ factor.

4. More than one RNA polymerase molecule may transcribe the same sense strand of a gene.

5. Mammalian cells have some DNA-dependent RNA polymerases. Each of these is responsible for the transcription of different sets of genes. The antibiotic *rifampin* inhibits the binding of prokaryotic DNA-dependent RNA polymerase to promoter sites of genes.

The overall process is given below.

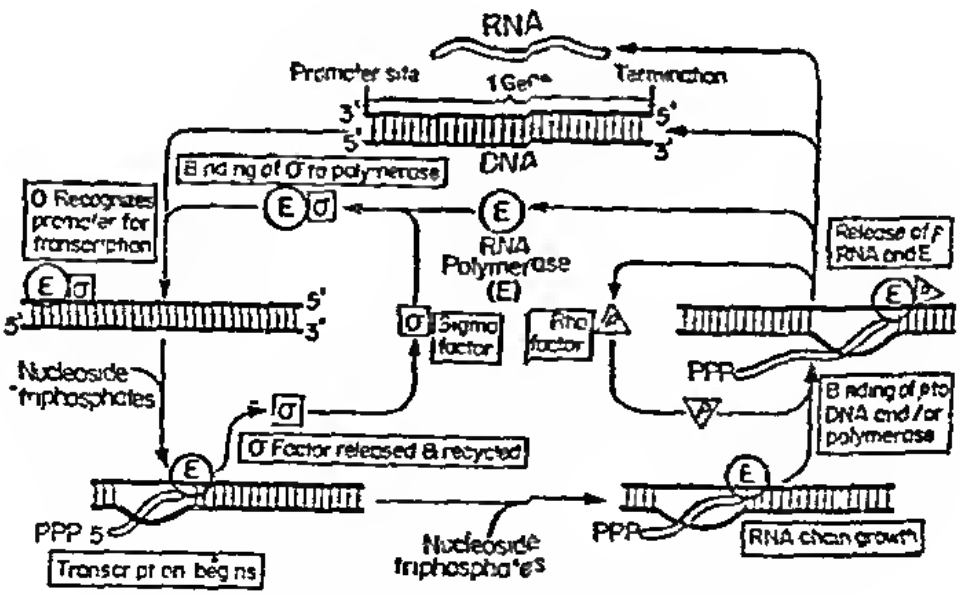


Fig. 24.7. The process of RNA synthesis

Inhibitors of DNA and RNA synthesis

Many antibiotics and nucleotide analogs inhibit the synthesis of DNA and RNA

Inhibitor	*Inhibition*
Actinomycin D	RNA and DNA chain elongation
Mitomycin (reduced)	DNA replication
Anthramycin	DNA and RNA synthesis
Rifampicin (Refampin)	RNA initiation
5 hydroxyuridine	RNA and DNA synthesis

Catabolism of RNA :

1. Ribonucleases specifically hydrolyze ribonucleic acids

2. Endonucleases cleave internal phosphodiester bonds to produce a 3'-hydroxyl and a 5'-phosphoryl or a 5'-hydroxyl and a 3'-phosphoryl terminus

3 Exonucleases are capable of hydrolyzing a nucleotide only when it is present at a terminus of a molecule

BIOLOGICAL IMPORTANCE OF NUCLEIC ACIDS :

1 Nucleic acids are able to reproduce their own kind or to store, express and transmit genetic information

2 They undergo mutation

3 In cell division, the nucleic acid chain is duplicated preserving in each daughter cell the information contained in the parent cell So the double helix unravels and each of the two original strands then serves as a template for the synthesis of another complementary chain

4 DNA produces a messenger RNA which helps in placing amino acids in the code for protein synthesis

5 RNA functions primarily in the cytoplasm of the cell as a template in connection with the synthesis of proteins as well as in the ribosomes The formation of RNA template is directed by nuclear DNA

6 Ribosomal RNA and tRNA are also involved in protein synthesis

7 RNA can be synthesized by RNA polymerase which is dependent on the presence of DNA acting as a template

8 *Adenylic acid in combination with two molecules of phosphoric acid is* the biochemical unit of energy exchange in all cells It is called ATP

9 Biological oxidation reduction involves the transport of hydrogen atoms or electrons through organized systems of substances called hydrogen acceptors or electron transport agents These hydrogen acceptors are nucleotides such as as NAD, FAD etc

10 According to the "pairing rule" in DNA, adenine can combine only with thymine and guanine only with cytosine The newly synthesized strand will be exactly constituted in its nucleotide sequences as was the original complementary strand of the parent strand The result is the synthesis of two pairs of strands

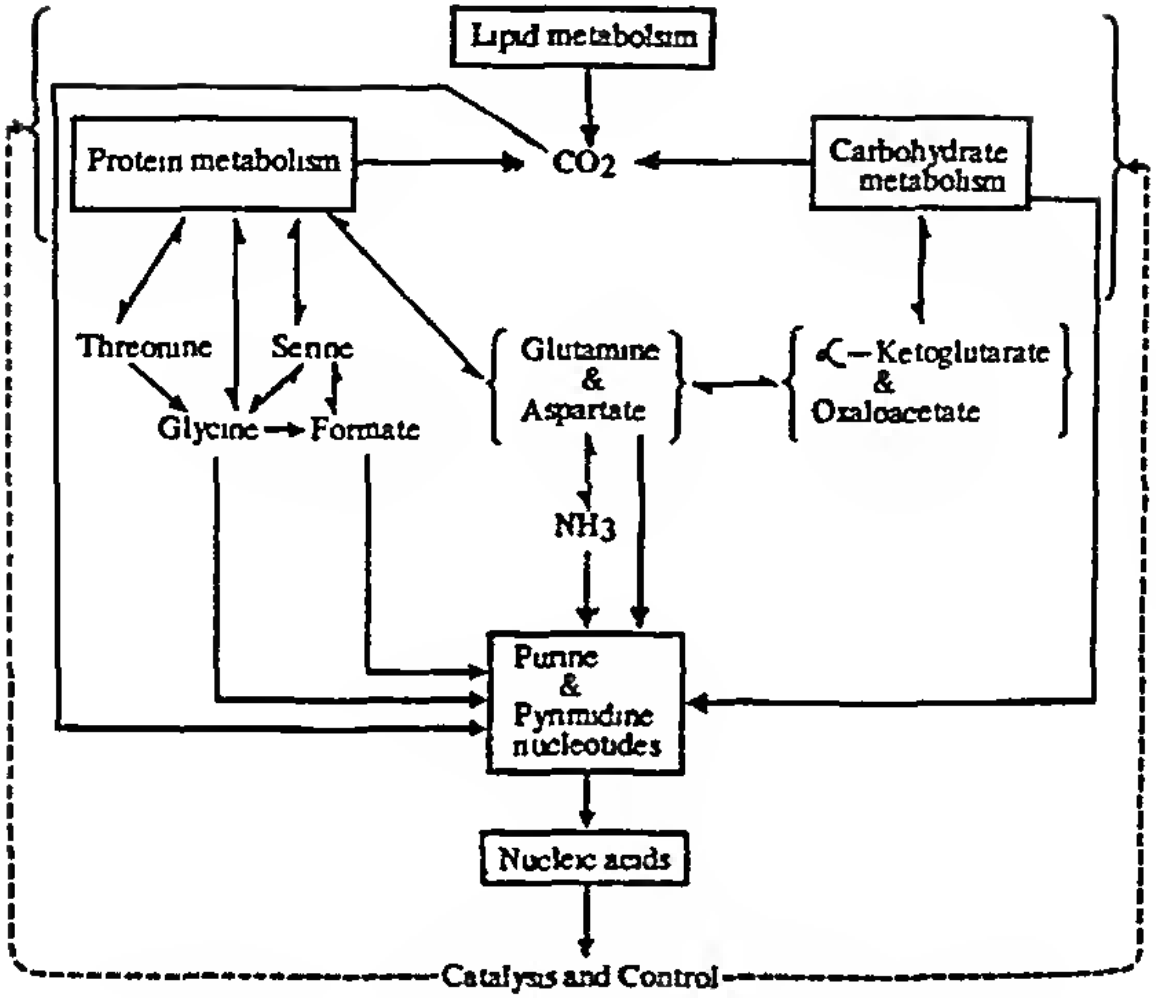


Fig. 24.8 Metabolic interrelations of nucleic acids with other foodstuffs

Exercise

1 Discuss the biological importance of nucleic acids (M U 72A ; R. U 68A)
2 Describe in short the metabolism of nucleic acid in man (R. U 71A)

CHAPTER 25

PROTEIN SYNTHESIS AND THE GENETIC CODE

In the nucleotide sequence of the mRNA molecule, code words exist for each amino acid This is referred to as the *genetic code* The adapter molecules which translate the code words into the amino acid sequence of a protein are the *transfer RNA (tRNA)* molecules The cell possesses the machinery necessary to translate information from the nucleotide sequence of an mRNA into the sequence of amino acids of the corresponding specific protein This process is said to be *translation*

The *Ribosome* is the cellular component on which the various functional entities interact to assemble the protein molecule Many of the ribosomes aggregate to translate a single mRNA molecule and form *polyribosomes* The rough endoplasmic reticulum is the factory of polyribosomes

Twenty different amino acids are required for the synthesis of proteins and there must be at least 20 genetic code There are only 4 different nucleotides in mRNA and each code word must consist of more than a single nucleotide Code words consisting of 2 nucleotides each can provide for only 16 (4×4) specific code words

Matthaei and Nirenberg initially observed that each code word, termed a *codon*, consists of a sequence of 3 nucleotides, i e it is a triplet code These 64 triplet codes in mRNA with 4 nucleotides are to provide for 20 amino acids Three codons do not code for specific amino acids and these have been termed *nonsense codon* Only a single amino acid is indicated for any specific codon , the genetic code is *unambiguous*

The recognition of specific codons in the mRNA by the tRNA adapter molecules is dependent upon their *anticodon region* and the base-pairing rules During the process of protein synthesis, the reading of the genetic code does not involve any overlap of codons Once the reading is started at a specific codon there is *no punctuation* between codons

The processes of protein synthesis :

1 The process of recognition and attachment (charging) is carried out in 2 steps by one enzyme for each of the 20 amino acids. These enzymes are termed *aminoacyl-tRNA synthetases* They form an activated intermediate of aminoacyl-AMP-enzyme complex which recognizes a specific tRNA to which it attaches the aminoacyl moiety The amino acid remains attached to the specific tRNA in an ester linkage

Activation of amino acids :

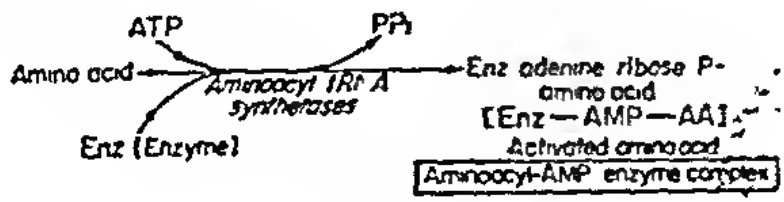


Fig 25 1 Activation of amino acid

Formation of aminoacyl-tRNA :

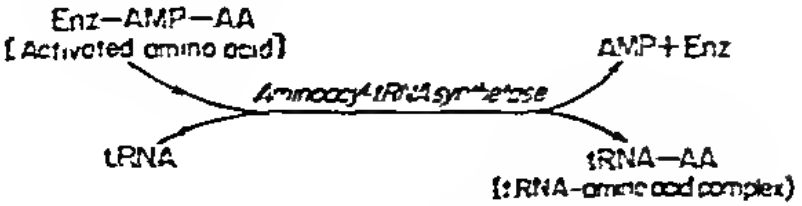


Fig 23.2. Formation of the aminoacyl-tRNA.

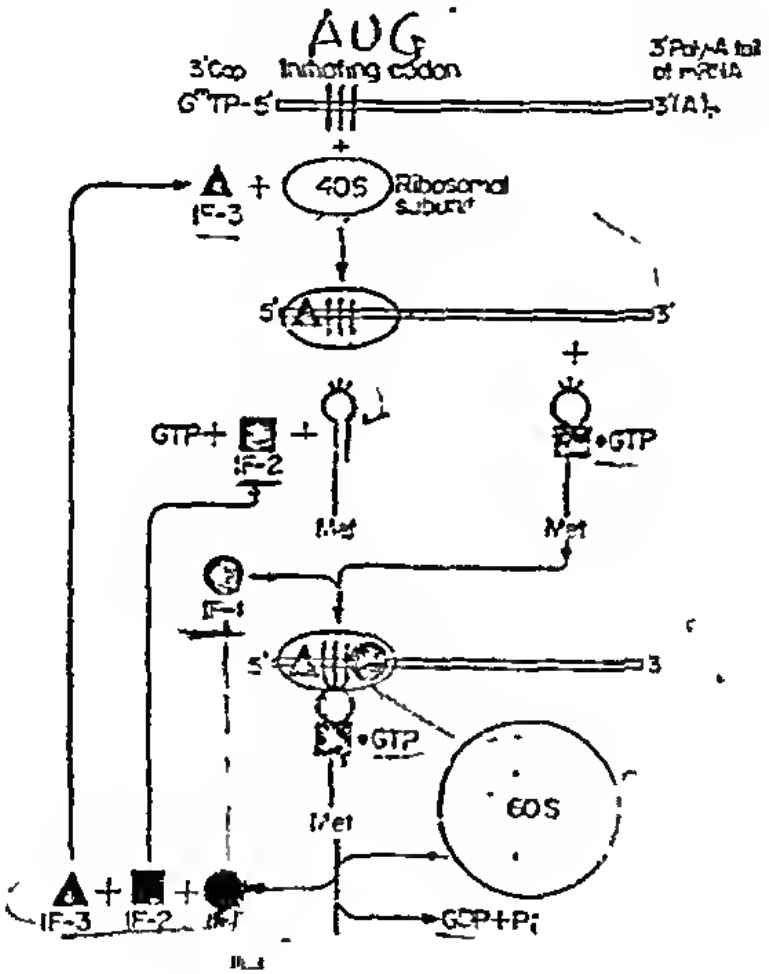


Fig. 23.3. Initiation of
and 3' poly (A)

2. Initiation :

(i) The 5′ termini of most mRNA molecules are "capped" This methyl-guanosyltriphosphate cap is necessary for the binding of many mRNA molecules to the 40S ribosomal subunits, the first codon A U G is intended from the capped 5 terminus The 18S ribosomal subunits binds to a region of the mRNA that precedes the first translated codon The binding of the mRNA to the 40S ribosomal subunit requires the presence of a protein factor, initiation factor 3 (IF 3)

(ii) The aminoacyl tRNA interacts with GTP and initiation factor 2 (IF 2) to form a complex This complex in presence of initiation factor 1 (IF 1) attaches the anticodon of tRNA to the first codon of mRNA to form a complex This again attaches the 60S ribosomal subunits with the release of IF 1, IF-2 IF 3 and the hydrolysis of GTP to GDP+Pi The initiation factors are recycled

(iii) The complete ribosome contains 2 sites—the peptioyl (P site) site contains the peptidyl tRNA attached to the codon on the mRNA and the A site contains the aminoacyl tRNA attached to its respective codon on the mRNA

3 Elongation :

(i) In the complete 80S ribosome formed during the process of initiation the A site is free The binding of the proper aminoacyl tRNA in the A site requires proper codon recognition Elongation factor 1 forms a complex with GTP and the entering aminoacyl tRNA This complex then allows the aminoacyl tRNA to enter the A site with the release of EF 1, GDP and phosphate EF 1 and GDP then recycle

(ii) The alpha amino group of the new aminoacyl tRNA in the A site has got an attack on the esterified carboxyl group of the peptidyl tRNA occupying the P site The reaction is catalyzed by peptidyl transferase of the 60S ribosomal subunits forming a growing peptide chain to the tRNA in the A site

(iii) The tRNA in the P site is discharged and quickly vacates the P site The newly formed peptidyl tRNA at the A site is translocated into the vacated P site by the elongation factor 2 (EF 2) and GTP The A site becomes free for another cycle of aminoacyl tRNA codon recognition and elongation GTP is hydrolyzed to GDP and Pi and also EF 2 is released

4 Termination :

After multiple cycles of elongation the nonsense or terminating codon of mRNA appears in the A site Releasing factors are capable of recognizing that a termination signal resides in the A site The releasing factor hydrolyzes the bond between the peptide and the tRNA occupying the P site On hydrolysis and release, the 80S ribosome dissociates into its 40S and 60S subunits which are then recycled. The releasing factors are proteins.

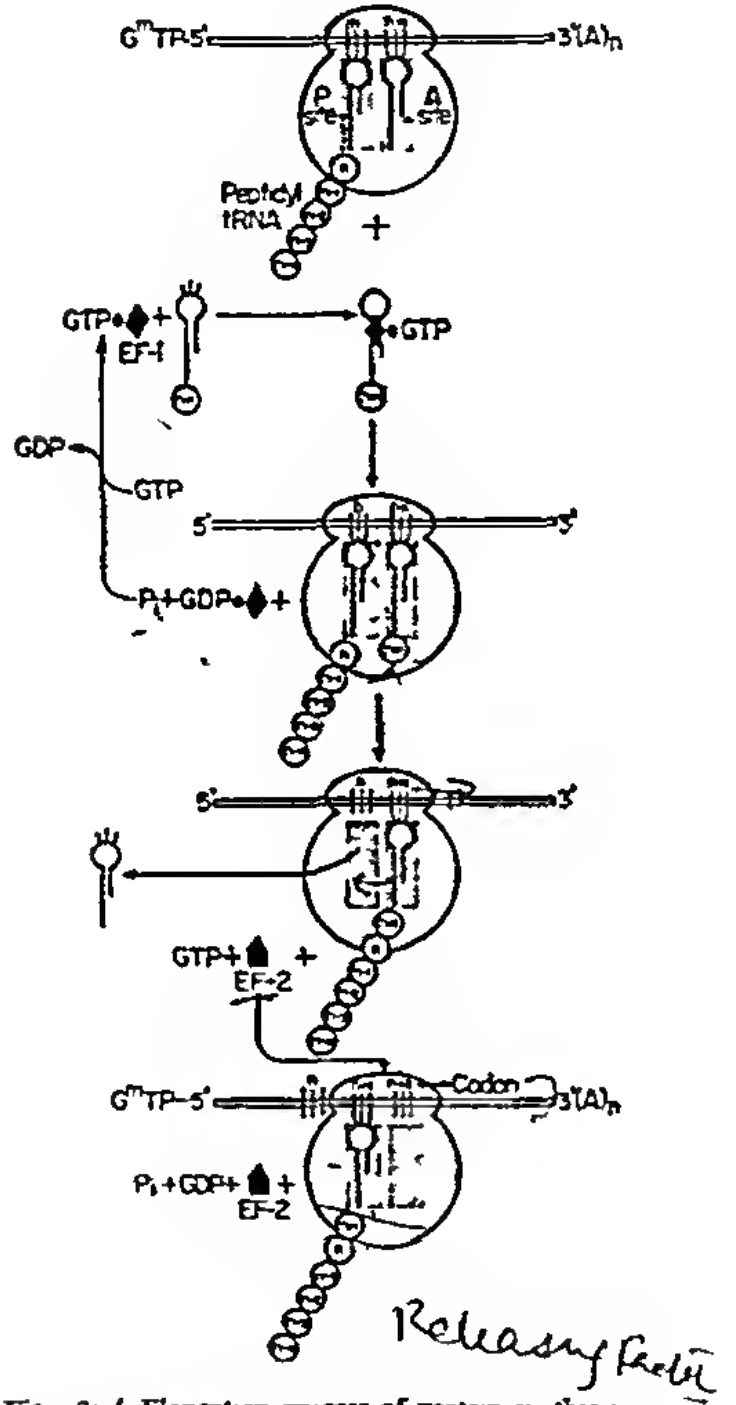


Fig. 2.4 Elongation process of protein synthesis

Inhibitors of protein synthesis :

1 The antibiotic, puromycin has structural similarity with that of tyrosinyl-tRNA is incorporated via the A site on the ribosome into the carboxyl terminal position of a peptide but causes the premature release of the polypeptide It, therefore, effectively inhibits protein synthesis

2 Diphtheria toxin catalyzes the ADP ribosylation of EF-2 in mammalian cells This modification inactivates EF-2 and thereby inhibits mammalian protein synthesis

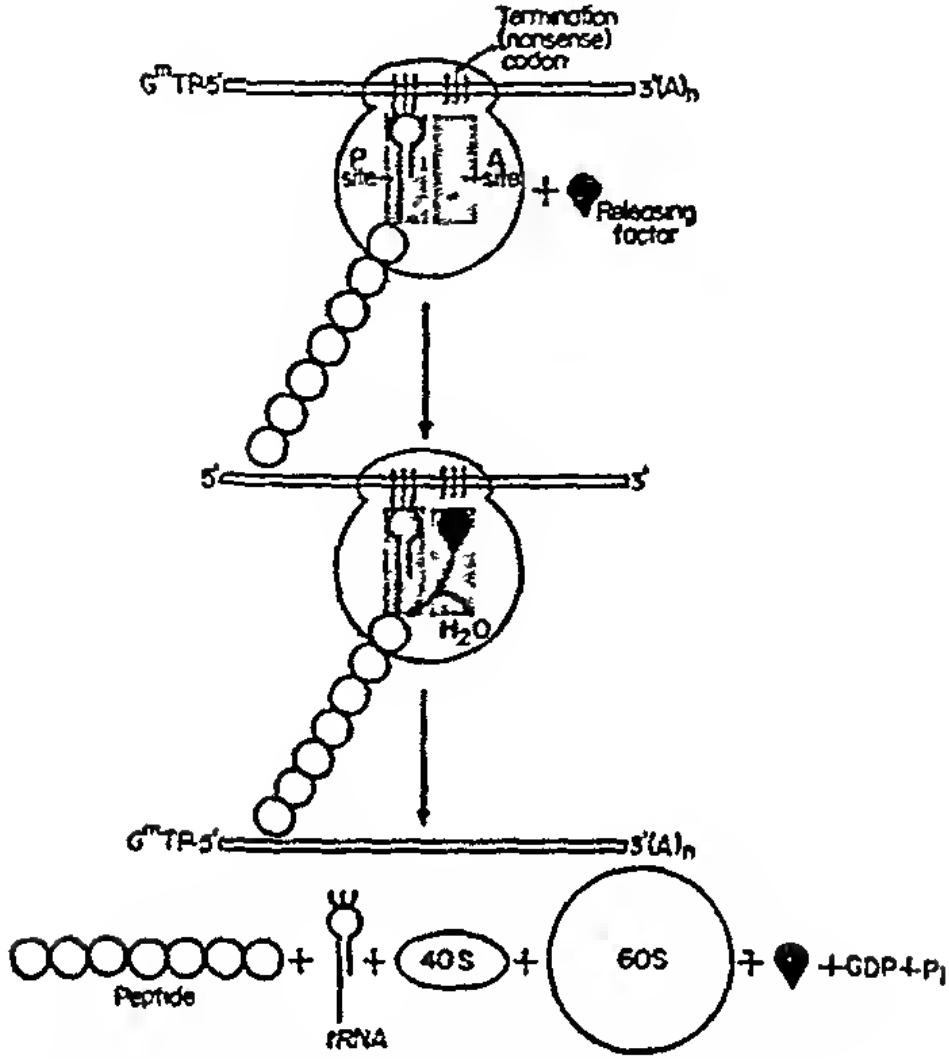


Fig. 25 5 The termination process of protein synthesis

Regulation of Gene Expression :

A single ribosome is capable of translating about 400 codons in 10 seconds into a protein with a molecular weight of 40,000

REGULATION OF GENE EXPRESSION

Mammalian cells possess only about 1000 times more genetic information than does the bacterium E Coli Much of this additional genetic information is probably involved in the regulation of Gene expression

There are only two types of gene regulation *Positive regulation* and *negative regulation.* When the expression of genetic information is quantitatively increased by the presence of a specific regulatory element, regulation is said to be *positive,* whereas when the expression of genetic information is diminished by the presence of a specific regulatory element, regulation is said to be *negative* The element or molecule mediating the negative regulation is said to be a negative regulator, that mediating positive regulation is a positive regulator A *double negative* has the effect of acting as a *positive* Thus, an effector that inhibits the function of a negative regu

lator will appear to bring about a positive regulation. In many regulated systems that appear to be induced, they are depressed at the molecular level.

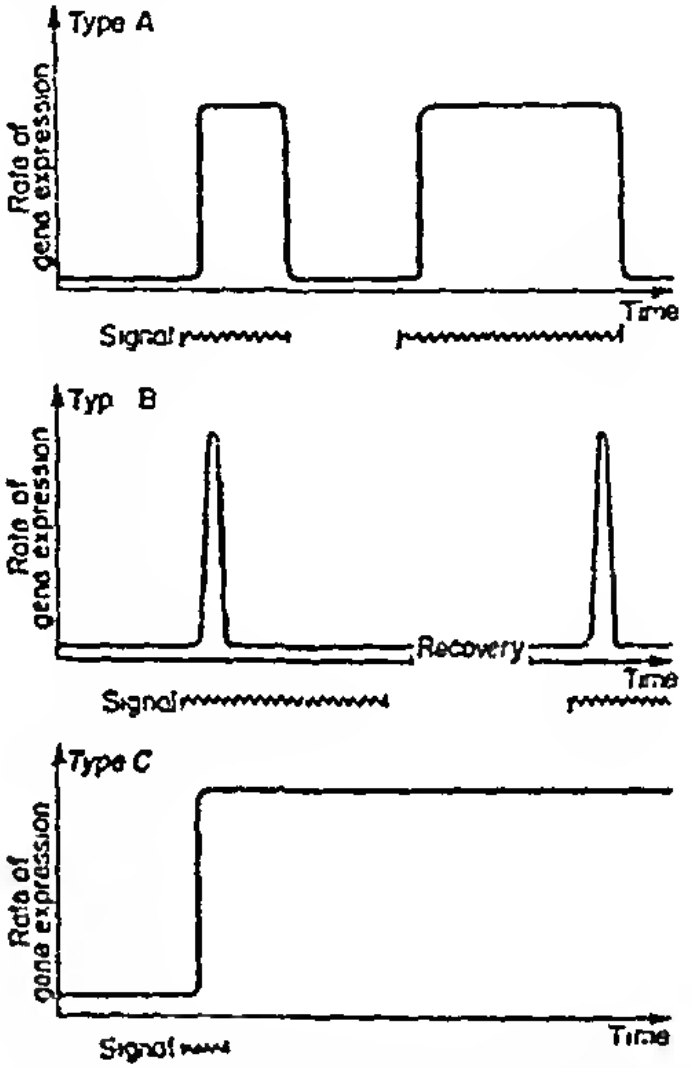


Fig. 23 6. Diagrammatic representations of the responses of the rate of expression of a gene to specific regulatory signals such as a hormone.

In biologic organisms, there are three types temporal responses to a regulatory signal. These three responses are shown in the fig. 23 6 above as rate of gene expression in temporal response to an inducing signal.

A Type A response is characterized by an increased rate of gene expression that is dependent upon the continued presence of the inducing signal. When the inducing signal is removed, the rate of gene expression diminished to its basal level, but the rate repeatedly increases in response to the reappearance of the specific signal. This type of response is commonly observed in many higher organisms after exposures to inducers such as steroid hormones.

A Type B response exhibits an increased rate of gene expression that is transient even in the continued presence of the regulatory signal. After the regulatory signal has terminated and the cell has been allowed to recover, a second transient response to a subsequent regulatory signal may be observed. This type of response may commonly occur during development of an organism when only the transient appearance of a specific gene product is required although the signal persists.

The Type C response exhibits an increased rate of gene expression that persists indefinitely even after the termination of the signal The signal acts as a trigger in this pattern Once the gene expression is initiated in the cell, it cannot be terminated even in the daughter cells, it is, therefore, an irreversible and inherited alteration

The *gene* or *cistron* is the unit of genetic information It is the smallest segment of the DNA molecule containing about 600 base pairs The genetic message is carried in the sequence of bases along the *DNA strand* These are arranged in orderly manner along the length of the DNA molecule in the chromosomes When a pair carries genes with the same characteristics say tallness then the individual is said to be homozygous with respect to that character When one of the pair tallness and the other gene shortness, the individual is heterozygous

THE LAC OPERON

Lac operon is nothing but structural gene+operator gene

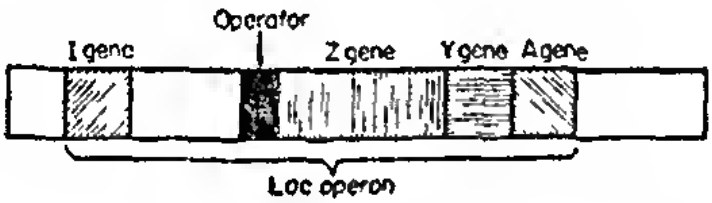


Fig 25 7 The positional relationship of the structural and regulatory genes of the lac operon

A lactose analog which is capable of inducing the lac operon while not itself serving as a substrate for β galactosidase is called a *gratuitous inducer*

The expression of some genes is *constitutive* i e they are expressed at a reasonably high rate in the absence of any specific regulatory signal

The regulator gene controls the activity of the operator gene The regulator gene induces the synthesis of protein macromolecules (probably RNA protein) called *repressors*

The operon becomes active because the repressor system is itself inactivated The phenomenon is said to be *Derepression*

The *operator locus* is a region of double stranded DNA of 27 base pairs long with a 2-fold rotational symmetry in a region that is 21 base pairs long The minimum effective size of an operator for lac repressor binding is 17 base pairs The binding occurs mostly in the *major groove* without interrupting the base paired, double helical nature of the operator DNA The binding of the inductor to the repressor molecule involves the amino acid residues in positions 74 and 75 The *operator locus* lies between the *Promoter site*, at which the DNA-dependent RNA polymerase attaches to commence transcription, and the beginning of the *Z gene* When attached to the operator locus, the repressor molecule prevents the transcription of the operator locus and of the distal structural genes, Z, Y, A Thus, the repressor molecule in a *negative regulator* and in its presence the expression of the Z, Y, and A genes is prevented Normally 20-40 repressor molecules are present and one or two operator loci per cell are also found to be present

The translation of the polycistronic mRNA can occur even before the transcription is completed The depression of the lac operon allows the cell to synthesize the enzymes necessary to catabolize lactose as an energy source Only for the RNA polymerase to attach at the promoter site, there must be the presence of the catabolite gene activation protein (CAP) to which cAMP is attached The

bacterium accumulates cAMP only when it is starved for a source of carbon In the presence of glucose or glycerol in concentrations sufficient for growth, the bacteria will lack sufficient cAMP to b nd to CAP Therefore, in the presence of glucose on glycerol, cAMP-saturated CAP is lacking, so that the DNA-dependent RNA polymerase cannot begin the transcription of the lac operon In the presence of the CAP-cAMP complex or the promoter site, transcription then takes place. Therefore, the CAP-cAMP regulator is acting as a *positive regulator*

When the i gene is mutated so that its product, the lac repressor, is not capable of binding to operator DNA, the organism will exibit *constitutive expression* of the lac operon An organism with an i gene mutation that prevents the binding of an inducer to the repressor will remain repressed even in the presence of the inducer molecule, because the inducer cannot bind to the repressor on the operator locus in order to depress the operon

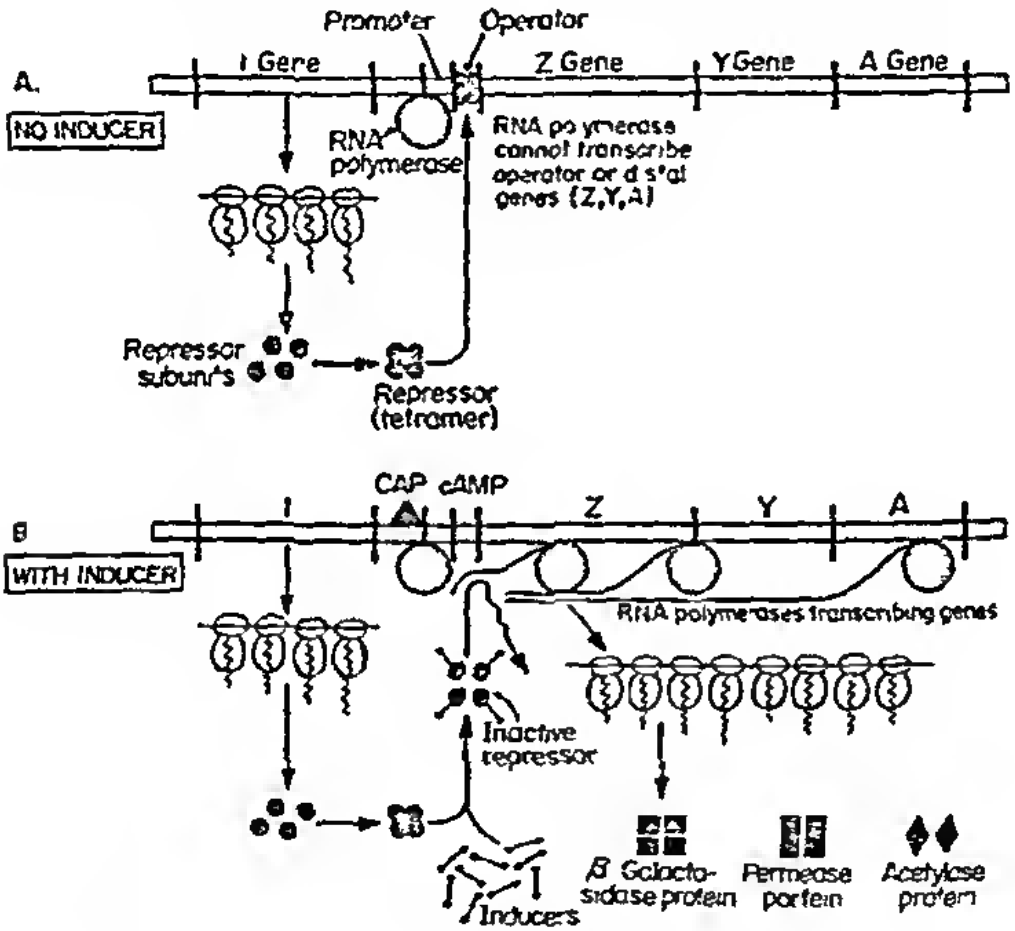


Fig 25 8 The Mechanism of repression and derepression of the lactose operon

Bacteriophase Lamda (λ)

When a lamda infects a sensitive E Coli, it injects its 45,000 base-pair, double-stranded, linear DNA molecule into the cell (Fig 25 9) Depending upon the nutritional state of the cell, the lamda DNA will either integrate into the host genome (Lysogenic Pathway) and remain dormant until activated, or it will commence replicating until it has made about 100 copies of complete, Protein-Packaged virus at which point it effects lysis of its host (Lytic Pathway) The newly generated virus articles can then infect other sensitive host

When integrated into the host genome in its dormant state, lamda will remain in such a state until activated by exposure of its lysogenic bacterial host to DNA—damaging agents. In response to such a noxious stimulus, the dormant bacteriophage becomes "induced" and begins to transcribe aod subsequently trauslate those genes of its own genome which are necessary for its excision from the host chromosome, its DNA replication, and its protein cost and lysis enzymes. This event acts like a trigger or type C responses.

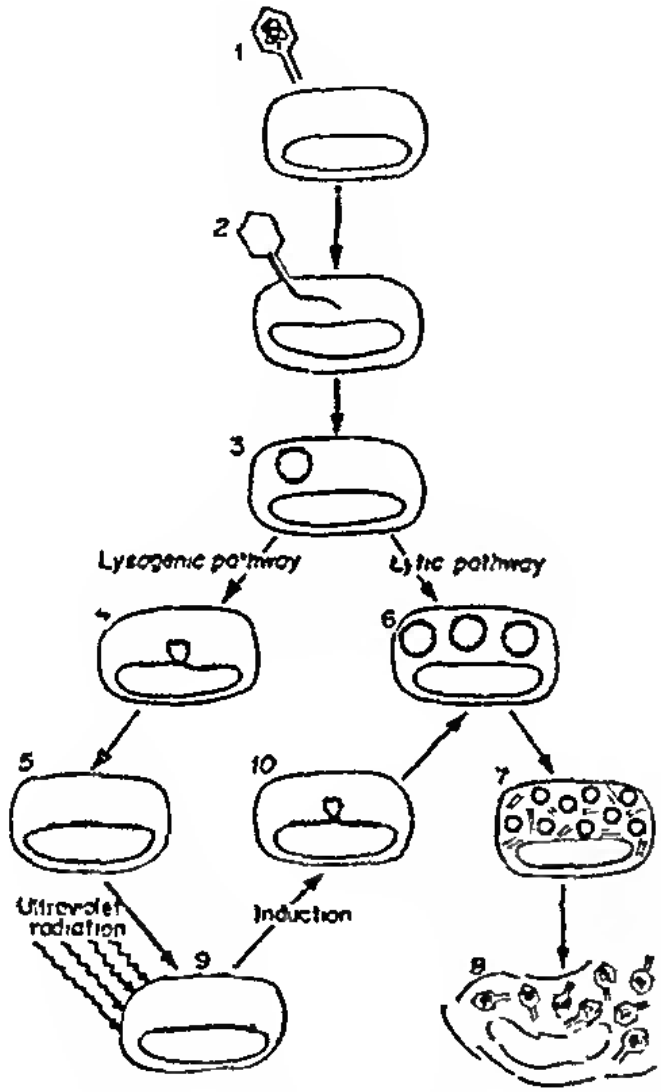


Fig. 25.9. Infection of the bacterium E Coli by the lamda virus

The switching event in lamda is centered around an eighty base pair region in its double-stranded DNA molecule referred to as the "right operator" (OR) Fig. 23.10A. The *right operator* is flanked on its left side by the structural gene for the lamda *repressor* and on its right side by the structural gene for another regulatory protein called ero. When lamda is in its prophage state, the *repressor gene* is the only lamda gene that is expressed. When the bacteriophage is undergoing lytie growth, the repressor gene is not expressed, but the ero gene, as well as many other genes in lamda, is expressed. That is, when the *repressor gene is on*, the *ero gene is off*, and when the *ero gene is on*, the *repressor gene is off*. These two genes regulate each other's expression.

The operator region can be subdivided into three discrete sites, each consists of 17 base pairs of similar but not identical DNA Sequence (Fig 25 10B) Each of these 3 subregions, (OR1, OR2, and OR3) can bind either repressor or cro proteins in the major groove of the DNA double helix. The DNA region between the cro and repressor genes also contains two promoter sequences that direct the binding of RNA polymerase in a specified orientation where it commences transcribing the adjacent genes. One promoter directs RNA polymerase to begin transcription in the *rightward direction* and, thus, to transcribe cro and other distal genes, while the other promoter directs the transcription of the *repressor* gene in the *leftward direction* (Fig 25 10B)

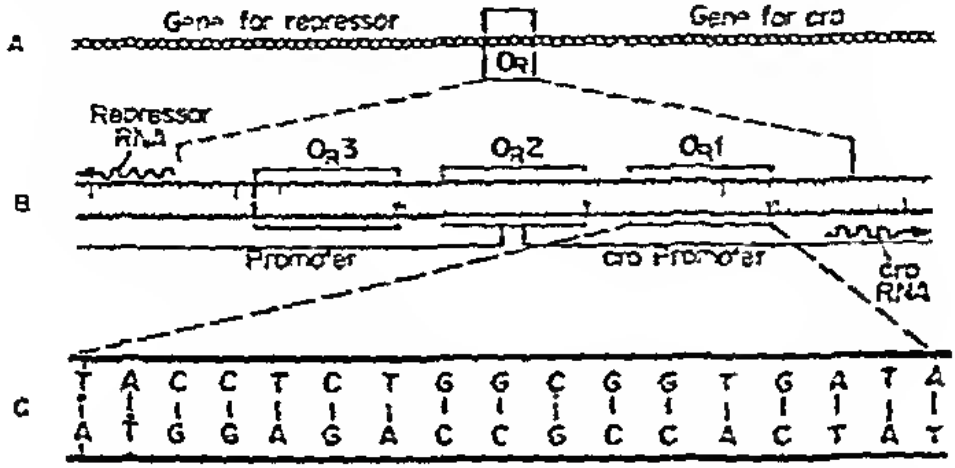


Fig 2 10 Right operator (OR) is shown in increasing detail in this series of drawings.

The product of the repressor gene, the 236 aminoacid *repressor protein*, exists as a 2-domain molecule in which the *aminoterminal domain binds to operator DNA* and the *carboxyterminal domain promotes the association* of one repressor protein with another to form a dimer A *dimer* of repressor molecules binds to *operator DNA* much more tightly than does the monomeric form (Fig 25 11A to C.)

The product of the cro gene, the 66-amino acid *Cro Protein*, has a single domain but also binds the operator DNA more tightly as a dimer (Fig 25 11D)

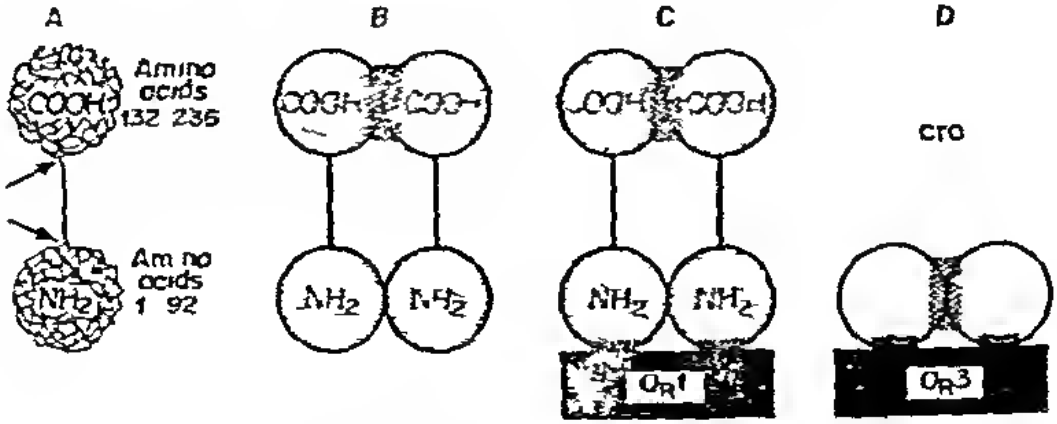


Fig 25 11 Lamda repressor protein is a chain of 236 amino acids The chain folds itself into a dumbbell shape with two substructures

In a lysogenic bacterium i e., a bacterium containing a lamda propbage, the lamda repressor dimer binds preferentially to OR1 but in so doing by a cooperative interaction, enhances the binding of another repressor dimer to OR2 (Fig. 25.12). The affinity of repressor for OR3 is the least of the three operator subregions. The binding of repressor to OR1 has two major effects. The occupation of OR1 by repressor *blocks the binding of RNA polymerase to the rightward* Promoter and thereby prevents the expression of the cro gene. Secondly, repressor dimer bound to OR1 enhances the binding of repressor dimer to OR2. The binding of repressor to OR2 has the important effect of *enhancing the binding of RNA polymerase to the leftward promoter* that overlaps OR2 and thereby enhances the transcription and subsequent expression of the repressor gene. Thus, the lamda repressor is both a *negative regulator* by preventing transcription of the cro gene, and a positive regulator, by enhancing the transcription of its own gene, the repressor gene. This dual effect of repressor is responsible for the stable state of the dormant lamda bacteriophage.

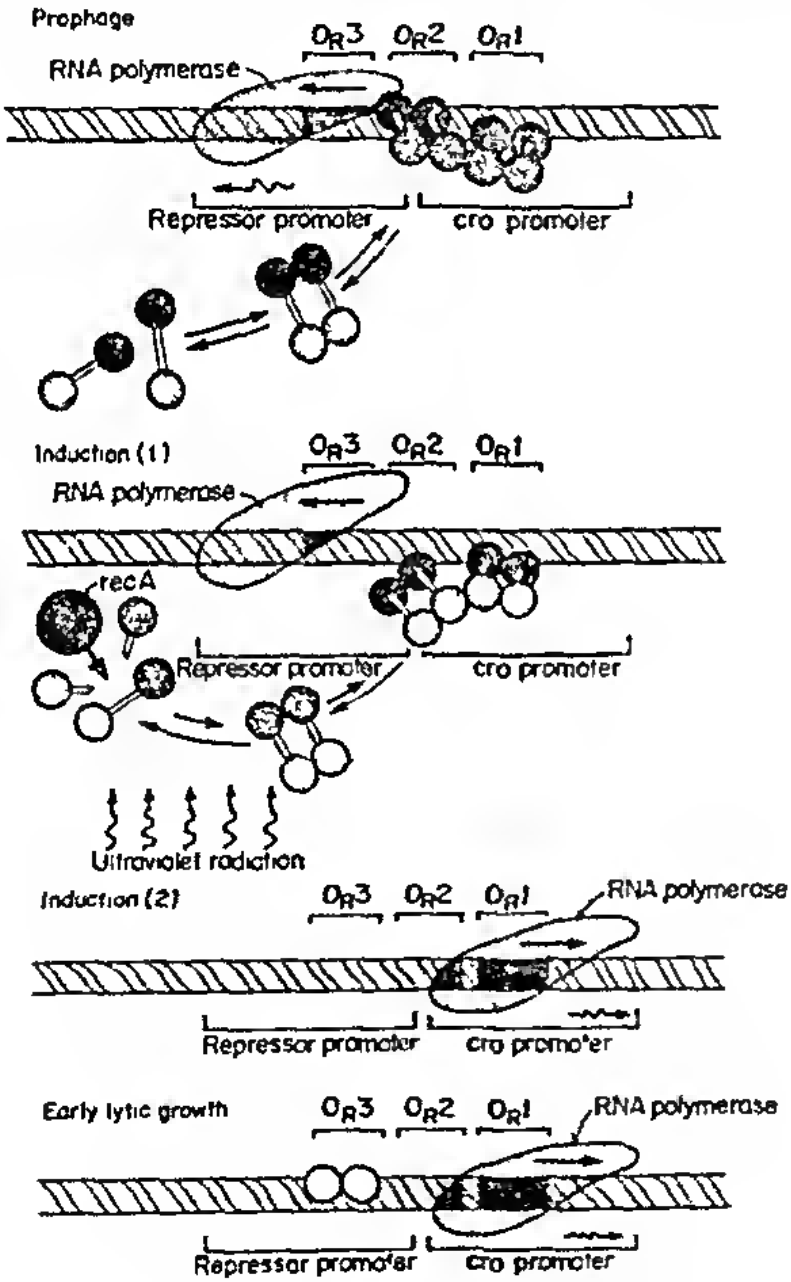


Fig 25 12. Configuration of the switch is shown at four stages of lamda's life cycle

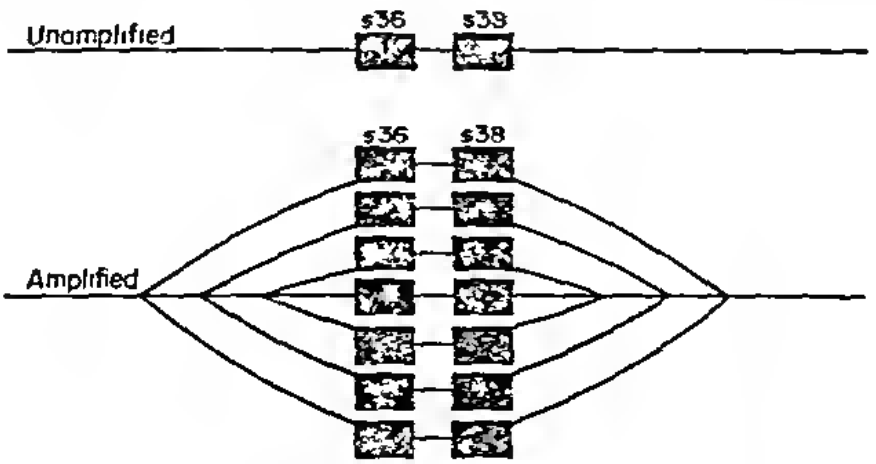
When a DNA damaging signal, such as ultra violet light, strikes the lysogenic host bacterium, fragments of signal-stranded DNA are generated that activate a specific *Protease* coded by a bacterial gene and referred to as *rec A* The activated rec A Protease hydrolyzes the portion of the repressor protein that connects the amino-terminal and carboxy-terminal domains of that molecule Such cleavage of the repressor domains causes the *repressor dimers to dissociate*, which in turn causes a *dissociation of the repressor molecules from OR2* and eventually from OR1 The effects of removal of repressor from OR1 and OR2 are speculated RNA polymerase immediately has access to the right ward promoter and begins transcribing the *cro gene*, and the enhancement effect of the repressor at OR2 on leftward transcription is lost.

The cro protein translated from the newly transcribed cro gene also binds to the operator region as dimers, but its order of preference is the opposite of that of repressor That is, *cro binds most tightly to OR3*, but there is no cooperative effect of cro at OR3 on the binding of Cro to OR2 At increasingly higher concentrations of Cro, the protein will bind to OR2 and eventually to OR1

The occupancy of OR3 by Cro immediately turn off the transcription from the leftward promoter and hence, *prevents any further expression of the repressor gene* Therefore, the switch is completely effected The Cro gene is now expressed and the repressor gene is fully turned off When Cro repressor concentration becomes very high, it will eventually occupy OR1 and in so doing turn down the expression of its own gene, a process that is necessary to effect the final stages of the lytic cycle

Gene Amplification During Development

During early development of metazoans, there is an abrupt increase in the need for specific genetic molecules such as ribosomal RNAs and messenger RNA molecules for proteins that make up such organs as the eggshell One way to increase the rate at which such molecules can be formed is to increase the number of genes available for transcription of these specific molecules Among the repetitive DNA sequences are thousands of copies of ribosomal RNA genes and tRNA genes These genes preexist repetitively in the genomic material of the gametes and, thus, are transmitted in high copy number from generation to generation In some specific organisms such as the fruit fly (Drosophila), there occurs during oogenesis an amplification of a few pre-existing genes, such as these for the chorion (eggshell) proteins, S36 and S38 Subsequently, these amplified genes, presumably generated by a process of repeated initiations during DNA synthesis provide multiple sites for gene transcription



In recent years, it has been possible to promote the amplification of specific genetic regions in cultured mammalian cells In some cases, a several thousand fold increase in the copy number of specific genes can be achieved over a period of time involving increasing doses of selective drugs In fact, it has been demonstrated in patients receiving methotrexate for treatment of cancer that malignant cells can develop *drug resistance* by increasing the number of genes for dihydrofolate reductase, the target of methotrexate

Immunoglobulin Gene Rearrangement.

1 The Coding segments responsible for the generation of specific protein molecules are frequently *not contiguous in the mammalian genome* Immunoglobulin molecules consist of two types of polypeptide chains, light (L) and heavy (H) chains The L and H chains are each divided into N terminal variable (V) and carboxyterminal constant (C) regions The V regions are responsible for the recognition of antigens (foreign molecules) and the constant regions for effector functions that determine how the antibody molecule will dispense with the antigen

2 There are three unlinked families of genes responsible for immunoglobulin molecule structure Two families are responsible for the light chains (λ and κ chains) and one family for heavy chains

3 Each *light chain* is encoded by three distinct segments, the variable (VL), the joining (JL), and the constant (CL) elements The mammalian haploid genome contains over 500 VL segments, five or six JL segments, and 10 or 20 CL segments

4 A VL segment is brought from a distant site on the same chromosome to a position closer to the region of the genome containing the JL and CL segments during the differentiation of a lymphoid B cell This *DNA rearrangement* then allows the VL JL, and CL segments to be transcribed as a single mRNA Precursor and subsequently processed to generate the mRNA for a specific antibody light chain The immunity system can generate a diverse library of antigen specific immunoglobulin molecules by *rearrangement* of the various VL, JL, and CL segments in the genome The DNA rearrangement is referred to as *V J joining* of the light chain

5 The *heavy chain* is encoded by four gene segments the VH, the D (Diversity), the JH, and the CH DNA segments The variable region of the heavy chain is generated by joining the VH with a D and a JH segment The resulting VH D JH DNA region is in turn linked to a CH gene These CH genes ($C\mu$, $C\delta$, $C\gamma3$ $C\gamma1$, $C\gamma2b$, $C\gamma2a$ $C\alpha$, and $C\varepsilon$) determine the immunoglobulin class or subclass—IgM IgG, IgA, etc —of the immunoglobulin molecule

6 A B cell that secretes antibody to a *specific antigen* will secrete antibodies of different classes having the same antigen specificity but different biologic roles during the differentiation The different classes of immunoglobulins contain the same light chains and VH regions but different CH regions Thus, a single B cell and its derivatives can undergo "class switching" which is the result of second type of *DNA rearrangement* occurring during differentiation of the immunity system The *V—J joining* for light chain expression and the *V D J joining* for heavy chain expression *precede the class switching DNA rearrangement*

V-J joining

1 In the undifferentiated cell (e g germ line cell), the K J gene (J_κ) is closely linked to the C_κ gene, but the gene segment for the κ variable region (V_κ) is quite distant on the same chromosome

2 Both the V_K–J_K and the similar Vλ Jλ gene rearrangements seem to involve two short conserved sequences that exist in the direction 3 *to the V segment* and 5 *to the J segment*, closed to the point of recombination.

3 There are two striking features of these conserved sequences. First, the conserved *sequences of the JL segments are inverse complements of the conserved sequences in the VL segments* Second, the length of the nonconserved sequence separating the heptamers and the nonomers is highly conserved.

4 The variable region of the heavy chain involves three DNA segments, VH, D and JH which must be joined in a process involving two DNA rearrangements since all three segments are separated.

Class Switching

1 Since differentiation proceeds and immunoglobulin production switches from IgM to IgA the V D J region of the parent B cell must be rearranged with Cα gene to permit the transcription of an mRNA precursor for an α chain containing the same antigen-specific variable region.

2 The temporal order of the class switching is unidirectional within the physical order from left to right. Rearrangement of the CH genes seems to involve deletion of those CH genes 5 to the CH gene joined to the V D J region.

The switch sites are different for different class switches but involve conserved sequences occurring in the appropriate regions of the genes to be rearranged.

Transcription Control

1 Glucocorticoids regulate gene expression. Once they enter the mammalian cell, they bind to a steroid specific receptor molecule that undergoes a conformational change in the cytoplasm and enters the nucleus. The glucocorticoid receptor complex in the nucleus binds to a specific receptor recognition site on DNA, a few hundred base pairs 5' upstream from the transcription start site, for steroid responsive genes The receptor recognition site on DNA influences the efficiency of utilization of the promoter by RNA polymerase and thereby influences the expression of the steroid responsive gene.

2 When an organism or its cultured cells are exposed to metal ions such as zinc or cadmium, there is an accelerated rate of transcription of the metallothionein gene and a subsequent increase in the metallothionein protein to bind the potentially toxic heavy metal Another structural gene, such as that for thymidine kinase, can be ligated to this '*metallothionein promoter region*' and the synthetic construct introduced to cultured cells, a small number of which will integrate the DNA into its own genome. When those cells are exposed to heavy metals, the metallothionein promoter region effects an induction of thymidine kinase

3 There are many more primary transcripts in the nucleus than are represented as messenger RNA molecules in the cytoplasm There must exist regulatory decisions as to which transcripts will ultimately be expressed and which will be discarded There is no information available regarding the mechanism of this process

Exercise

1 What is codon ? Describe the biosynthesis of proteins (Mdu 75A)

2. Write notes on Codon. (M U 72A)

CHAPTER 26

WATER AND MINERAL METABOLISM

Distribution of fluids in the body

1 Water makes up 50 to 70 per cent of the weight of the adult human body and varies inversely as the fat content

2 This mentioned amount of water is distributed throughout the body as the major component of the intracellular and extracellular fluids

3 The intracellular fluids amount to about 50 per cent of the body weight in a lean individual and much less in an obese person

4 The extracellular fluids represent about 20 per cent of the body weight

5 Of the extracellular fluids interstitial fluid amounts to some 15 per cent and blood plasma about 5 per cent of the body weight

6 Relatively small volumes are represented by specialized fluids such as cerebrospinal fluid, ocular fluid, lymph, and synovial fluids etc

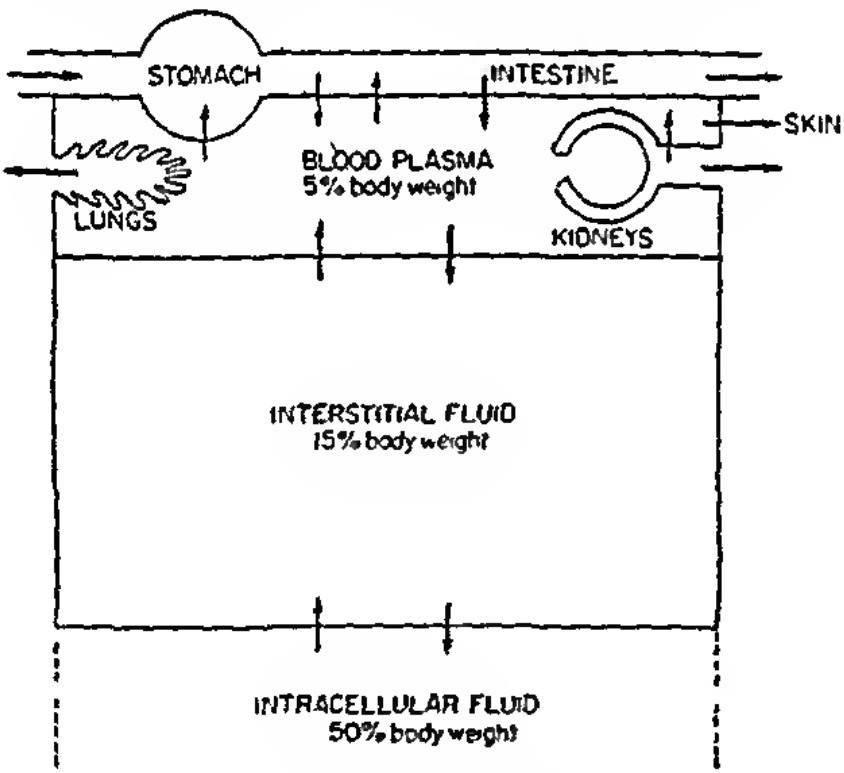


Fig 26.1 Distribution of fluids

Extracellular fluid

1 All body cells exist in an environment of fluid collectively designated extracellular fluid This includes the blood plasma, interstitial fluid and lymph

2 7 per cent protein is present in plasma, slightly less in hepatic lymph and 0 1 per cent protein in subcutaneous interstitial fluid

3 They are solutions mainly of NaCl and $NaHCO_3$ with small amounts of Ca, Mg K, H, phosphate, sulphate and organic acid ions, some nonelectrolytes (glucose, urea, lipids etc) and with p^H values ranging from 7 35 to 7 45 under normal conditions

4 The total concentration of the ionic constituents is about 310 m mol per liter of plasma

5 Much of the intracellular magnesium is not in the ionic form but is bound to protein and other smaller organic molecules

6 Several components of the extracellular fluid are important in preserving osmotic, anion-cation balance and hydrogen ion regulation

7 The cations (K^+, Ca^{++} Mg^{++} and H^+) although present in comparatively very low concentrations exert profound influences on physiological processes

8 The interstitial fluid contains a higher total concentration of diffusible anion and a lower concentration of cation than does the plasma

Intracellular fluid

1 Less amounts (5 10 m mol /liter) of sodium are present in the intracellular fluid which also contains little but extremely biologically important calcium.

2 The chief cations of this fluid are potassium (about 160 m mol /liter) in muscle and magnesium (about 26 m mol /liter) in muscle

3 The intracellular fluids contain much more phosphate and sulphate ions as well as protein than the extracellular fluid. Chloride ion is practically absent from this fluid except in the case of erythrocytes and cells of the kidney tubules, stomach and intestines Both Na and K ions are able to cross the membrane more freely under certain physiological and pathological conditions.

4 Much of the magnesium is present as undissociated compounds of protein and organic phosphate and therefore is not in ionic form

Exchanges between fluid compartments

Many of the substances entered into the body and produced by the cells, are distributed to other tissues or excreted The exchange systems are outlined as follows

1 Alveolar air Blood plasma This system provides for entrance oxygen into and loss of CO_2 and water from the body

2. Plasma Erythrocytes This system provides for ready exchange of oxygen CO_2 water and certain anions (particularly Cl and HCO_3^-) in both directions Cations are exchanged very slowly

3 Plasma Interstial fluid These two media are separated by the capillary walls which are permeable to water inorganic ions and small organic molecules (glucose amino acids urea etc) but not to large organic molecules such as proteins

4 Interstitial fluid Intracellular fluid These two compartments are separated by the cell membranes across which gases in solution water and small unchanged molecules can diffuse The small molecules e g glucose is not subjected to simple diffusion but is carried across cell membrane by active

transport processes The permeability of electrolytes follows biological pump mechanisms These membranes are also relatively impermeable to large molecules such as proteins except in special situations namely the liver

WATER METABOLISM

Distribution of water in the body

Water is the large constituent of the body The average body water is 60 to 70 per cent of the total body weight Females contain a little less amount of water than males Body water is distributed as follows

1 *Intracellular fluid* (the fluid within the cells) contains 50 per cent of the total body weight

2 *Extracellular fluid* (all of the fluid outside the cell) contains 20 per cent of the total body weight This 20 per cent can be further divided into the following compartments

(i) Plasma (the extracellular fluid of the blood)—4 5 per cent

(ii) Interstitial and lymph fluids—8 per cent

(iii) Dense connective tissue cartilage and bone—6 per cent

(iv) Transcellular fluid (found in salivary glands, pancreas liver, thyroid gland, gonads, skin, mucous membranes of the respiratory and gastro intestinal tracts and the kidneys as well as the fluids in spaces within the eye, CSF etc)—1 5 per cent body weight

Factors influencing the distribution of body water

The distribution of water is continuously changing Osmotic forces are the principal factors for controlling the amount of fluid in the various compartments of the body These are maintained by the solutes in the body water The solutes are divided into three categories

1 Organic compounds of small molecular size (glucose urea, and amino acids etc) Since these substances diffuse freely across the cell membrane, they are not important in the distribution of water But if they are present in large amounts, they can help in retaining water

2 Organic substances of large molecular size (proteins) These substances can throw effect in the transfer of fluids from one compartment to the other

3 The inorganic electrolytes These inorganic electrolytes are the most important both in the distribution and in the retention of body water

Intake and loss of body water

A Water intake : Water is supplied to the body by the following processes
1 Water taken orally
2 Along with food
3 Oxidation of food stuffs—Fats, proteins and carbohydrates yield water after combustion Fats produce 107 ml /100 gm, proteins 41 ml /100 gm and carbohydrates 56 ml /100 gm

B Water losses Water is lost from the body by 4 routes
1 Evaporation from skin and lungs
2 Kidneys as urine
3 The intestines, in the feces
4 Perspiration

C. Additional water losses in disease 1 Water loss is more in diarrhea and vomiting and these losses can be fatal in infants

2 In kidney disease renal water loss is more

3 In fever insensible losses may rise much higher than normal.

4 Patients in high environmental temperatures also sustain extremely high extrarenal water losses

Water balance An equilibrium persists between the intake and output of water in the body In addition to other factors certain hormones such as ADH, vasopressin oxytocin and aldosterone influence the regulatory mechanism of body water The balance sheet of water is given below

Balance sheet of water [per day]

Water intake		*Water loss*	
Drinks	1350 ml	Lungs	500 ml.
Solid food	900 ml	Skin	700 ml.
Oxidation of food	450 ml	Urine	1400 ml
		Feces	100 ml
	2700 ml		2700 ml

There is a continuous excretion of water in the form of digestive juices from the body into the alimentary canal and that water is reabsorbed along with the water of food and drink The amount of this internal secretion is 7 to 10 liters per day All of this amount is reabsorbed except about 100 ml which is excreted in feces The secretion of saliva experiences the fact when we have a sore throat

Physiological functions of water

1 *Specific heat* More heat is required to raise the temperature of 1 gram of water through 1°C than almost any other solid or liquid This clearly explains that heat produced as a result of chemical reactions in the cell can cause the minimum rise in body temperature

2. *Latent heat of evaporation* Water has the highest latent heat of evaporation than any other liquid. A certain amount of water can cause maximum cooling by evaporation so that the body temperature does not rise

3 *Solvent power* Water can form true solutions as well as colloidal solutions Even the water insoluble substances are made water soluble by the hydrotropic action Therefore it is the most suitable solvent for cellular components water thus brings various substances in contact for chemical reactions to proceed

4 *Dielectric constant* Oppositely charged particles can coexist in water Therefore it is a good ionizing medium Thus increases the chemical reactions.

5 *Catalytic action* A large number of chemical reactions in the body are accelerated by water due to its ionizing power All chemical reactions in the body proceed in presence of water only

6 *Lubricating action* · Water acts as a lubricant in the body to prevent friction in joints, pleura, conjunctiva and peritoneum

Regulation of passage of water :

1 . If the capillary pressure is increased, more water will flow into the tissues

2 A fall in blood pressure helps the passage of water from the tissues to the blood

3 If the plasma proteins are decreased, water will flow into the tissues

4 Dilution of blood by excessive ingestion of water can lower the osmotic pressure of the plasma proteins and thus may increase capillary pressure

DEHYDRATION

Definition :

When the losses of water exceeds the intake, the body's water content is reduced That is, the body is in negative water balance and the condition is known as dehydration

Causes :

1 **Primary dehydration.** (a) Simple deprivation of water from desert travel, extreme weakness and mental patients refusing to drink causes dehydration It occurs more quickly in fever or in the high temperature of the environment

(b) Excessive water loss due to vomiting, prolonged diarrhea, excretion of large quantities of urine or sweat

In water depletion the concentration of extracellular fluid increases Water is drawn from the cells and both extracellular and intracellular compartments shrink Extreme thirst results, the individual complains of hot and dry body, dry tongue

2 **Secondary dehydration :** The concentration of electrolytes of the body fluids is maintained constant through the elimination or retention of water The reduction or increase in the total electrolytes, which affects chiefly the basic radicals Na (extracellular) and K (intracellular) and the acid radicals HCO_3 and Cl_2 is accompanied by a corresponding increase or decrease in the volume of body water This causes intracellular edema There is slowing of circulation and impairment of urinal function The individual becomes weaker

3 **Dehydration due to injection of hypertonic solution :** When a highly concentrated sugar or salt solution is injected into the body, the osmotic pressure of blood will increase This results in the flow of fluid from the tissues into the blood until equilibrium sets in Consequently, the blood volume increases This increased blood volume soon returns to normal by the loss of excess material through excretion This causes a net loss of body water producing dehydration

Effects of dehydration :

1 Loss of weight due to the reduction in tissue water
2 Disturbances in acid-base balance
3 Rise in the nonprotein nitrogen of blood
4 Rise in the plasma protein concentration and of chloride
5 Rise in body temperature due to reduction in circulating fluid
6 Increased pulse rate and reduced cardiac output
7 Dryness, wrinkling and looseness of skin
8 Exhaustion and collapse.

Correction of dehydration :

1 Ordinarily sodium chloride solution may be given parenterally to repair the losses

2 In the case of the removal of a fluid high in sodium and hicarbonate during fluid and electrolyte losses originating from the intestinal tract (as in prolonged diarrhea, pancreatic or biliary fistulas etc), a mixture of two-thirds isotonic saline solution and one third sodium lactate solution (M/6) should he administered intravenously

3 But dehydration is a problem in diabetes mellitus, addison's disease, uremia, extensive burns and sbock. This type of dehydration can not he corrected by the application of above two processes

Water intoxication .

This condition is caused hy the excess of water retention in the body and can occur due to renal failure, hypersecretion of ADH and excessive administration of fluids parenterally Symptoms of water intoxication are headache, nausea and muscular weakness

MINERAL METABOLISM

The mineral elements present in the animal body are supplied by the diet In poor diets consumed hy a large majority in India and other developing countries, there occurs commonly the deficiencies of calcium and iron Iodine deficiency occurs in people living in certain hilly tracts in India and in some other countries, where the soil and water are deficient in iodine In tropical countries, addition of sodium chloride in the diet is of great importance, because of the loss of NaCl in sweat. The deficiencies of other minerals do not occur normally in average diets

The mineral elements can be classified as *principal elements* (*macronutrients*) and *trace elements*

Principal mineral elements (Macronutrients) :

Seven essential elements Calcium, Magnesium, Sodium, Potassium, Phosphorus, Sulphur and Chlorine

1 Sodium, potassium and chlorine are involved mainly in the maintenance of acid base balance and osmotic control of water metabolism.

2. Calcium, phosphorus and magnesium as constituents of bones and teeth.

3 Phosphorus as constituent of body cells of soft tissues such as muscles, liver etc.

4 Sulphur in cysteine and methionine, thiamine, biotin, lipoic acid and CoA

Trace elements .

These elements are present in living tissues in small amounts They are subdivided into three groups—Essential, possibly essential and nonessential

1 Essential trace elements (micronutrients) Iron, iodine, copper, zinc, manganese, cobalt, molybdenum, selenium, chromium and fluorine

2 Possibly essential trace elements Nickel, tin, vanadium and silicon

3 Nonessential trace elements Aluminium, boron, germanium, cadmium, arsenic, lead and mercury

Physiological importance :

(i) Iodine is required for thyroxine formation.

(ii) Iron aod copper are required for hemoglobin formation

(iii) Zinc is a constituent of carbooic anhydrase and insulin

(iv) Cobalt is a constituent of vitamin B_{12}

The ratio of one element to the other in the tissues is of physiological importance e g, oormal ossification demaods a proper ratio of calcium to phosphorus

CALCIUM

Physiological functioos

1 Calcium along with phosphorus is essential for the formation and development of booes aod teeth

2 Ionized calcium is required io blood coagulation proeess

3 It regulates the excitability of nerve fibres aod oerve centres

4 It is esseotial for nerve impulses and muscular contraction

5 It regulates the permeability of membranes

6 It is esseotial for maiotaining the integrity of intracellular material

7 Calcium in the normal ratio with potassium maintains the normal activity of muscles

8 It is required for the activation of several enzymes such as succinate dehydrogenase, ATPase aod certaio proteolytic enzymes

Sources

Richest sources—Milk aod cheese

Good sources—Egg yolk nuts, figs, beans cabbage, cauliflowers, turnip greens aod asparagus

Daily requirements :

Adult males aod females	800 mg
Womeo during pregnancy aod lactation	1 2 grams.
Infants uoder 1 year	360-540 mg
Children 1-18 years	0 8 1 2 grams

Absorptioo :

Calcium is taken in diet as calcium phosphate, carbonate, tartarate and oxalate Calcium is actively absorbed in the upper small iotestine The active transport process is regulated by 1, 25-dihydroxycholecalciferol a metabolite of vitamin D which is produced in the kidney in response to low plasma Ca^{++} conceotratioos The absorption of it is influenced by the following factors

1 Vitamio D promotes absorption of calcium.

2 Acidic p^H favours calcium absorption , because calcium salts, particularly phosphates and carbonates are quite soluble in acid solutions and are relatively insoluble in alkaline solutions. Hence alkali decreases its absorptioo

3 Organic acids, lactose and basic amino acids in the diet favour calcium absorption

4 Higher levels of proteins in the diet help to increase the absorption of calcium

5 Calcium Phosphorus ratio of 1 1 (2 1 or 1 2) is the most convenient for the absorption of both

6 High concentration of magnesium in the diet decreases absorption.

7 Phytic acid (inositol hexaphosphate), which occurs in cereal grains, forms insoluble salts (phytin) with calcium and magnesium resulting in the impaired absorption of calcium

8 Fatty acids interfere with calcium absorption because of the formation of insoluble calcium salts of fatty acids which are excreted in the feces.

9 Presence of excess fibres in the diet interferes with the absorption of calcium

10 Oxalic acid in the diet forms insoluble calcium oxalate which is excreted in the feces decreasing calcium absorption

11 The percentage of calcium absorption decreases as its intake increases.

12. Parathyroid hormone increases the intestinal absorption of calcium.

13 After the age of 55 to 60 years, there is gradual diminution of intestinal transport of calcium

14 Adrenalglucocorticosteroids diminish intestinal transport of calcium.

Distribution of calcium :

Serum	9-11 mg./100 ml.
C.S.F.	4.5-5 mg./100 ml.
Muscle	70 mg./100 gm.
Nerve	15 mg./100 gm.

Blood calcium :

There is virtually no calcium in erythrocytes. The calcium content of plasma (usually determined in serum) is 9 to 11 mg./100 ml. During infancy and very childhood, the average values approach the upper limit of this range and fall with advancing age Calcium exists in the plasma in three fractions ionized or diffusible calcium, protein bound (nondiffusible) and complexed (citrate and phosphate) In the usual determination of calcium, all these fractions are measured together

About 2 mg. of the total calcium occurs in ionized form, about 5 mg. occurs in nonionized form and about 2 mg. in the complex form.

Factors influencing blood calcium level :

1 *Parathyroid hormone*—In the fasting state (i.e. no absorption from the intestine) the normal plasma calcium concentration is maintained primarily by its rate of excretion and its mobilization from the bones through the action of the parathyroid hormone.

2. *Vitamin D*—It enhances absorption of calcium from the intestine and thus maintains the normal plasma calcium concentration.

3 *Plasma proteins*—Half of the blood calcium (nondiffusible fraction) is bound to plasma protein (chiefly albumin) and thus any decrease in these proteins will be accompanied by a decrease in the total calcium level.

4 *Plasma phosphate*—A reciprocal relationship exists between the concentration of calcium and phosphate ions in plasma. The marked increase in serum phosphate causes a fall in serum calcium concentration.

5 *Calcitonin*—An increase in the ionized calcium levels in the plasma is the stimulus for the production of calcitonin which then causes a deposition of calcium in bone

Excretion

Calcium is excreted in the urine, bile, and digestive secretions. Much of that is excreted in the feces which has escaped absorption. Under optimal conditions, 75 per cent of dietary calcium is absorbed. The remainder is the fecal calcium which is unabsorbed.

In man, about 10 grams of calcium are filtered in 24 hours by the renal glomeruli. Only about 200 mg. appear in the urine which is in the ionic state as well as in the complexes with citrate and other organic anions.

70–90 per cent of the calcium eliminated from the body is excreted in the feces.

A very small amount is excreted into the intestine after absorption.

The daily loss of calcium in sweat is about 15 mg. Vigorous physical exercise increases the loss of calcium by the way of sweat.

Disease state

A. Effects of Parathyroid

1. In *hyperparathyroidism*, the following changes occur:
   (i) Hypercalcemia (serum calcium 12–22 mg./100 ml.)
   (ii) Decrease in serum phosphate
   (iii) Diminished renal tubular reabsorption of phosphate
   (iv) Increased phosphatase activity
   (v) Raised urinary calcium and phosphorus from bone decalcification and demineralization
   (vi) The extra calcium and phosphorus is lost from soft tissues and bones by increased bone-destroying activity.
2. In *hypoparathyroidism*, the following changes occur:
   (i) The concentration of serum calcium may drop below 7 mg./100 ml.
   (ii) Increased serum phosphate and decreased urinary excretion of calcium and phosphorus
   (iii) Normal or occasionally raised serum phosphatase activity
   (iv) Normal acid-base equilibrium
   (v) Probably increased bone density

B. Tetany

Decreased ionized fraction of serum calcium causes tetany. This may be due to:
   (i) An increase in the p^H of blood
   (ii) Poor absorption of calcium from the intestine
   (iii) Decreased dietary intake of calcium
   (iv) Increased renal excretion of calcium as in nephritis
   (v) Parathyroid deficiency
   (vi) Increased retention of phosphorus as in renal tubular disease

Symptoms (i) Muscles lose tone and become flabby.
(ii) Affects the face, hands and feet.

C. Rickets

This disease is characterized by faulty calcification of bones in children showing serum phosphate values 1 to 2 mg./100 ml. This may be due to:
   (i) Vitamin D deficiency

(ii) A deficiency of calcium and phosphorus in the diet or a combination of both

(iii) Poor absorption of calcium from the intestine

(iv) Parathyroid deficiency

(v) Increased serum alkaline phosphatase activity

D Dsteoporosis

The disease occurs in adults due to the following causes

(i) Decalcification of bones as a result of calcium deficiency in the diet.

(ii) Hypoparathyroidism

(iii) Low vitamin D content of the body

Symptoms (i) Fractures of the brittle bones occur even after minor accident.

(ii) Pain due to fractures of vertebrae which may radiate round the trunk, to the buttocks or down the legs

E Renal Rickets

Renal Rickets are more accurately designated *familial hypophosphatemic rickets* which is an inherited disease Affected males show hypophosphatemia and severe rickets Hypophosphatemic rickets is caused by the following reasons

(i) Defective transport of phosphate by the intestine and by the renal tubules.

(ii) Lowered serum phosphorus and hyperphosphaturia.

(iii) Reduced intestinal absorption of calcium and phosphorus

Vitamin D in ordinary doses does not relieve the disease Hence it is some times referred to as *vitamin D resistant rickets*

PHOSPHORUS

Physiological functions

1 It is essential for the formation and developmeot of bones and teeth along with calcium.

2. It is required for the formation of phospholipids nucleic acids, and phosphoproteins

3 It is involved in the formation of organic phosphates such as hexose phosphate triose phosphate and creatine phosphate etc.

4 It is required for the formation of energy rich compouods such as ATP

5 It forms coenzymes such as NADP, ADP, AMP & B_1—PO_4 etc

6 It functions in the huffering system in cells

7 It is required in the ahsorption of glucose by phosphorylation.

Sources Milk cheese egg yolk, meat fish and nuts etc.

Daily requirements

Infants	240-400 mg.
Children	800-1200 mg
Adults	800 mg
Women during pregnancy and lactation	1.2 gms

Distribution :

Serum (inorganic phosphorus) of

(a)	Children	4-7 mg /100 ml
(b)	*Adults*	3-4 5 mg /100 ml
	Muscle	170-250 mg /100 gm
	Nerve	360 mg /100 gm
	Bones and teeth	22,000 mg /100 gm

Absorption :

1 Moderate amounts of fat or acid favour absorption of phosphorus

2 High calcium diet and phytic acid (present in cereals) decrease phosphorus absorption

3 The absorption is enhanced when the calcium and phosphorus ratio is 1 · 1 (2 1 or 1 2)

Blood phosphorus :

The normal inorganic phosphate of plasma is 3 to 4 5 mg /100 ml in adults and is 4 5 to 6 5 mg /100 ml in children It is somewhat higher in summer than in winter It decreases during increased carbohydrate metabolism due to increased utilization for phosphorylation

Phosphorus occurs in the blood in the following forms

1	Inorganic phosphorus	2 5 mg /100 ml
2	Organic phosphorus	14-29 mg /100 ml
3	Phospholipids	8-18 mg /100 ml

Excretion :

Inorganic phosphorus is excreted in the urine and feces The source of urinary inorganic phosphorus is mainly that of plasma On a balanced diet, urine phosphate constitutes about 60 per cent of the total excretion The rest is excreted in the feces The "renal threshold for phosphate excretion is about 2 mg /100 ml of plasma The reabsorption of phosphorus is inhibited by the parathyroid hormone

Disease state :

1 In rickets, serum phosphate level is as low as 1-2 mg /100 ml

2 There is a temporary decrease in serum phosphate during absorption of carbohydrate and some fats

3 A lower concentration of organic phosphorus but a higher concentration of inorganic phosphorus in the serum has been estimated in diabetes mellitus

4 Phosphate retention causes the acidosis in severe renal disease The resultant is the increase in serum phosphorus level

5 Serum phosphorus levels are increased in hypoparathyroidism

6 Blood phosphorus levels are decreased in hyperparathyroidism and in celiac disease

7 In renal rickets there is low blood phosphorus level with an increased alkaline phosphatase activity

8 The deficiency of vitamin D is the cause of the low serum phosphorus and the defects in the calcification of bones

MAGNESIUM

Physiological functions :

1 70 per cent of the total magnesium content (21 g) of the body is combined with calcium and phosphorus in the complex salts of bone. The remainder is in the soft tissues and body fluids It is the principal cation of the soft tissue

2. Magnesium ions act as activators for many of the phosphate group transfer enzymes

3 It is found in certain enzymes, such as co-carboxylase

4 It functions as a cofactor for oxidative phosphorylation

Sources It is present in milk, eggs, cabbage, cauliflowers and fruits etc

Distribution .

Whole blood	2-4 mg./100 ml
C. S F	3 mg /100 ml.
Muscle	21 mg./100 gm

Daily requirement :

Infants	100-150 mg
Children	150-200 mg
Adults	200-300 mg

Blood magnesium :

The normal level of magnesium in blood is 1-3 mg /100 ml

Absorption .

1 A greater part (40 to 50 per cent) of the daily ingested magnesium (200 to 300 mg) is not absorbed

2. Very high intake of fat, phosphate, calcium and alkalis diminish its absorption

3 Parathyroid hormone increases its absorption

Excretion

Two-third of the total excreted magnesium is excreted in the feces and the remaining one-third into the urine

Disease state :

1 Magnesium deficiency causes depression, muscular weakness, and liability to convulsions The serum magnesium level is below 1 mg /100 ml

2 Its deficiency has also been observed in chronic alcoholics with the low serum magnesium and muscular weakness

3 In cases of kwashiorkor, the serum magnesium level is low causing weakness

4 Low values for serum magnesium have been reported in uremia, normal and abnormal pregnancy, rickets, growth hormone treatment, hyper-calcemia and the recovery phase of diabetic coma.

SODIUM

Physiological functions .

1 It is the major component of the cations of the extra-cellular fluid and exists in the body in association with the anions chloride, bicarbonate, phosphate and lactate

2 It is largely associated with chloride and bicarbonate in regulation of acid-base equilibrium

3 It maintains the osmotic pressure of the body fluid and thus protects the body against excessive fluid loss

4 Sodium ion plays an important role in the absorption of glucose and galactose as well as amino acids from the small intestine

5 It maintains the normal water balance and distribution

6 Sodium ion is involved in initiating and maintaining the heart beat

7 It maintains the normal neuromuscular function

8 It functions in the permeability of the cells

Sources : The *main source* of sodium is the sodium chloride used in cooking and seasoning

Rich sources : Bread, cheese, wheat germ, whole grains and oysters etc

Good sources : Carrots, cauliflowers, eggs, milk, nuts, spinach and turnips etc

Distribution : About one third of the total sodium content of the body is present in the inorganic portion of the skeleton Most of the sodium is found in the extracellular fluid

Plasma	330 mg /100 ml
Cells	85 mg /100 gm
Muscle	60-160 mg /100 gm
Nerve	312 mg /100 gm

Daily requirement :

For adults the daily requirement is 5 to 15 gms In temperate region, the sodium chloride intake is less but in tropical countries, the intake is more A person suffering from hypertension should not take more than 1 gm of sodium

Absorption : Normally, sodium is practically completely absorbed from the gastrointestinal tract. Less than 2 per cent of ingested sodium is eliminated in the feces In subjects with diarrhea, large amounts are lost in the feces

Blood sodium . The normal level of sodium is 310-340 mg /100 ml In man, erythrocytes contain little or no sodium Aldosterone increases plasma sodium level

Excretion : The daily losses of sodium are as follows

Urine	5- 35 mg
Stool	10-125 mg
Skin (not sweating)	25- 25 mg
TOTAL	40-185 mg

About 95 per cent of the sodium leaving the body is excreted in the urine since sodium is readily absorbed in the intestine Therefore, feces contain very little except in diarrhea There is the variable loss of sodium by way of the sweat Heavy exercise, environmental heat and high fever can cause excessive losses of sweat which leads to more sodium losses Loss of sodium by excessive sweating causes *heat cramps* with the intense and painful contractions of skeletal muscle of men working hard in hot humid climates

Disease state :

1 Adrenocortical steroids regulate the metabolism of sodium. In the insufficiency of adrenocortical steroids, the serum sodium level is decreased with an increase in sodium excretion

2. In chronic renal disease when acidosis exists, sodium depletion occurs due to poor tubular reabsorption of sodium as well as to the loss of sodium in the buffering of acids

3 In case, a person is not adapted to a high environmental temperature, large amount of sodium is lost in the sweat due to extreme sweating developing muscular cramps of the extremities and abdomen, headaches, nausea and diarrhea.

4 In byponatremia, the serum sodium level is below normal. This causes severe dehydration, reduced blood pressure, decreased blood volume and circulatory failure The following clinical conditions will develop :

(i) Prolonged vomiting and diarrhea resulting in excessive loss of digestive juices rich in sodium ion.

(ii) Chronic renal disease with acidosis due to poor reabsorption of sodium in the tubules

(iii) Adrenocortical deficiency leading to Addison's disease.

(iv) Loss of weight due to loss of water also

5 In hypernatremia, the serum sodium level is higher than normal. This occurs in (i) hyperactivity of adrenal cortex as in Cushing's syndrome (ii) Prolonged treatment of cortisone and ACTH as well as sex hormones The symptoms of hypernatremia are

(i) Increased retention of water in the body

(ii) Increase in blood volume

(iii) Increase in blood pressure

6 In certain stages of pregnancy, the steroid hormones cause the retention of sodium as well as water which results in gain in weight.

POTASSIUM

Physiological functions :

1 Potassium is largely present in the intracellular fluid and it is also present in small amounts in the extracellular fluid because it influences cardiac muscle activity

2. It plays an important role in the regulation of acid-base balance in the cell.

3 It maintains osmotic pressure

4 It functions in water retention

5 It is essential for protein biosynthesis by ribosomes

6 The glycolytic enzyme pyruvate kinase requires K^+ for maximal activity.

Sources : The high content of potassium is found in chicken, beef liver, bananas, the juices of oranges, pineapples, yams, winter squash and potatoes etc.

Distribution :

Plasma	20 mg./100 mL
Cells	440 mg./100 gm.
Muscles	250-400 mg./100 gm.
Nerves	530 mg./100 gm.

Daily requirement :

The normal intake of potassium in food is about 4 gms It is so widely distributed in food that its deficiency is rare except under pathological conditions

Blood potassium :

The normal level of potassium in serum is 14-20 mg /100 ml In man, erythrocytes contain large amounts of potassium. Therefore, care must be taken to avoid hemolysis for the determination of serum potassium The serum potassium decreases during increased carbohydrate utilization following administration of glucose or insulin Aldosterone decreases serum potassium level

Absorption :

Normally, potassium is practically completely absorbed from the gastrointestinal tract and less than 10 per cent of potassium is eliminated in the feces In subjects with diarrhea, large amounts of potassium are lost in the feces

Excretion :

Potassium is normally eliminated almost entirely in the urine and a small amount in the feces Aldosterone exerts an influence on potassium excretion In the presence of normal kidney function, potassium is very promptly and efficiently removed from the blood

Disease state :

1 Potassium not only filtered by the kidney but is also secreted by the renal tubules The excretion of potassium is greatly influenced by changes in acid base balance and also by the activity of the adrenal cortex The capacity of the kidney to excrete potassium is very great and therefore, hyperkalem a does not occur even after the ingestion of potassium if the kidney function is unimpaired Potassium should not be given intravenously unless circulatory collapse and dehydration are corrected

2 *Hyperkalemia* occurs in patients in the following conditions (i) Renal failure, (ii) Severe dehydration, (iii) Addison's disease due to decreased excretion of potassium by the kidney, (iv) Intravenous administration of excessive amounts of potassium salts, (v) Shock

The *symptoms* of hyperkalemia are cardiac and central nervous system depression The heart signs include bradycardia and low heart sounds followed by peripheral vascular collapse leading to cardiac arrest The other symptoms are mental confusion, numbness, weakness of respiratory muscles and flaccid paralysis of the extremities

The symtoms are corrected by administration of deoxycorticosterone which helps the excretion of potassium

3 Prolonged *hypokalemia* causes injury to myocardium and kidneys Potassium deficits occur in chronic wasting diseases with malnutrition, prolonged negative nitrogen balance, gastrointestinal losses and in metabolic alkalosis

The clinical conditions exhibiting hypokalemia are

(i) Prolonged diarrhea and vomiting with the loss of digestive juices

(ii) Intravenous administration of potassium-free fluid to replace digestive juices lost by prolonged vomiting

(iii) Overactivity of adrenal cortex (Cushing's syndrome) which causes increased excretion of potassium in urine

(iv) Prolonged use of diuretics

(v) Heart failure treatment with digitalis

(vi) Diabetic coma treatment with insulin

(vii) In familial periodic paralysis, a rare disease, potassium is withdrawn from extracellular fluid and retained in the cells.

The *symptoms* of hypokalemia are muscular weakness irritability, paralysis, tachycardia and dilatation of the heart with gallop rhythm and changes in the electrocardiogram (ECG)

CHLORINE

Physiological functions

1 As a component of sodium chloride, chloride ion is essential in acid base equilibrium

2. As chloride ion, it is also essential in water balance and osmotic pressure regulation

3 It is also important in the production of hydrochloric acid in the gastric juice

4 Chloride ion is important as an activator of amylase.

Sources It is mainly available as sodium chloride

Distribution

Plasma	365 mg./100 ml
Cells	190 mg./100 gm
C. S F	440 mg./100 ml.
Muscle	40 mg./100 gm.
Nerve	171 mg./100 gm

Daily requirement

The requirements of NaCl depend on the climate and occupation and on the salt content of the diet. Foods of animal origin contain more NaCl than those of vegetable origin. The daily requirement in tropical countries are given below

Adults	10-20 gms.
Children	5-10 gms
Women during pregnancy and lactation	10-15 gms.

Excessive consumption of NaCl causes edema in protein deficiency and increases blood pressure in hypertension patients

Blood Cl The normal level of Cl in serum is 96-105 m mol/liter In man, erythrocytes contain smaller amounts of Cl. The distribution of Cl^- between plasma and erythrocytes is related to that of HCO_3^- The Cl content of whole blood is relatively high in anemia and relatively low in polycythemia. The serum Cl may fall during active gastric secretion of HCl

Absorption Normally Cl is practically completely absorbed from the gastrointestinal tract

Excretion Cl is chiefly eliminated in the urine It is also excreted in the sweat. It is lost more during excessive sweating in hot climates under hard work. Its concentration in sweat is decreased by aldosterone

Disease state

1 Chloride deficit also occurs when losses of sodium are excessive in diarrhea, sweating and certain endocrine disturbances

2 There is a loss of chloride in the loss of gastric juice by vomiting or in pyloric or duodenal obstruction

3 Hypochloremic alkalosis may develop in Cushing's disease or after the administration of ACTH or cortisone

SULFUR

Physiological functions :

1 Sulfur is present primarily in the cell protein in the form of cysteine and methionine

2 The cysteine is important in protein structure and in enzymic activity

3 Methionine is the principal methyl group donor in the body The "activated" form of methionine, S adenosylmethionine, is the precursor in the synthesis of large number of methylated compounds which are involved in intermediary metabolism and detoxification mechanism

4 Sulfur is a constituent of coenzyme A and lipoic acid which are utilized for the synthesis of acetyl CoA and S acetyl lipoate respectively

5 Sulfur is a component of other organic compounds, such as heparin, glutathione, thiamine, biotin, ergothioneine, taurocholic acid, sulfocyanides, indoxyl sulfate, chondroitin sulfate, insulin, penicillin, anterior pituitary hormones and melanin

Sources :

Sulfur intake is mainly in the form of cystine and methionine present in proteins Other compounds present in the diet contribute small amounts of sulfur

Sulfur in blood :

The normal concentration of sulfur in the serum is as follows

Inorganic sulfur	0 5-1 1 mg /100 ml
Ethereal sulfate	0 1-1 0 mg /100 ml
Neutral sulfur	1 7-3 5 mg /100 ml

Absorption :

Inorganic sulfate is absorbed as such from the intestine into the portal circulation A small amount of sulfide may be formed in the bowel by the action of bacteria, but, if absorbed into the blood stream, this is rapidly oxidized to sulfate

Excretion :

Sulfur is excreted in the urine in three forms The total sulfate excretion may be diminished in the presence of renal functional impairment and is increased in conditions accompanied by excessive tissue protein breakdown, such as high fever and increased metabolism.

Disease state :

1 The serum sulfate concentration is increased in the presence of renal functional impairment, pyloric and intestinal obstruction and leukemia.

2 Marked sulfate retention in advanced glomerulonephritis cause the development of acidosis

3 An increase in the blood indican concentration (indoxyl potassium sulfate) may occur in uremia

TRACE ELEMENTS

Essential Trace Elemeots

IRON

The total iron content of the normal adult is about 4 to 5 gms Aboot 60 to 70 per cent of the total iron is present in hemoglobio , about 51 per cent is in storage as ferritio , 3 per cent as myoglobin and only about 0 1 per ceot is carried in the plasma in combination with the β globulin transport protein transferrin The hemoproteio and flavoprotein enzymes together make up less than 1 0 per cent of the total iroo Large amounts are present as bemosiderin

Physiological functions :

1 Iron fuoctioos mainly in the transport of oxygen to the tissues (hemoglobio)

2. It is also involved io the processes of cellular respiration

3 It is an essential componeots of hemoglobin, myoglobio, cytochromes and the respiratory enzyme systems (cytochrome oxidase, catalase and peroxidase)

4 The nooheme iron is completely protein-bound which exists in the form of storage aod traosport.

5 The nonheme iroo is also utilized in the structure of xanthioe dehydrogenase and succinate dehydrogenase aod also in the iron sulfur proteins of the respiratory chain

Sources :

Rich sources : Liver, heart, kidney, spleen

Good sources : Egg yolk, fish, nuts, figs, dates, beans, spinach, molasses, apples, bananas etc

Poor sources : Milk, wheat flour, polished rice and potatoes etc. Human milk contains 0 3 to 0 6 μg iron/ml

Daily requirement : About 10 per cent of the iogested iroo is only absorbed.

Infants	10-15 mg.
Children (1 to 3 years of age)	15 mg.
,, (4 to 10 years of age)	10 mg.
Older children and adults (Males)	
(11 to 18 years of age)	18 mg
(after 19 years of age)	10 mg
Females	
11-50 years of age and duriog pregnancy or lactatioo	18 mg
After 51 years of age	10 mg.

In adult women, the average loss of blood duriog a menstrual period which is a monthly loss of 16-32 mg of iron or an additional average loss of 0 5-1 0 mg. per day This amount is easily obtained from the diet

In excessive menstrual blood loss and in chronic iron-deficiency anemia, a supplemeot of 100 mg of iron per day is sufficient to respond. So during growth, preenancy and lactation, iron demand is more

In healthy adult male or in healthy women after menopause, the dietary requiremeot is negligible unless any deficiency or loss of iroo occurs

The iron deficiency occurs as a result of malabsorption from the gastrointestinal tract. A defect in hemoglobin synthesis in anemia is commonly found in copper deficiency

Iron in the blood : The normal concentration of iron in blood is 75-175 mcg /100 ml

Distribution :

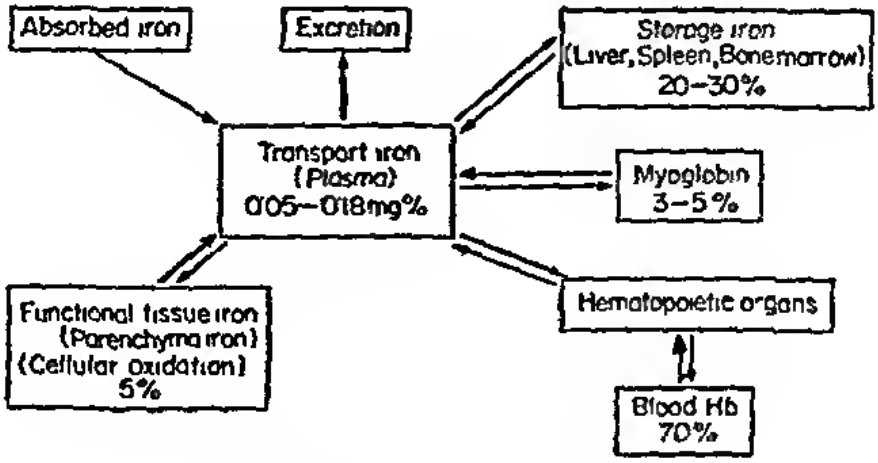


Fig 26.2 Approximate distribution of iron in the body

Absorption :

Under normal conditions, very little dietary iron is absorbed (less than 10 per cent), the amount excreted in the urine are minimal Infants and children absorb a higher percentage of iron from foods than adults Iron deficiency in infants is due to a dietary deficiency Iron-deficient children absorb twice as much as normal children

Most of the iron in food occurs in the ferric (Fe^{+++}) state either as $Fe(OH)_3$ or as ferric organic compounds These compounds are broken down into free ferric ions or loosely bound organic iron The gastric hydrochloric acid and the organic acids of the foods are both important for this purpose Reducing substances in foods, such as cysteine and ascorbic acid convert ferric ion into the ferrous (Fe^{++}) state In this form it is more soluble and therefore readily absorbed Iron absorption is enhanced by proteins of low molecular weight digestive products (peptides, amino acids) forming iron chelate Heme enters the mucosal cells without being released from the porphyrin ring In humans, dogs and rats, heme is broken down in the mucosa and iron appears in the plasma transferrin

Factors effecting iron absorption :

1 Absorption of iron occurs mainly in the stomach and the duodenum Impaired absorption takes place in patients who have total removal of stomach or a removal of the considerable amount of the intestine

2 A diet high in phosphate causes decreased absorption due to the formation of insoluble ferric phospbate ($FePO_4$) Very low phosphate favours increased absorption of iron

3 Phytic acid (present in cereals) and oxalates interfere absorption

4 Vitamin C increases absorption

5 Gastric acidity increases absorption by converting $Fe(OH)_3$ to Fe^{+++}, Achlorhydria and administration of alkali decrease absorption

6 Proteins of low molecular weight favour absorption

7 Copper deficiency also causes decrease in absorption

8 Alcohol ingestion favours iron absorption

Mechanism of absorption :

Previously, "Mucosal Block" theory was considered to be the controlling of iron absorption The iron-binding protein, *apoferritin*, in the mucosal cells, was the controlling factor Ferrous ion being oxidized to ferric ion combines with apoferritin to form iron containing protein.

Ferritin It was believed that the absorption depended on the formation of ferritin When apoferritin was saturated with iron, no further uptake of iron could take place

More recently, evidences show that ferritin is involved in the major regulation of iron absorption Iron taken into the mucosal cell is bound to specific carriers which regulate its passage across the cell to the blood. Intestinal ferritin, therefore, acts as a storage compound rather than the controlling of absorption

Transport in the plasma :

All the iron released from the mucosal cell enters the portal blood in the ferrous state In the plasma, ferrous is oxidized to ferric state by ceruloplasmin (a copper binding plasma protein) exerts a catalytic activity (serum ferroxidase) in plasma Human serum also contains a yellow cuproprotein (ferroxidase II) which catalyzes the oxidation of ferrous ions. Ferric ion is then incorporated into a specific iron binding protein, *transferrin* or *siderophilin*, which is a glycoprotein of molecular weight 76 000 containing 5 3 per cent carbohydrate. Transferrin can bind 2 atoms of ferric ions per molecule of protein to form a red ferric protein complex. Iron release from the mucosal cell is facilitated by a low degree of transferrin saturation by iron

Under normal circumstances, almost all of the iron bound to transferrin is taken up readily by bone-marrow Only the reticulocytes can utilize the ferric ion bound to transferrin, although reticulocytes and the mature erythrocytes can take up unbound ferric ion The iron with transferrin makes a complex which is not filtrable by the kidney The total iron binding capacity in both sexes is about 300-360 μg/dl

Losses of iron into the urine occur in proteinuria. In nephrosis, iron (1 5 mg./day) with protein may be excreted in the urine In hepatic disease, both the bound iron and the total iron binding capacity of the plasma is low

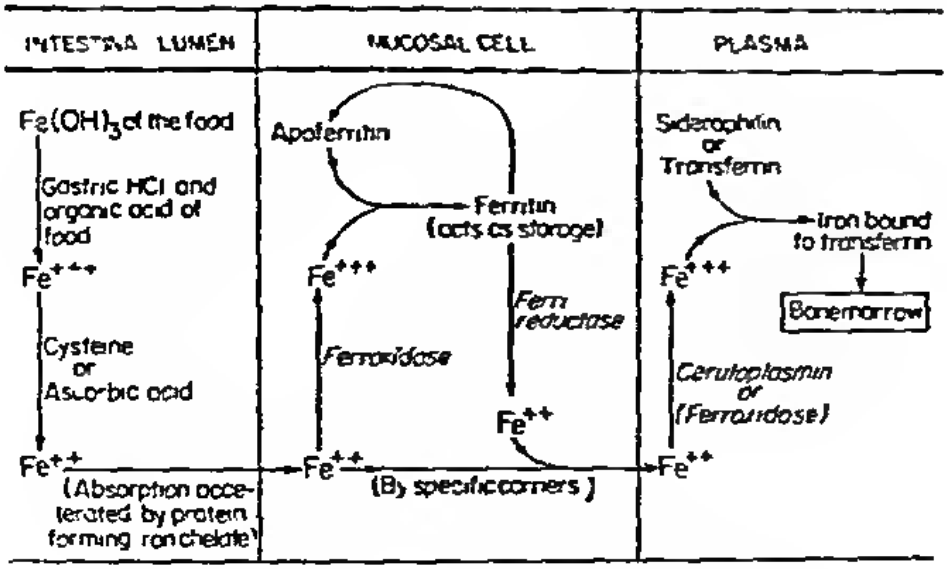


Fig 26.3 Iron absorption and transport mechanism

Excretion :

The body stores of iron are conserved very efficiently Only minute amounts being excreted in the urine, feces and sweats Relatively large amounts are lost in the menstrual flow The bulk of the iron of the feces is unabsorbed food iron In the tropics, iron loss is often much greater During pregnancy, iron is lost to the fetus Iron is also lost from the skin by means of sweat, hair loss and nail clippings

The daily excretion of iron is as follows

Adult male	0 5 1 5 mg in the urine
„ women during menstruation	double the above in the urine
Adult (Both Male & Female)	0 5-1 0 mg in the sweat
„ „ „ „	0 3 0 75 mg in the feces

Abnormal iron metabolism .

Ferritin and hemosiderin, the storage forms of iron, act as an internal iron reserve to protect against sudden losses of iron by bleeding *Ferritin* is present not only in the intestine but also in liver (about 700 mg), spleen and bonemarrow If more iron is administered parenterally exceeding the capacity of the body to store as ferritin, it accumulates in the liver as *hemosiderin,* a form of colloidal iron oxide in association with protein The iron content of hemosiderin is 35 per cent by weight

Iron metabolism is disturbed mainly by the following causes

1 Decreased formation of hemoglobin
2 Decrease in circulating hemoglobin
3 Abnormalities in the serum iron concentration
4 Abnormal deposition of iron-containing pigment in the tissues

The disorders of iron metabolism are · 1 Siderosis, 2 Nutritional siderosis, 3 Hemochromatosis

1 **Siderosis :** When excessive amounts of iron are released in or introduced into the body beyond the capacity for its utilization the excess is deposited in the various tissues, mainly in the liver This may occur due to repeated blood transfusion, excessive breakdown of erythrocytes in hemolytic types of anemia and inadequate synthesis of hemoglobin as in pernicious anemia

2 *Nutritional siderosis : This disorder is found among* Bantus in South Africa Bantus cook their food in large iron pots and consume iron rich food *The absorption of iron appears to be high leading to the development of* nutritional siderosis Livers of the Bantus contain large amounts of iron

3 **Hemochromatosis :** Hemochromatosis is a rare disease in which large amounts of iron are deposited in the tissues, especially the liver, pancreas, spleen and skin producing various disorders Accumulation of iron in the liver, pancreas and skin produces hepatic cirrhosis, bronze diabetes and bronze-state pigmentation respectively

Iron deficieney anemia :

Iron deficiency anemia is widely prevalent among children, adolescent girls and nursing mothers The hemoglobin content of the blood is 5 to 9 g /100 ml

Women of child bearing age The clinical symptoms are breathlessness on exertion, giddiness and pallor of the skin In severe cases, there may be edema of the ankles

Weaned infants and young children · The hemoglobin level is 5 to 9 g /100 ml blood The children are dull, and inactive and show pallor of the skin The appetite is poor and growth and development are retarded

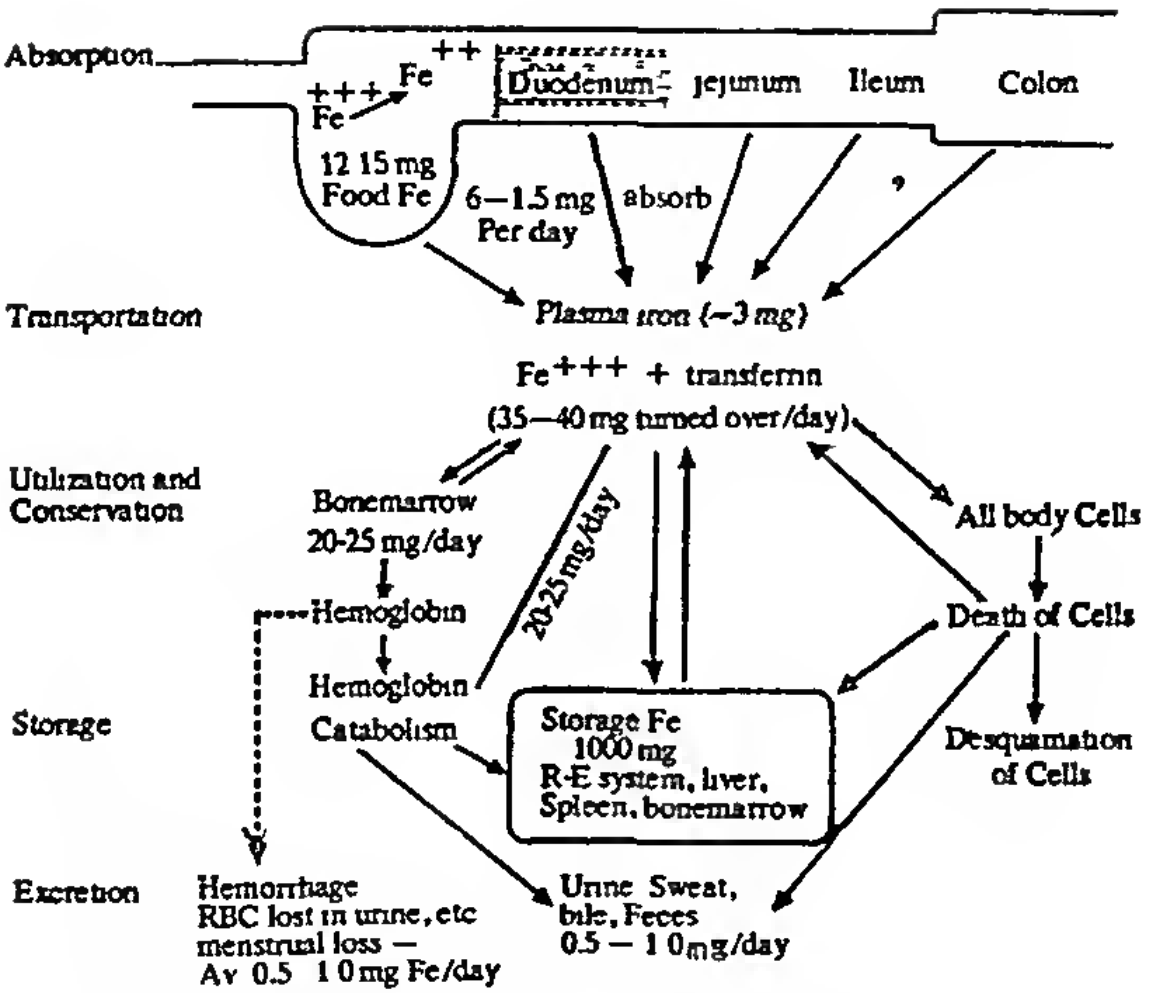


Fig 26 4 Schematic representation of iron metabolism in man
RBC = Red blood cells RE system = Reticuloendothelial system

Treatment Anemic women should take ferrous sulphate tablets For a child of below 12 months a mixture of ferrous ammonium citrate sweetened with glycerine and for children of 1 to 5 years ferrous ammonium citrate mixture should be given in curing

COPPER

Physiological functions .

1 It has important role in hemoglobin synthesis

2 It is required for melanin formation phospholipid synthesis, and collagen synthesis

3 It has role in bone formation and in maintenance in the integrity of myelin sheath

4 It is a constituent of several enzymes, such as tyrosinase, cytochrome oxidase, ascorbic acid oxidase uricase, ferroxidase I (ceruloplasmin) and ferroxidase II

5 It is a constituent of superoxide dismutase, amine oxidase and dopamine hydroxylase

6 Three copper containing proteins namely cerebrocuprein, erythrocuprein and hepatocuprein are present in brain, RBC and liver respectively

Sources :

Richest sources Liver, kidney, other meats, shellfish, nuts and dried legumes

Poor sources Milk and its products

Cow's milk	0 015 to 0 18 mg /L
Human milk	1 05 mg /L (at the beginning of lactation)
„ „	0 15 mg /L (at the end of lactation)

Distribution The adult human body contains 100-150 mg of copper

Muscles	64 mg of the total
Bones	23 mg „ „ „
Liver	18 mg „ „ „

The concentration of copper in the fetal liver is 5 10 times higher than that in liver of an adult

Daily requirements :

Adults	2 5 mg
Infants and children	0 05 mg /kg body weight

A nutritional deficiency of copper has never been demonstrated in man, although it has been suspected in case of nephrosis

Blood copper The normal concentration of copper in serum is 90 μg/100 ml Both the red blood cells and serum contain copper 80 per cent of the red blood cell copper is present as superoxide dismutase (erythrocuprein) The copper in plasma occurs in firmly bound and loosely bound forms The firmly bound copper consists of ceruloplasmin The loosely bound copper is known as 'direct reacting' copper and is loosely bound to serum albumin The plasma copper levels increase in pregnancy because of their estrogen content Oral contraceptives have a similar effect

Absorption Absorption of copper occurs in the human duodenum 30 per cent of the normal daily diet of copper is absorbed in the duodenum

Excretion : Only 10 to 60 μg of copper is excreted in normal urine in 2 hours. The daily biliary excretion of copper is 0.5 to 1.3 mg. and 0.1 to 0.3 mg is excreted across the intestinal mucosa into the bowel lumen.

Effects of copper deficiency :

1. Although iron absorption is not disturbed but the release of iron into the plasma is prevented due to the decreased synthesis of ceruloplasmin. As a result, hypoferremia occurs which leads to the depressed synthesis of heme developing anemia in severe deficiency of copper.

2. The experimental animals on a copper-deficient diet lose weight and die.

3 In copper-deficient lambs, low cytochrome oxidase activity results in neonatal ataxia.

4 Copper deficiency produces marked skeletal changes, osteoporosis and spontaneous fractures.

5 Elastin formation is impaired in the deficiency of copper. Because a copper containing enzyme plays an important role in the connective tissue metabolism, especially in the oxidation of lysine into aldehyde group which is necessary for cross-linkage of the polypeptide chains of elastin and collagen.

6 Copper deficiency results in myocardial fibrosis in cows. It is suggested that reduction in cytochrome oxidase activity may lead to cardiac hypertrophy.

Disorders of copper metabolism :

Wilson's Disease (*Hepato-lenticular degeneration*) :

Wilson's disease is a rare hereditary disorder of copper metabolism. The following disorders have been observed in this disease :

1 The absorption of copper from the intestine is very high (about 50 per cent) , whereas 2 to 5 per cent copper is absorbed in normal subjects.

2. Ceruloplasmin formation is very less. Hence, a greater part of serum copper remains loosely bound to serum protein—notably albumin and, therefore, copper can be transferred to the tissues, such as brain and liver or to the urine.

3 Excessive deposition of copper in the liver and the kidney causes hepatic cirrhosis and renal tubular damage respectively. The renal tubular damage results in the increased urinary excretion of amino acids, peptides and glucose.

Clinical symptoms :

1 Progressive hepatic cirrhosis of a coarse nodular type gradually leads to portal hypertension and finally to hepatic failure.

2. There is dysfunction of the lenticular region of the brain necrosis and sclerosis of the corpus structures cause basal ganglion syndromes in adolescence.

3 Defects in renal tubular reabsorption and glomerular filtration.

4 Copper deposition in Descemet's memorane of the eye causes a golden brown, yellow or green ring round the cornea. This lesion is called Kayser-Fleischer ring.

5 There is occasional pigmentation of the nails and skin.

Treatment : Improvement can be achieved by removing the excess of tissue copper by administering the copper chelating agent *penicillamine*. This brings about marked increase in the urinary secretion of the metal Increased values of serum ceruloplasmin occur in many acute and chronic infectious diseases, hepatic and biliary tract disease, leukemia and other forms of malignancy, iron deficiency anemia, hyperthyroidism, myocardial infarction, and certain neurological diseases.

Ceruloplasmin :

1 It is a copper binding plasma protein

2 Its molecular weight is about 151,000

3 It contains about 8 atoms of copper per mole

4 Normal plasma contains 30 mg of this protein per dl

5 It functions as a ferroxidase enzyme during iron transport

Superoxide dismutase .

1 It is an enzyme which catalytically scavenges the toxic free radical superoxide ion (O_2^-) formed during aerobic metabolism

2 Its molecular weight is about 32,000 and consists of two identical subunits

3 It contains one Cu^{++} and one Zn^{++} per subunit

4 Recent studies have shown that the copper proteins erythrocuprein, hepatocuprein and cerebrocuprein present in RBC, liver and brain respectively are identical with this enzyme

IODINE

Physiological functions

Iodine is required for the formation of thyroxine and triiodothyronine hormones of the thyroid gland These thyroid hormones are involved in cellular oxidation, growth, reproduction and the activity of the central and autonomic nervous systems Triiodothyronine is more active than thyroxine in many respects

Sources :

Rich sources are sea water, marine vegetation, sea foods, and vegetables as well as fruits grown on the seaboard Plants (and animal tissues) grown at high altitudes are *deficient* in iodine because of its low concentration in the water In such regions, iodide is commonly added to the drinking water or table salt in concentration of 1 5000-1 200 000

Daily requirements :

Adults	100-150 μg
In adolescence and in pregnancy	200 μg

Distribution :

The body normally contains about 10 to 20 mg of iodine 70 to 80 per cent of this is present in the thyroid gland Muscles contain large amount of iodine The concentration of iodine in the salivary glands, ovaries, pituitary gland, hair and bile is greater than that in muscle

All the iodine in saliva is inorganic, but most tissues contain less amount of iodine in the inorganic form and most of the iodine is present in the organic form

Blood iodine .

Practically all of the iodine in the blood is in the plasma The *normal concentration* of iodine in the plasma or serum is 4 to 10 μg/100 ml

0 08 to 0 60 μg/100 ml is in the inorganic form and 4 to 8 μg/100 ml is in the organic form The organic form is bound to protein and precipitated by protein precipitating agents 90 per cent of the organic form consists of thyroxine and the remainder is tri and diiodothyronine About 0 05 per cent of the thyroxine is in the free state Erythrocytes contain no organic iodine

Absorption :

Iodine and iodides are absorbed most readily from the small intestine Organic iodine compounds (diiodotyrosine and thyroxine) are partly absorbed as such and a part is broken down in the stomach and intestines with the formation of iodides Absorption also takes place from other mucous membranes and the skin

Storage :

90 per cent of the iodine of the thyroid gland is in organic combination and stored in the follicular colloid as "thyroglobulin", a glycoprotein of molecular weight 650,000 containing thyroxine, diiodotyrosine and smaller amounts of triiodothyronine

On demand, these substances are mobilized and thyroxine as well as triiodothyronine are passed into the systemic circulation They undergo metabolic degradation in the liver

Excretion :

1 Inorganic iodine is mostly excreted by the kidneys, liver, skin, lungs and intestine and in milk

2. About 10 per cent of circulating organic iodine is excreted in feces This is entirely unabsorbed food iodine

3 40 to 80 per cent is usually excreted in the urine , of which 20 to 70 μg. daily in adults and 20 to 35 μg. in children. The urinary elimination is largest when the intake is lowest

4 Urine iodine is increased by exercise and other metabolic factors

Iodine deficiency in human beings :

1 *In adults*, the thyroid gland is enlarged producing the disease *goitre* If treatment is started very early, the thyroid becomes normal If treatment is delayed, the enlargement of the gland persists

2 *In children*, severe iodine deficiency results in the extreme retardation of growth which is known as *cretinism*

Prevention of goitre . Goitre can be prevented by the regular use of iodide salt or iodide added to the drinking water in the concentration of 1 · 5000 to 1 200,000

Goitrogenic substances in foods :

Foods, such as cabbage, cauliflower and radish contain substances 1 to 5, vinyl 2 thio oxazolidone which react with the iodine present in the food and make it unavailable to the body These substances are known as ' Goitrogenic" substances

FLUORINE

Physiological functions :

1 Fluoride, in trace quantities, is essential for the development of teeth and bones

2 It is, in combination with vitamin D, required for the treatment of osteoporosis

3 Sodium fluoride is a powerful inhibitor of the glycolytic enzyme enolase.

4 Fluoroacetate acts as a powerful inhibitor of aconitase activity, responsible for the conversion of citrate to cis aconitate, of the citric acid cycle

5 Fluoride ions inhibit the metabolism of oral bacterial enzymes and diminish the local production of acids which are important in the production of dental caries

6 Fluorine forms a protective layer of acid resistant fluoroapatite with hydroxyapatite crystals of the enamel

Sources :

For humans, drinking water is the main source of fluoride

Daily requirement :

Fluoride is present in small amounts in normal bones and teeth Drinking water containing 1 to 2 ppm meet up the requirement of the body and prevent dental caries without producing any ill effect

Distribution :

It occurs in many tissues, notably the bones, teeth and kidneys The amounts of fluoride in the soft tissues are very low and do not increase with age It remains mostly in the extracellular water

***Absorption* :**

Soluble fluorides are rapidly absorbed from the small intestine

Excretion .

It is excreted in the urine, in the sweat, and by the intestinal mucosa Most of the fluoride that escapes retention by the bones and teeth is excreted rapidly into the urine

***Abnormalities* :**

1 Intake of excessive amounts of fluoride (3 to 5 parts per million) in childhood causes "*dental fluorosis*" (*mottled enamel*) The enamel of the teeth loses its lustre and becomes rough Chalky white patches with yellow or brown staining are found over the surface of the teeth The enamel becomes weak and in severe cases there occurs a profound loss of enamel with 'pitting' which gives the both surface a corroded appearance

2 Highly excessive intake of fluorine (over 10 parts per million) results in increased density and hypercalcification of the bone of spine, pelvis and limbs In addition, the ligaments of the spine become calcified and the collagen in the bone is also calcified Neurological disturbances are common Such individuals are crippled and cannot exhibit simple daily tasks, such as bending squatting etc , as the joints become stiff

3 Drinking water containing less than 0 5 ppm fluorine causes dental caries in children

Prevention of fluorosis :

Fluorosis can be prevented by removing fluorides from the water by treatment with activated carbon or by some other suitable absorbents

ZINC

Physiological functions :

1 Zinc is an essential constituent of many enzymes, such as carbonic anhydrase, alkaline phosphatase, pancreatic carboxy peptidases, and cytosolic superoxide dismutase

2 The retina contains a zinc metalloenzyme, *retinene reductase* which is required for the formation of retinene

3 It maintains normal concentration of vitamin A in plasma.

4 It is required for the mobilization of vitamin A from the liver

5 It is required for the preparation of insulin and increases the duration of insulin action when given by injection Zinc is used in the β-cells of the pancreas to store and release insulin as required.

6 It is concerned with the healing of wounds.

7 It is essential for the normal growth and reproduction of animals

Sources :

Rich sources	Oysters and herrings.
Good sources	Meat, eggs, liver, and milk
Fair sources	Cereals, pulses, nuts, oilseeds, vegetables and fruits

Distribution .

It is widely distributed in the tissues of the body The whole body (70 kg weight) contains 1 4 to 2 3 gms zinc. 20 per cent of the total is present in skin A certain amount is also present in the bones and teeth High concentration of zinc are present in spermatozoa, prostate and epididymis The highest concentration occurs in the choroid of the eye

Blood zinc :

1 Zinc is present in higher concentration in erythrocytes than in plasma

2 Normal plasma contains about 20 per cent of the zinc present in whole blood

3 The concentration of zinc of human blood, plasma and erythrocytes are 0 8 mg , 0 12 mg and 1 44 mg /100 ml respectively

4 About 3 per cent of zinc ion is contained in leukocytes In certain types of chronic leukemia, there is a marked fall in the zinc content of peripheral leukocytes

5 Most of zinc in erythrocyte is present in carbonic anhydrase

6 The plasma concentration of zinc of human falls to 10 per cent of the normal level during later part of pregnancy and among those taking oral contraceptives

Daily requirement :

Breast fed newborn Baby	0 7 5 mg.
Infants	3-5 mg
Children	10 mg
Adolescents	13 mg.
Adults	15 mg.
Pregnancy	30 mg
Lactation	25 mg

Absorption :

1 Zinc present in animal foods are well absorbed in the small intestine especially from the duodenum.

2 Zinc present in cereals, pulses, nuts and oilseeds are poorly absorbed due to the presence of phytic acid which interferes in its absorption

Excretion .

1 Zinc given orally or by injection is mostly excreted in the feces

2 Endogenous zinc is secreted into the small intestine in the pancreatic juice

3 90 per cent of zinc intake by healthy adult human is lost in the feces, about 5 per cent is excreted in the urine and 5 per cent retained in the body

4 Excessive sweating in the hot climate causes excessive loss of the metal

Deficiency of zinc :

1 Zinc deficiency in man results in dwarfism and hypogonadism (retarded genital development)

2 There is loss of taste acuity

3 There is also poor growth, loss of appetite and hypogeusia in young malnourished children with subnormal hair zinc levels

4 The deficiency of zinc causes hepatosplenomegaly, delayed closure of the epiphyses of the long bones and anemia

COBALT

Physiological functions :

1 Cobalt is an essential component of vitamin B_{12}, which is necessary for normal red blood cell formation

2 Certain enzymes, such as methylmalonyl-CoA mutase, methyltetrahydrofolate oxidoreductase, homocysteine methyltransferase, and ribonucleotide reductase require vitamin B_{12} for activity

Sources :

It is highly available in food

Distribution :

It is present in all tissues in small amounts The total body content of cobalt is about 1 1 mg The highest concentration occurs in liver, kidneys and bones Most of the cobalt is present in vitamin B_{12}

Daily requirement :

Its requirement for man is very less It is, if required, required as vitamin B_{12} As little as 1 to 2 μg of B_{12}, containing 0 045 to 0 09 μg cobalt, is sufficient to maintain normal bone marrow function in pernicious anemia

Absorption :

Cobalt is readily absorbed from the small intestine (70 to 80 per cent) Only minute amounts are present in the tissues, cobalt administered orally as a soluble salt is poorly absorbed and therefore largely eliminated in the feces

Excretion :

About 65 per cent of the amount ingested is excreted in the urine, the remainder in the feces Injected isotopic cobalt is eliminated rapidly and almost completely by the kidneys into the urine

Cobalt in ruminant nutrition :

Nutritional anemia in cattle and sheep living in cobalt poor soil areas can be treated successfully with cobalt Microorganisms in the rumens of these animals use cobalt to synthesize vitamin B_{12}

Cobalt toxicity :

Cobalt administered in large amounts to man or animals becomes toxic It develops a condition known as polycythemia (increased number of erythrocytes in blood)

MANGANESE

Physiological functions :

1 Manganese is essential for normal bone structure, reproduction, and the normal functioning of the central nervous system

2 Manganese ions activate glycosyltransferase which is concerned with the synthesis of the mucopolysaccharides of cartilage and also associated with the synthesis of glycoproteins (e g prothrombin)

3 Pyruvate carboxylase and superoxide dismutase contain tightly bound manganese

4 Arginase is activated by manganese ions

5 It activates isocitrate dehydrogenase and phosphotransferases

6 Manganese ions act as cofactor along with glucose-6-phosphate dehydrogenase

7 Manganese ions inhibit lipid peroxidation reactions

Sources

Rich sources Nuts and whole grains

Good sources Vegetables and fruits

Poor sources · Meats, poultry, seafoods, and fish

Distribution .

The body of a normal adult (70 kg weight) contains 12-20 mg manganese It is present in all tissues of the body The kidney and the liver are the main storage organs for manganese Mitochondria are the principal intracellular sites of manganese uptake

Daily requirement :

In humans, deficiency of manganese is not known The average dietary intake of 2 5-7 0 mg is quite sufficient

Manganese in blood :

Normal blood contains 4-20 μg /100 ml In human serum, manganese is found to a specific β globulin

Absorption :

Manganese is readily absorbed in the small intestine Only 3 to 4 per cent of manganese present in the diet is absorbed

Excretion :

95 to 96 per cent dietary manganese is excreted in the feces Only traces of manganese is excreted in the urine

Manganese deficiency in animals :

1 In manganese deficiency, the animals give birth to young ones which develop ataxia In more severe deficiency, sterility results In poultry, egg production and hatchability are decreased even in mild deficiency of the metal

2 The livers of manganese-deficient rats contain large amount of fat This fat accumulation is prevented by manganese or chlorine

3 Liver arginase activity and blood phosphatase activity are reduced in manganese deficiency

4 Bone deformities also occur in all animals in its deficiency

Manganese toxicity :

Mioers wbo inhale large amount of manganese suffer from chronic mangaoese toxicity There is the development of hepatolenticular degeneratioo resembliog Parkinson's disease

MOLYBDENUM

Physiological functions :

1 Molybdenum is an essential component of xanthine oxidase, aldebyde oxidase, and sulfite oxidase.

2 It is also present in nitrate reductase in plants, and nitrogenase, which functions in nitrogen fixation by microorganisms

3 Traces of molybdenum are required for tbe maintenance of normal levels of xaotbine oxidase in animal tissues

Sources :

Good sources Liver, kidney, puls, cereals, and some greeo leafy vegetables
Poor sources Other vegetables and fruits

Distribotion :

Small amounts of molybdenum are present in all tissues Liver and kidoey eontain larger amouots than other tissues

Daily requiremeots :

Adequate amounts of molybdenum are piesent in averoge diets Therefore, exact requirement is unknown

Absorption and excretion :

About 50 to 70 per cent of the intake is readily absorbed in the small intestine Half of the absorbed molybdenum is excreted in urine

Toxicity :

1 Molybdenum rich diet consumption causes severe diarrbea and ill health in cattle

2 Rats, on high molybdenum diet, lose body weigbt with marked anorexia

SELENIUM

Pbysiological functions :

1. Seleoium is essential for normal growth, fertility and for the prevention of a wide variety of diseases in animals, although not known as essential for humaos

2 Glutathione peroxidase, a selenoprotein, catalyzes the peroxidation of glutathione This enzyme is tbe protective agent against accumulatioo of H_2O_2 and organic peroxides within cells

3 It is involved io immune mechanisms, ubiquioone synthesis, and mitochondrial ATP biosynthesis

4 Selenoprotein nlso functions in tbe reductive deamination of glycine

Sources :

Selenium is largely available in different foodstuffs The variation depends on the differences in soil selenium content

Distribution :

It is widely distributed in the animal body and highest concentration is present in renal cortex, paocreas, pituitary, and liver

Daily requirement :

Since average diet contains adequate amounts of selenium the requirement of it is not known.

Selenium deficiency :

1 Selenium deficiency produces necrosis of the liver of rats

2. Calves and lambs suffer from muscular dystrophy in selenium deficiency

3 Chicks, on selenium-deficient diet, fail to grow and develop a diseased condition known as exudative diathesis

Relationship of selenium to vitamin E :

Both selenium and vitamin E are essential for curing certain diseases in experimental animals When animals are given adequate amounts of vitamin E, selenium deficiency causes the following signs and symptoms.

1 Retardation of growth and muscular wasting in *rats*

2. Retardation of growth and fertility in *chicks*

These symptoms may be cured by the administration of both selenium and vitamin E because of their close metabolic relationship

Toxicity :

1 Chronic selenium poisoning develops 'alkali disease" The symptoms of alkali disease are dullness, lack of vitality, roughness of coat, loss of hair from the body and tail, stiffness and lameness, cirrhosis of liver and anemia.

2. Acute selenium poisoning produces in animals salivation, grating of teeth, paralysis and blindness Death results due to respiratory failure.

CHROMIUM

Physiological functions :

1 Chromium potentiates the action of insulin in accelerating utilization of glucose in animal and humans.

2. It is effective in improving glucose tolerance in some patients suffering from diabetes mellitus

3 It maintains the normal cholesterol level in blood of rats

4 It regulates the incorporation of certain amino acids in heart muscle in rats.

Sources :

It is highly available in dietary foods

Distribution :

The chromium content of adult human body is estimated to be 6 mg. It is widely distributed in tissues.

Chromium in blood

Normal blood contains about 0 009 to 0 055 parts per million

Requirements

Since average diet meets up the requirement the exact necessity is unknown

Absorption and excretion :

It is readily absorbed in the small intestine It is mobilized from the tissues in response to glucose administration.

Chromium is mainly excreted in urine, a small amount is lost in bile and feces.

Deficiency :

Its deficiency is characterized by impaired growth, disturbances in glucose, lipid and protein metabolism.

Toxicity :

Excessive amounts of chromium produces growth depression, liver and kidney damage in some experimental animals

Exercise

1. Give an account of the distribution of fluids in the human body. Describe how water metabolism is regulated (R. U. 70A ; Lucl . 69S).
2. State the distribution of the water in the body. Mention how extracellular fluid differs from intracellular fluid in composition Describe the mechanism of exchange of fluids in the body. (P. U. 75S)
3. Describe the electrolyte composition of intracellular and extracellular compartments of body Describe the importance of potassium ions and give the mechanism of its regulation (P. U 76A)
4. Describe the metabolism of calcium in the body (P. U. 68S, 71A)
5. Describe the sources, requirements and physiological functions of calcium (Mith. 71A)
6. What part does calcium play in bodily functions ? How is the level of calcium in the blood regulated ? (R. U. 71S)
7. Mention the sources of iron in the diet. Describe the mechanism of iron absorption from G I T Mention how iron is transported and stored in our body. (M. U 75A)
8. State briefly the sources of iron in our diet Mention the functions of iron in the body Discuss the metabolism of iron (R. U. 71S)
9. Write the metabolism of iron in the body. Describe the physiological functions of iron. Give the sources and daily requirements of iron (M. U 72A)
10. Discuss how iron is absorbed from the intestine and is utilized in the body. (P. U. 72A)
11. Discuss the metabolism of sodium in the body. (R. U. 72S)
12. State how copper is metabolized in the body. (Mith. 76A)
13. Discuss the metabolism of sulphur in the body. (R. U. 74S)
14. Write notes on :
    (a) Composition of extracellular fluid (R. U. 66A)
    (b) Functions of fluorine in the body. (Mith. 72S)
    (c) Water intoxication (Bh. U. 75A)
    (d) Functions of Manganese in the body. (Mith. 77S)

CHAPTER 27

INBORN ERRORS OF METABOLISM

Inhorn errors of metabolism nre a group of metaholic 'diseases' which can be formed as n result of the lack of a single enzyme in n single metaholic pathway. These disorders are hereditary nnd tend to occur only in closely-related family groups.

Sir Archihald Garrod (1902) first identified only n small number of such metaholic disorders. A large numher of such conditions which can be termed inhorn errors have heen recognized with the increase in knowledge of metaholic pathwnys and the improvement of more sensitive nnd senrching methods of hiochemical investigation.

HEREDITARY ANEMIAS

Sickle cell anemia and thalassemia. See page 117 (in hemoglohin chapter).
Wilson's disease. See page 448.

CARBOHYDRATE METABOLISM

Galactosemia. See page 286.

Glycogen storage diseases (Glycogenoses). See page 287.

Pentosuria. See page 288.

Fructosuria. See page 288.

Essential pentosuria. See page 285.

Hereditary fructose intolerance. See page 285.

Hurler's syndrome (Mucopolysaccharidosis I).

This is a hereditary disorder in which acid mucopolysaccharides are excessively deposited in tissues. This disorder is due to a defect in catabolism rather than to excessive synthesis. The most important symptoms of this disorder are the followings :

1. Skeletnl deformities and severe mental retardntion.
2. Deafness of n conductive type and corneal opacity.
3. The voice is harsh and behnviour is ape-like.
4. Dermatan sulfnte and heparan sulfnte are present in urine and tissues.
5. Dilatation of the venticles.
6. Cardiovascular findings are prominent.

Hunter's syndrome (Mucopolysaccharidosis II).

This is also n hereditary disorder in which ncid mucopolysaccharides are excessively deposited in tissues. This disorder is also due to n defect in catabolism rather than to excessive synthesis. The most important symptoms nre the followings :

1. Severe skeletal deformities nnd moderate mental deterioration.
2. Deafness occurs early hut there is no corneal opacity.

3 Dermatan sulfate and heparin sulfate are present in urine and tissues
4 Dwarfing and abnormal facies are present
5 Cardiovascular changes, obstruction and restriction of chest movement
6 Death frequently occurs from cardiac troubles at an early age

LIPID METABOLISM

Gaucher's disease :

In this disease, the cerebroside content of the reticuloendothelial cells (e g spleen) is very high In the cerebroside molecule, the kerasin is characterized by glucose replacing galactose The concentrations of cerebrosides are much higher in medullated than in nonmedullated nerve fibers The symptoms of this disease are mentioned below The disease is caused by the deficiency of glucosyl seremide hydrolase or glucocerebrosidase

1 The spleen is significantly increased and there are signs of leukopenia and thrombocytopenia
2 The liver is enlarged and the marrow cavity is widened
3 Eyes show a yellow brown wedge shaped elevation.
4 In early infantile form, the disease starts early and death occurs before attaining the age of 2 years Neurological symptoms are usually present

Niemann Pick disease .

In this disease excessive amounts of sphingomyelin are deposited in spleen, brain and liver This results in the deficiency of *sphingomyelinase* The hereditary disease is found in infancy and death occurs within the first two years of life The clinical findings are

1 Enlarged liver and spleen
2 Mental retardation and fatal in early life
3 Anemia and leukocytosis
4 The nervous system is affected
5 A cherry red spot may he seen over the retina
6 Cholesterol deposits in the tissues are increased

Tay-Sach's disease :

This disease is characterized hy the increased accumulation of Gangliosides (GM_2) in hrain and spleen The defect is caused by the deficiency of the enzyme *Hexosaminidase A* in tissues The characteristic clinical symptoms are

1 Mental retardation, blindness and muscular weakness
2 A cherry red spot nppears in the muscular region of the eye within the first years of life
3 At ahout two years of age, the circumference of the head hecomes 50 per cent greater than normal
4 There is repeated respiratory tract infections of the patient and the patient expires at the third and fourth years

Fabry's disease :

In this disease large amounts of ceramide trihexoside are accumulated in the kidney The deficiency of the enzyme ceramide trihexosidase causes this disease The clinical findings nre

1 Skin rash and kidney failure.

2. Male patients generally die due to progressive renal failure in the fourth or fifth decade of life

3 Cardiac enlargement and edema of the extremities

4 Some patients suffer from excessive pain in the joints.

5 Corneal opacities and vascular dilatation are frequent.

Refsum's disease

This disease occurs due to the accumulation of large amounts of phytanic acid (3, 7, 11, 15-tetramethyl hexadecanoic acid) The deficiency of the enzyme phytanic acid oxidase causes the disease The clinical symptoms are the following

1 The early symptoms are signs of chronic polyneuropathy with distal muscular atrophy

2. Severe pain in the knees.

3 The deep tendon reflexes are weak or absent.

4 Night blindness and narrowing of the visual fields

5 Deafness and anosmia.

6 Cardiac involvement may lead to tachycardia.

7 The cerebrospinal fluid protein is always increased while the cell count is normal

Krabbe's disease *

This disease results in the deficiency of *galactocerebrosidase* which catalyzes the hydrolysis of galactocerebroside to form ceramide and galactose Galactocerebroside is the important component of myelin

The clinical manifestation of this disorder is severe mental retardation in infants There is nearly total absence of myelin in the central nervous system which is replaced by gliosis and 'globoid bodies appear in the white matter Diagnosis of the patients depends on the determination of the galactocerebrosidase activity in leukocytes.

PROTEIN METABOLISM

Albinism

1 This condition appears in the total absence of tyrosinase inside the melanocytes in the skin

2. The black pigment melanin is not formed in the skin, eyes and hair

3 This inherited condition occurs to a greater or less extent in all types of organism.

4 The diagnostic advice is for the prevention of exposure to sunlight and protection of the eyes by wearing dark glasses.

Tyrosinosis .

1 This syndrome is due to the absence either of hepatic *P hydroxyphenyl-pyruvate hydroxylase* or of *tyrosine transaminase* activities.

2. The patient excretes large quantities of tyrosine in the urine

3 Diet rich in tyrosine causes the excretion of other P hydroxyphenyl acids including 3, 4-dihydroxyphenylalanine (dopa) and P hydroxyphenyl lactic acid.

Tyrosinemia

A *Neonatal tyrosinemia*

1 Neonatal tyrosinemia occurs in the new born

2 Tyrosine, P-hydroxyphenylpyruvic acid P hydroxyphenyl lactic acid and P hydroxyphenyl acetic acid appear in the urine

3 A transient deficiency of P hydroxyphenylpyruvic acid oxidase causes this condition and the condition lasts for a few weeks

4 Administration of ascorbic acid and reduction of protein intake brings about the normal condition

B *Hereditary tyrosinemia*

1 This disorder is similar to neonatal tyrosinemia but the amount of P hydroxyphenyl lactic acid in the urine is greater

2 This disorder is not controlled by the administration of ascorbic acid

3 It is due to the inherited deficiency of *P hydroxyphenyl pyruvic acid oxidase*

4 Liver failure and death can occur six months after birth and the infant has a characteristic odour

5 Other patients develop the clinical findings including hepatosplenomegaly, a nodular cirrhosis of the liver, abnormalities of tyrosine and methionine metabolism, multiple defects in renal tubular reabsorption, rickets, hyperphosphaturia, proteinuria and amino aciduria

6 Diet containing low in tyrosine and phenylalanine improves renal function and also retards degenerative liver changes

Phenylketonuria

1 This inherited disorder appears in the absence of phenylalanine hydroxylase which is responsible for the conversion of phenylalanine to tyrosine As a result, alternative catabolites of phenylalanine are produced, these include phenylpyruvic acid deamination product of phenylalanine, phenyl lactic acid, the reduction product of phenylpyruvic acid, and phenyl acetic acid, the decarboxylation and oxidation product of phenylpyruvic acid Much of the phenylacetylglutamine which is excreted in the urine

2 Mental retardation develops among infants and children

3 Patients with phenylketonuria tend to have a deficiency of serotonin This may be connected with the defect of myelin synthesis

4 The accumulation of phenylalanine also impairs melanin synthesis and children with this defect tend to have fair skin and fair hair

5 Excess of phenylalanine in the blood leads to excretion of the amino acid into the intestine Here it competes with the tryptophan for absorption and tryptophan is subjected to the action of intestinal bacteria

6 Early diagnosis (shortly after birth) and extreme restriction of phenylalanine intake is effective in preventing this disorder

Alkaptonuria

1 This condition is characterized by the excretion of homogentisic acid (dihydroxyphenyl acetic acid) in the urine owing to the lack of homogentisic acid oxidase

2 This abnormal condition is often found in infancy Over 600 cases have been reported The incidence of alkaptonuria is 2-5 per million live births

3 The most clinical manifestation is the dark urine due to the oxidation of homogentisic acid in air

4 In this disorder, several grams of homogentisic acid are excreted daily

5 This condition is present at birth and persists throughout life

6 In later life, accumulation of dark pigment in cartilages and tendons gives rise to the condition known as ochronosis, which is accompanied by arthritic changes

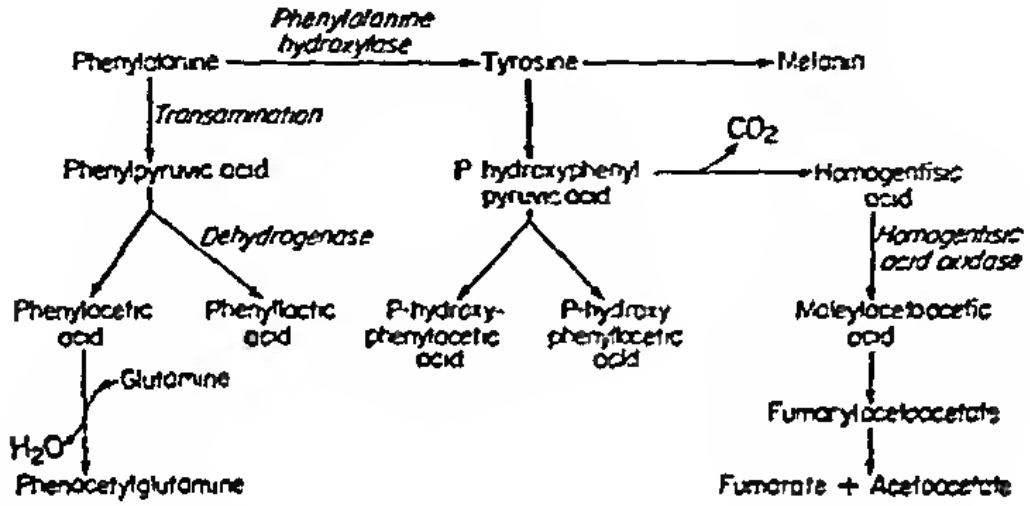


Fig 27 1. Overall representation of tyrosinosis albinism, tyrosinemia, phenyl ketonuria and alkaptonuria

Maple syrup urine disease :

1 This syndrome is characterized by the absence of the enzymes required for the oxidative decarboxylation of the keto acids derived from the branched chain amino acids—valine, leucine and isoleucine As a result, these keto acids are accumulated in the blood and excreted in urine The urinary excretion of these keto acids produce an odour like that of maple syrup or of burnt sugar

2 This familial disorder is recognized by central nervous system manifestations of convulsions and attacks of flaccidity and apnea.

3 The patients may be well treated with amino acid mixtures low in the above three amino acids

4 The infant is difficult to feed and may vomit. The patient suffers from a significant degree of lethargy

5 Death may occur by the end of the first year of life without treatment.

Hartnup's disease :

1 It is a hereditary disease characterized by a pellagra like skin rash and mental deterioration in the abnormal metabolism of tryptophan

2 The urine of the patients contain significantly increased amounts of indole acetic acid as well as tryptophan

3 The urinary excretion comes to normal after administration of broad-spectrum antibiotics.

Glycinuria

1 This condition is associated with the excess urinary excretion of glycine with a tendency to form oxalate renal stones in spite of the normal amount of excretion of oxalate in the urine

2 The plasma content of glycine is normal in glycinuric patients while the urinary excretion of glycine ranges from 600-1000 mg /d

3 It is supposed that glycinuria is due to a defect in renal tubular transport of glycine and the decreased reabsorption of glycine by the renal tubule permits the amino acid to pass into the urine in high concentration

Cystinuria :

1 It is an inherited metabolic disease in which lysine, arginine, ornithine and cystine are excreted in the urine in large amounts

2 Cystinuria is due to renal transport defect

3 Cystinuria is a misnomer, so that cystine-lysinuria may be preferred as the descriptive term for this disease

4 Cystine is an insoluble amino acid which may precipitate in the kidney tubules to form cystine calculi in cystinuric patients This is a major complication of this disease

Homocystinuria

1 In this abnormal condition homocystine (300 mg /d) together with S adenosylmethionine in some cases is excreted in the urine and plasma methionine levels are elevated

2 The clinical findings of this disease are the occurrence of thrombosis, osteoporosis, dislocated lenses in the eyes and frequently mental retardation

3 This condition appears due to the lack of *cystathionine synthetase* in the liver due to which both homocystine and methionine are accumulated in blood and urine

4 A low methionine and a high cystine diet effectively prevent this condition if treated earlier

Histidinemia

1 It is an inherited disorder of histidine metabolism in which the amounts of histidine in the blood and urine are increased There is also increased excretion of imidazole pyruvic acid

2 The metabolic block of histidine is due to the insufficient activity of liver *histidase* which impairs the conversion of histidine to urocanic acid

3 Development of speech in this condition is retarded Mental development is also retarded

4 Histidine excretion is also increased during normal pregnancy but not in the toxaemia of pregnancy This increase is not due to metabolic defect

5 These histidinemic patients are treated well with a diet containing protein hydrolysate free from histidine instead of intact protein

Hypervalinemia

1 In this abnormal condition, the infants suffer from stunted growth, muscle wasting and vomiting

2 The valine content of blood and urine is very high

3 A diet containing protein hydrolysate low in valine prevents this condition effectively

NUCLEIC ACID METABOLISM

Lesch-Nyhan syndrome

1 This condition is characterized by the complete deficiency of *phosphoribosyl transferase*, which causes hypoxanthine or guanine to form a nucleotide with PRPP (5 phosphoribosyl 1 pyrophosphate) These purines are thus available for the formation of uric acid

2 This disorder is X linked in its inheritance.

3 This appears in childhood as a severe neurological syndrome, which is sometimes accompanied by gout The urinary uric acid amount is five to six times the normal

4 Hypothyroidism, hypo- and hyperparathyroidism are accompanied by hyperuricemia

5 Hypertension is accompanied by increased plasma uric acid Such patients show an increased tendency to myocardial infarction, which is also a cause of hyperuricemia.

6 It can be prevented or diminished by the administration of *allopurinol*, an analogue of hypoxanthine Allopurinol inhibits xanthine oxidase due to which uric acid cannot be formed

Hereditary xanthinuria

1 In this rare genetic disorder, there is the deficiency of xanthine oxidase which leads to the diminished level of blood uric acid (1 mg.100 ml or less)

2. The urinary excretion contains large amounts of xanthine with lesser amounts of hypoxanthine

3 Urinary calculi composed of xanthine may be produced

Orotic aciduria

1 This is an inherited disorder which causes the excessive production of orotic acid This occurs by the deficiency of orotate phosphoribosyl transferase.

2. The urinary excretion consists of large amounts of pyrimidine nucleotide precursor

3 The urine becomes cloudy on cooling with the deposition of needle-shaped crystals of orotic acid

4 Children affected by this condition develop a severe megaloblastic anemia with physical and mental retardation

5 Administration of uridine improves this condition significantly

Exercise

Brief reason

1	Alkaptonuria	(R U 72S, P U 71S, Mith. 62S)
2.	Phenylketonuria.	(Maz. 75A Mith 61A, R U 70A)
3	Niemann-Pick desease.	(R U 70A, Mith. 75S)
4	Hunter s syndrome	(M U 76S)
5	Glycogen storage disease	(P U 72A, R U 65S)
6	Fructosuria.	(M U 74S, Mith. 71A)
7	Galactosemia.	(R U 64A)
8	Gaucher's disease.	(P U 68A, R U 6-S)
9	Refsum s disease	(Mith. 72S)
10	Tyrosinosis	(R U 72A, Bb U 74S)
11	Maple syrup urine disease	(P U 69S, R U 73S)
12.	Homocystinuria.	(Bb U 73S)
13	Lesch-Nyhan syndrome	(P U 74S).
14	Glycinuria.	(M U 76A)

CHAPTER 28

DETOXIFICATION

'Detoxification' occurring in the body converts toxic substances introduced into or formed in the body into less toxic or non toxic substances which are easily excreted out by the excretory routes

The substances introduced into the body are the drugs used for treatment of diseases and the chemicals used for diagnostic purposes The toxic substances are formed in the body by the bacterial action in the large intestine e g indole, skatole, phenol, histamine etc The other toxic substance bilirubin is formed in the body by the breakdown of hemoglobin

Liver is the principal organ for the detoxification processes to take place The detoxification processes can be grouped as follows

1 Oxidation, 2 Reduction, 3 Hydrolysis, 4 Conjugation

1 Oxidation :

Alcohols, aldehydes, amines and amine derivatives, hydrocarbons and sulphur compounds are detoxified by the process of oxidation

Alcohols : Alcohols are oxidized to the corresponding acids via aldehydes

Methyl alcohol ——→ Formic acid

Ethyl alcohol ——→ Acetic acid

Benzyl alcohol ——→ Benzoic acid

Aldehydes : Aldehydes are oxidized to the corresponding acids

Benzaldehyde ——→ Benzoic acid

Chloral ——→ Trichloracetic acid

Amines and amine derivatives Aliphatic amines are oxidized to the corresponding acids

Aliphatic amine ——→ Aliphatic acid + Urea

Aromatic amines are oxidized to P-phenols

Aniline ——→ P-amino phenol

Acetanilide ——→ P acetylaminophenol

Hydrocarbons : Aromatic hydrocarbons are oxidized to phenols

Benzene ——→ Phenols

Sulphur compounds : Sulphur compounds are oxidized to sulphuric acid

Organic sulphur ——→ Sulphuric acid

2 Reduction :

Less commonly reduction takes place in man Nitro or aldehyde groups are reduced

Chloral ——→ Trichlorethylalcohol

P nitro benzaldehyde ——→ P amino benzaldehyde

Picric acid ——→ Picramic acid

3 Hydrolysis :

The therapeutic activity of some therapeutic agents becomes less in the body due to their hydrolysis Some examples are mentioned below

Atropine ——→ Tropic acid + Tropine

Digitalis ——→ Aglycones + Sugars

Aspirin ——→ Salicylic acid + Acetic acid

Procaine ——→ P-aminobenzoic acid + Diethylaminoethanol

4 Conjugation :

It is the process in which the chemical to be detoxified combines with another chemical formed or supplied by the body Eight types of chemical substances are used by the body for conjugation reactions These are glucuronic acid, sulphuric acid, glycine, glutamine, cysteine, acetic acid, active methyl groups and thiosulphate

Glucuronic acid . This important conjugating agent is formed in the body from glucose It produces the following products by conjugation

Phenol + Glucuronic acid ——→ Phenyl glucuronide

Bilirubin + Glucuronic acid ——→ Bilirubin diglucuronide

Benzoic acid + Glucuronic acid ——→ Benzoyl glucuronide

Sulphuric acid : It detoxifies phenol, indoxyl etc.

Phenol + Sulphuric acid ——→ Phenol sulphate

Indoxyl + Sulphuric acid ——→ Indoxyl sulphate

Glycine . The amino group of glycine reacts with the carboxyl group of the compound to be detoxified

Benzoic acid + Glycine ——→ Hippuric acid

Nicotinic acid + Glycine ——→ Nicotinuric acid

Cholic acid + Glycine ——→ Glycocholic acid

Glutamine .

Phenylacetic acid + Glutamine ——→ Phenylacetyl glutamic acid

Dogs use glycine for detoxifying phenylacetic acid

Cysteine .

Bromobenzene + Cysteine ——→ P Bromophenyl mercapturic acid

Acetic acid .

Sulphanilamide + Acetic acid ——→ Acetyl sulphanilamide

Active methyl groups :

Pyridine + Active methionine ——→ N methyl pyridine

Nicotinic acid + Active methionine ——→ N methyl nicotinic acid

Thiosulphate . Inorganic cyanides are detoxified by thiosulphates converting the highly toxic cyanides into less toxic thiocyanate

Cyanide + Sodium thiosulphate ——→ Thiocyanate + Sodium sulphate

CHAPTER 29

IMMUNOCHEMISTRY

The lymphocyte is the primary cell for immune system The *T lymphocytes* T cells) are thymus derived. These are responsible for cellular immunity (i e delayed skin reactivity, antitumor immunity, cellular defense against fungi) The *B lymphocytes* (B cells) are derived from bone marrow in mammals These are responsible for humoral immunity which is expressed by the production of specific circulating plasma proteins termed antibodies or immunoglobulins B lymphocytes are mainly discussed in this chapter

STRUCTURE OF IMMUNOGLOBULINS

1. The basic unit of all immunoglobulin molecules consists of 4 polypeptide chains linked by disulfide bonds shown in the figure below

2 There are two identical heavy (H) chains (MW 53,000-75,000) and two identical light (L) chains (MW 23 000)

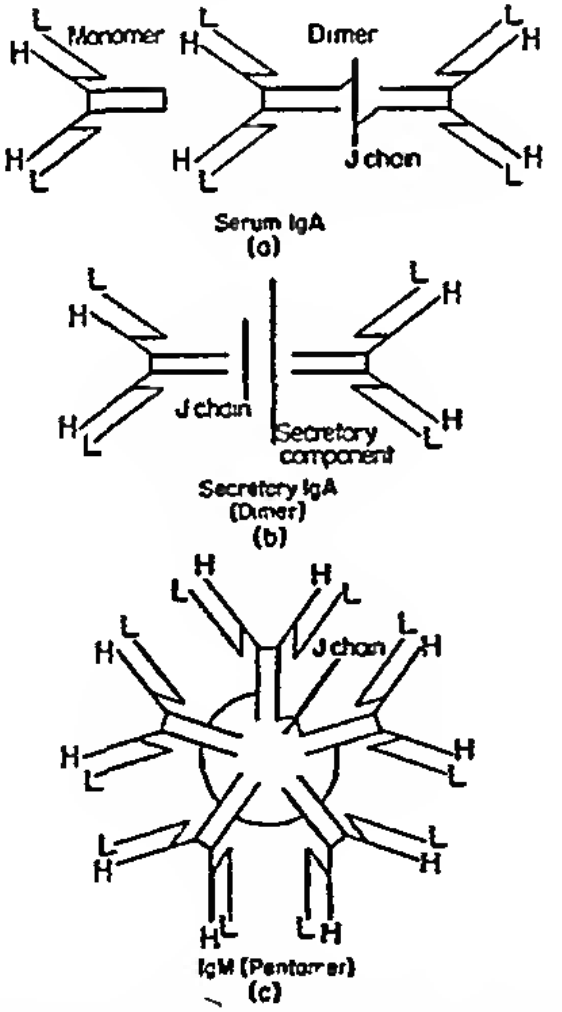


Fig 29 1. Polypeptide chains are represented by thick lines, disulfide bonds linkings different polypeptide chains are represented by thin lines

3 Immunoglobulins composed of more than one basic monomeric unit are termed polymers

Important examples are IgA dimers (2 units), IgA trimers (3 units) and IgM pentamers (5 units)

4 The polypeptide chains are not straight sequences of amino acids but are folded 3-dimensionally with disulphide bonds to form areas called domains

5 The part of the antibody molecule which combines with antigens is formed by a few amino acids in the F region of H and L chains

6 Papain cleaves IgG in the presence of cysteine into 3 fragments

2 Fab fragments (*MW 52,000*) and an *FC* fragment (*MW 48,000*) The Fab fragments bear the entire antibody combining site for the antigen

7 There are two major types of L chains in man, the Kappa (κ) and lambda (λ) chains About 70 per cent of the human immunoglobulin molecules carry κ light chains and 30 per cent carry λ light chains In IgG, the H chain is termed a gamma (γ) chain , in IgA, an alpha (α) chain , in IgM, a mu (μ) chain , in IgD, a delta (δ) chain , and in IgE, an epsilon (ϵ) chain

8 Carbohydrate residues are attached to the polypeptide chains 2 carbo hydrate units per molecule of IgG and 3 per molecule of IgM The carbohydrate residues include D mannose, D galactose L fructose D acetylneuraminic acid and glucosamine

9 Each chain is divided into specific domains or regions that have structural and functional significance The half of the *light chain* (L) toward the carboxy terminus is termed as the *constant region* (CL), while the amino terminal half is the *variable region* (VL) of the light chain About one quarter of the *heavy* (*H*) *chain* at the amino terminus is termed as variable region (VH), and the other three quarters of the heavy chain are referred to as the constant regions (CH^1, CH^2, CH^3) of that H chain

10 The portion of the *immunoglobulin molecule which binds the specific antigen* is formed by the aminoterminal portions (variable regions) of both the H and L chains, i e the *VH and VL domains* The domains of the Protein chains do not simply exist as linear sequences of amino acids but form globular regions with secondary and tertiary structure

Classification of immunoglobulins

On the basis of electrophoretic, immunologic and ultracentrifugal studies, the immunoglobulins have been divided into 5 groups

1 IgG :

(a) This is the major antibody-containing fraction which comprises 80 per cent of the gamma globulins

(b) It is a single basic immunoglobulin unit with γ heavy chains

(c) Its molecular weight is 150,000-160 000 and it contains 2-4 per cent carbohydrates

(d) It has the slowest electrophoretic mobility and is distributed in the extracellular fluid and is capable of crossing the placenta

2 IgA :

(a) It has a molecular weight of about 180,000-400,000 and its S rate is 6 6-13

(b) It has a higher content of carbohydrate (5 10 per cent)

(c) It is present in high concentrations in the blood, in saliva and tears and in the secretions of gastrointestinal tract

(d) It is a single basic immunoglobulin unit with heavy α chains Secretory IgA is made up of 2 basic units connected by a J chain

(e) A 60,000 MW molecule called *transport piece* (*t piece*) is attached to the Fc portion. This is necessary for the transport of IgA molecules into the lumens of exocrine glands Secretory IgA plays an important role in host defense mechanisms against viral and bacterial infectons IgA does not cross the placenta

3 IgM :

(a) It contains 576 amino acids and has a mass of 950,000 daltons

(b) It is the first antibody to be formed in a new born animal or human

(c) IgM (with IgD) is the major immunoglobulin expressed on the surface of B cells

(d) Its carbohydrate content is 10-12 per cent It is dissociated into subunits designated IgMs Each monomer is composed of two L chains and two H chains (μ) with 2 combining sites, so that the intact molecule has 10 combining sites

(e) IgM does not cross the placenta

(f) The basic units of it are connected by disulfide bond bridges and a small polypeptide J chain.

4 IgD : No antibody activity is associated with IgD

5 IgE :

(a) It is present in the serum in very low concentrations as a single basic unit with heavy E chains

(b) Its molecular weight is about 190,000 (8S) Half of patients with allergic diseases have increased serum IgE levels

(c) The specific interaction between antigen and IgE bound to the surface of mast cells results in the release of inflammatory mast cell products such as serotonin and histamine

ELECTROPHORETIC DETERMINATION OF IMMUNOGLOBULINS

In 1937, Tiselius performed the separation of proteins in electrical fields Owing to the relative complexity of his method, zone electrophoresis has replaced electrophoresis in a free solution

Zone electrophoresis :

(i) Serum or other biologic fluid samples are placed on the cellulose acetate and separated by electrophoresis for 90 minutes using alkaline buffer solutions.

(ii) The strips are then stained and scanned

(iii) This procedure separates normal serum proteins into 5 major electrophoretic bands (albumin, α_1-globulin, α_2-globulin, β-globulin, and γ-globulin)

(iv) This electrophoresis is very useful for the diagnosis of human paraprotein disorders such as mutiple myeloma and hypogammaglobulinemia In hypogammaglobulinemia, the decrease in serum γ-globulin is easily detected.

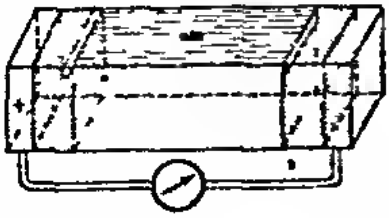


Fig 29.2a Electrophoresis of sample in electrolyte buffer is performed

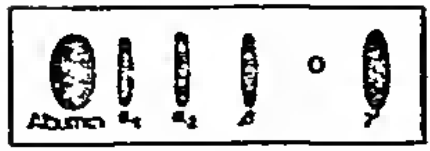


Fig 29.2b Separated protein bands are seen after being stained.

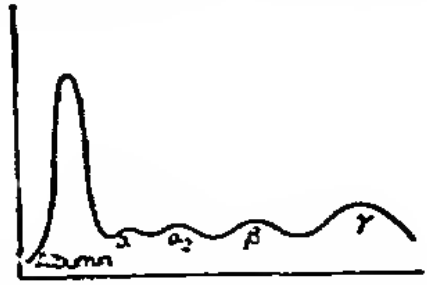


Fig. 29.2c Graphical representation of albumin, α_1-globulin, α_2-globulins, β-globulins and γ-globulins

Immunoelectrophoresis (IEP) -

(i) It consists of both electrophoretic separation and immune precipitation of proteins.

(ii) A glass slide covered with buffered molten agar (p^H 8.2) is required to perform the test

(iii) An antigen (serum) well and an antibody trough are cut in the agar after the agar is cooled

(iv) The serum sample is placed in the well and the various proteins are then separated in an electrical field

(v) Antiserum is next placed in the trough and allowed to diffuse for 18-24 hours in the direction of the separated proteins

(vi) When the various protein fractions meet the migrating antibody, precipitin lines are formed These lines are stained or photographed to make a *permanent record*

(vii) The absence of immunoglobulin classes or the presence of abnormal immunoglobulin molecules are detected by this process even in very low concentrations that are not apparent by zone electrophoresis.

QUANTITATIVE DETERMINATION OF IMMUNOGLOBULINS

(i) The radial diffusion technique is utilized for the quantitative determination of immunoglobulins

(ii) Wells are cut in an agar plate impregnated with a specific antiserum directed against a single human immunoglobulin class.

(iii) A circular precipitin ring will form after the human serum proteins placed in the well diffuse through the agar

(iv) The diameter of the precipitin ring is proportionate to the concentration of serum immunoglobulin

(v) The level is determined by comparing the diameter of the unknown serum to that of a standard containing known levels of immunoglobulins.

(vi) This test does not differentiate between normal and abnormal immunoglobulin molecules as does the immunoelectrophoresis procedure.

(vii) The normal concentrations of the 3 major immunoglobulin classes are as follows .

IgG : 710-1530 mg/dl (92-207 IU/ml)
IgA : 60-490 mg/dl (54-268 IU/ml)
IgM : 40-210 mg/dl (69-287 IU/ml)

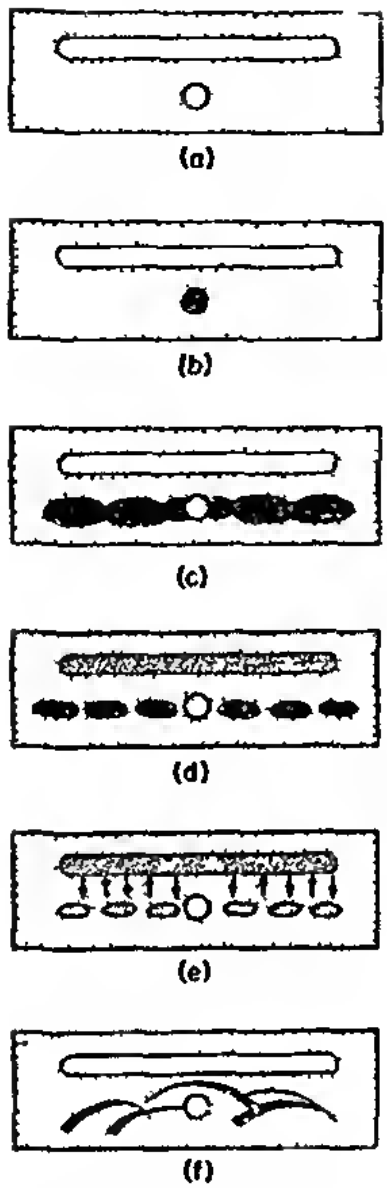


Fig 29 3

(a) Semi-solid agar poured into glass slide and ant gen well and antiserum trough cut out of agar
(b) Antigen well filled with human serum
(c) Serum separated by electrophoresis
(d) Antiserum trough filled with antiserum to whole human serum
(e) Serum and antiserum diffuse into agar
(f) Precipitin lines form for individual serum proteins

ANTIGENS

Definition : Substances which can give an immune response when introduced into an animal are called *immunogens or antigens*

Although most antigens are macromolecular proteins, polysaccharides, synthetic polypeptides and other synthetic polymers may also be immunogenic. Although the characteristics of antigens are complex, certain conditions must be satisfied in order that a molecule be immunogenic, as follows .

1 The molecules that are foreign to the host are immunogenic

2 Molecules smaller than MW 10 000 are only weakly immunogenic Macromolecular proteins with molecular weights greater tban 100,000 are the most potent immunogens

3 Immunogenicity increases with structural complexity Aromatic amino acids are more immunogenic than nonaromatic amino acids

4 The ability to respond to a particular antigen varies witb the genetic constitution of the animal

ANTIGENIC DETERMINANTS

The initiation of immunoglobulin production requires binding of the antigen to the lymphocyte surface The combining sites on the surface of the lymphocytes are antibody-like molecules called *antigen receptors*

The portions of antigenic molecules wbich are involved in actual binding with antibody combining sites are termed *antigenic determinants*

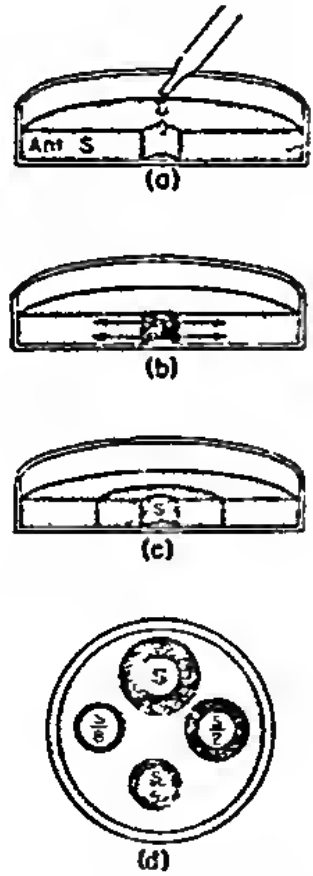


Fig 29 4

(a) Petridish is filled with semi-solid agar solution containing antibody to antigen S
(b) Antigen S is allowed to diffuse radially from the center well for 24–48 hours
(c) Antigen S meets corresponding antibody to S in the agar, precipitin results After reaction a sharp border is formed
(d) By serial dilution of a known standard quantity of antigen S-S/1, S/2 S/4, S/8 —rings of progressively decreasing size are formed The amount of antigen S in unknown specimens can be calculated and compared with standard

Haptens : (i) Karl Landsteiner prepared haptens by covalently coupling diazonium derivatives of aromatic amines to lysine tyrosine, and histidine residues of immunogenic proteins These proteins are called *carriers*

(ii) These are small, chemically defined substances which, although not immunogenic, react with antibodies

(iii) The *protein hapten conjugates form antihapten antibody* The conjugated haptens thus behave as the complete antigenic determinant of the molecules

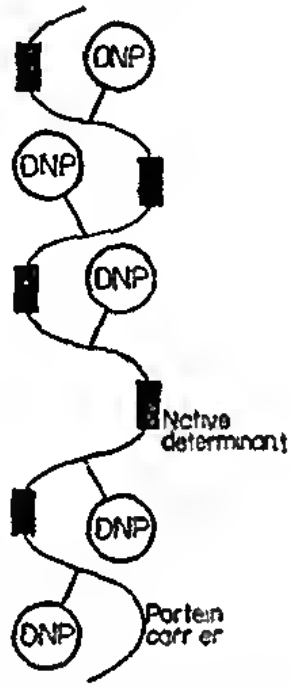


Fig 29.5. Hapten protein conjugate The conjugated dinitrophenyl (DNP) hapten introduces new antigenic determinants The protein has several native or integral antigenic determinants denoted by thickened areas

Size of antigenic determinants

1 Antigenic determinants and antibody combining sites are similar to a "lock and key arrangement The binding affinity between the antigen and antibody site is directly proportionate to the closeness of fit

2 The antigenic determinant size was performed using single sugar (glucose) polysaccharides (dextran) These single-chain polysaccharides with few branch points were used to produce antibodies

3 The hexasaccharide was the best inhibitor of the dextran antidextran precipitant reaction

IMMUNOPOTENCY

The ability of the region of the antigen molecule to act as an antigenic determinant and to induce the formation of specific antibodies is called *immunopotency* The following factors influence immunopotency

1 Exposure to the aqueous environment is important in immunopotency

2 Charge residues contribute significantly to the specificity of antigens Because charged groups are hydrophilic and are in close contact with the environment.

3 Genetic factors play an important role in the ability of different animals to produce antibodies of different specificities against the same antigen The dominant component of the antigenic determinant is termed *immunodominant*

CHAPTER 30

HORMONES

General characteristics of hormones :

The endocrine or ductless glands secrete hormones which catalyze and control metabolic processes The hormones differ from enzymes in the following ways :

1 The hormones are produced in an organ in which they ultimately perform their function

2 They are secreted into the blood prior to use

3 Structurally, they are not always proteins

The action of a hormone at a target organ is regulated by 5 factors :

(a) Rate of synthesis and secretion of the stored hormone from the endocrine gland.

(b) Specified transport systems in the plasma

(c) Conversion to a more active form

(d) Hormone specific receptors in target cell cytosol or membranes

(e) Ultimate degradation of the hormone, usually by the liver or kidneys

Mechanism of action of hormones :

The exact site of action of any hormone is not yet known, five general sites have been proposed

1 **Induction of enzyme synthesis at the nuclear level :**

(i) Steroid hormones initially act by binding to a specific high affinity receptor protein in the cytosol

(ii) The complex is then transported to the nucleus of the cell where it reacts with the nuclear chromatin

(iii) The combination formed thus influences the synthesis of messenger RNA (mRNA) which may act as a template directing the synthesis in the cytoplasmic endoplasmic reticulum of specific protein enzyme

(iv) Thyroid hormones act similarly to increase RNA and enzyme synthesis but do so by directly binding to specific receptor proteins in the nuclear chromatin Receptors in the cytosol are less effective in regulation

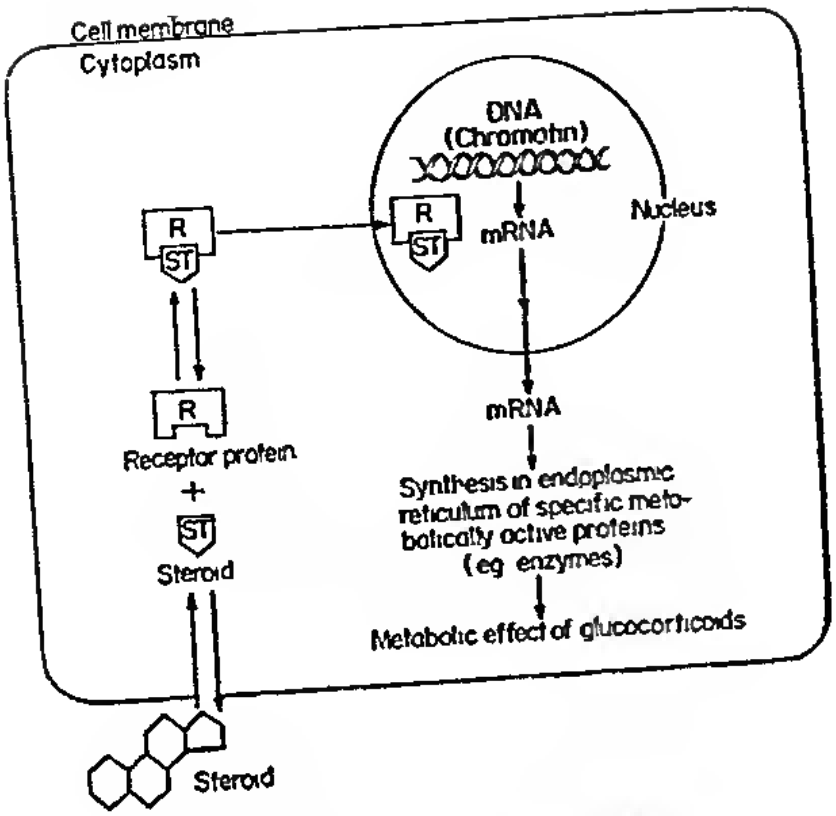


Fig 30 1 Steps in glucocorticoid action
ST=Steroid
R=Specific glucocorticoid receptor

2 Stimulation of enzyme synthesis at the ribosomal level :

Activity is at the level of translation of information carried by the mRNA on the ribosomes to the production of enzyme protein

3 Direct activation at the enzyme level :

Since cell membranes are usually required it is probable that the initiating hormonal event is activation of a membrane receptor.

4 Hormonal action at the membrane level :

(i) Many hormones are superficially involved in the transport of a variety of substances across cell membranes including carbohydrates, amino acids, cations, and nucleotides In general, these hormones specifically bind to cell membranes

(ii) Most protein hormones and catecholamines activate different membrane enzyme systems by direct binding to specific receptors on the cell membrane rather than in the cytosol

(iii) Increased insulin and thyroid hormone decrease their respective receptors

(iv) In many cases, binding capacity exceeds the hormone levels required for maximum biologic response suggesting that there is an excess of receptors or that nonfunctional receptors can exist

5 Hormonal action as it relates to the level of cyclic nucleotides :

(i) Cyclic AMP is a nucleotide which plays an important role in the action of many hormones Its level may be increased or decreased by hormonal action

(ii) Glucagon may cause large increases of cAMP in the liver but comparatively small increases in muscle But epinephrine produces a greater increase of cAMP in muscle than in liver. Insulin can decrease hepatic cAMP.

(iii) The hormones probably act at specific receptor sites in the different cell membranes which in turn activate the adenylate cyclase. Receptors for different hormones in a cell membrane activate a relatively common adenylate cyclase

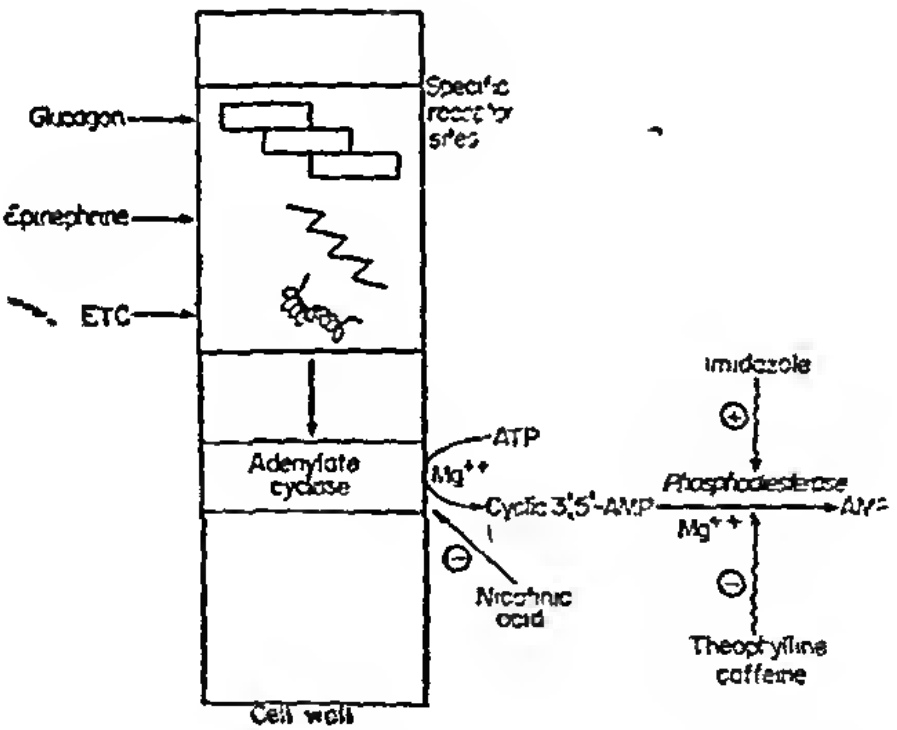
(iv) cAMP activation of phosphorylase is the result of a specific activation of the enzyme phosphorylase kinase which results ultimately in the conversion of inactive dephosphophosphorylase to active phosphorylase

(v) In adipose tissue, cAMP may activate lipolysis by a similar stimulation of protein kinase which causes increased lipase activity.

(vi) The hydrolysis of cAMP results in the liberation of 1 6 KCal/mol. more energy than the hydrolysis of a high energy bond from ATP.

(vii) The kinases not only phosphorylate enzymes but they also phosphorylate other proteins (e g nucleoproteins) Thus, cAMP may affect regulatory systems at the chromatin level of transcription and possibly at the ribosomal protein level of translation

(viii) cAMP is now recognized as important as ATP or AMP in controlling enzymic reactions



2. Factors involved in the production and degradation of cyclic 3', 5'-AMP (cAMP)

calcium in hormone action and secretion :

The action of most protein hormones is inhibited in the absence of even though ability to increase or decrease cAMP. Thus, calcium may re terminal signal for hormonal action than cAMP.

2 Ionised calcium of the cytosol is the important signal The source of this calcium may be the extracellular fluid or it may arise from mobilization of intracellular, tissue bound calcium, whereas cAMP primarily mobilizes tissue bound calcium

3 The secretion of almost all hormones stored in granules requires calcium Stimulators often increase uptake of calcium whether or not they increase cAMP Furthermore, cAMP can partially initiate or modulate the action of primary stimulators by mobilizing intracellular bound calcium

Assay of Hormones :

A Biologic Assays :

1 Biologic assays measure the levels of functional activity of hormones

2 These assays are not usually specific due to lack of sensitivity

B Chemical Assays :

1 These assays measure the absolute quantity of a given hormone

2 These are not generally applicable in case of protein hormones

C Radiodisplacement Chemical Assays .

1 These assays are largely used now a days for protein and nonprotein hormones

2 The binding protein is a specific antibody, membrane receptor or serum transport protein The unlabelled hormone, present as standard or unknown, displaces the labelled hormone and results in the increase in radioactivity in the unbound fraction These include electrophoresis and preferential salt precipitation

3 These assays are more sensitive than most bioassays because the concentrations of less than 1 ng/ml can be detected by these assays

CHEMISTRY AND FUNCTIONS OF THE HORMONES

The Pituitary gland :

1 The human pituitary is located in the brain just behind the optic chiasm as an extension from the floor of the hypothalamus and is about 10 mm in diameter

2 The average weight of human pituitary is 0 5-0 6 g in males and 0 6-0.7 g in females

3 It consists of two parts—(a) the adenohypophysis or anterior lobe , (b) the neurohypophysis or posterior lobe

Role of Hypothalamus :

1 The regulatory factors secreted by the hypothalamus control the secretion of hormones by the pituitary gland The regulatory factors are considered to be hormones

2 In addition to the secretion of regulatory hormones, neurophysial hormones are actually synthesized in the hypothalamus and migrate as granules down the nerve fibres and accumulate at the nerve endings from which they are secreted

ADENOHYPOPHYSIS OR THE ANTERIOR PITUITARY GLAND

1 The anterior lobe is the largest and most important portion of the pituitary

2 In man, this lobe is about 70 per cent of the total weight of the gland

3 This gland releases a number of hormones which are divided into two groups—(a) Tropic hormones Thyrotropic hormone (TSH), adrenocorticotropic hormone (ACTH), Lutemizing hormone (LH), Follicle stimulating hormone (FSH), (b) Growth hormone (GH, somatotrophin, prolactin and lipotrophins)

Thyrotropic Hormone, Thyroid Stimulating Hormone (TSH)

Chemistry :

1 It is a glycoprotein of molecular weight about 30,000

2 It consists of two polypeptide chains termed TSH-α and TSH-β

3 It is very rich in sulphur containing amino acids consisting of 11 disulphide residues and contains fucose, mannose, galactose, glucosamine and galactosamine

Functions.

1 It binds to specific membrane receptors and activates thyroidal adenylate cyclase causing an increased cellular cAMP

2 It increases the rates of the removal of inorganic iodide from blood by thyroid and incorporation of iodide in the thyroid hormone

3 It increases the release of thyroxine from the thyroid gland to circulation

4 It is used clinically to differentiate primary hypothyroidism (myxedema) from secondary hypothyroidism (Pituitary insufficiency)

Control of release :

The hypothalamus secretes thyrotropic releasing hormone (TRH) a regulatory factor It is a tripeptide containing pyroglutamic acid, histidine, and prolinamide This factor is abundant in the pineal gland and frog skin Inhibitors of protein synthesis have no effect on the synthesis of this factor from the hypothalamus which indicates that the process is nonribosomal Its action is calcium dependent This factor specifically acts on the thyrotropin secreting cells TRH also stimulates prolacting secretion

ACTH (Adrenocorticotropic Hormone) :

Chemistry :

1 It is a straight chain polypeptide of molecular weight 45,00 containing 39 amino acids

2 Only the first 23 amino acids are required for activity These 23 amino acids in the peptide chain is the same in all species, whereas the remaining biologically inactive 16 amino acid chain varies according to the animal source

Functions :

1 It stimulates the synthesis of corticosteroids by the adrenal gland and also enhances their release from that gland

2 It increases the total protein synthesis

3 It causes the synthesis of steroid hormone from cholesterol

4 It has a mild stimulating effect in dispersing melanin granules on the skin

5 The administration of ACTH causes (a) the increased excretion of nitrogen, potassium and phosphorus, (b) retention of sodium chloride and secondary retention of water, (c) Elevation of fasting blood sugar, (d) increase in circulating free fatty acids, (e) increased excretion of uric acid, (f) decline in circulating eosinophils and lymphocytes

6 Since it activates adenylate cyclase and increases intracellular levels of cAMP, it can increase lypolysis in adipose tissue and stimulate insulin secretion from the pancreas

7 Puromycin, the blocking agent of protein synthesis, inhibits the effect of ACTH on the adrenal gland

Control of ACTH Secretion

1 It is controlled by corticotropin releasing hormone (CRH) of the hypothalamus

2 Activation of the hypothalamic centers takes place via neurotransmitters in the central nervous system (e g acetylcholine and serotonin) Therefore, stresses such as cold, insulin hypoglycemia epinephrine, estrogens, psychic stimuli cause an increased production of ACTH leading to increased adrenal cortical activity High levels of ACTH inhibits the further synthesis of ACTH

Luteinizing Hormone (LH)

Functions .

1 In the female, it stimulates final maturation of the graafian follicle, ovulation, and the development of the corporalutea The secretion of estrogen and progestone is also stimulated

2 In the male, it stimulates testosterone production by the testis which maintains spermatogenesis and causes the development of accessory sex organs such as the vas deferens, prostate, and seminal vesicles

3 It increases cAMP Both cAMP and LH are blocked by puromycin

Follicle Stimulating Hormone (FSH)

Functions :

1 In the female, it stimulates the growth and maturation of graafian follicles and prepares them for ovulation and for the action of LH and enhances the release of estrogens This hormone is very active during menstrual cycle

2 In the male, it stimulates seminal tubule and testicular growth and also the early stages of spermatogenesis

3 The secretion of this hormone is inhibited by the administration of testosterone, progesterone and high concentration of FSH

Growth Hormone (GH, Somatotrophin)

Chemistry :

1. It is a single polypeptide with a molecular weight of about 21,500
2. The human growth hormone consists of 191 amino acids
3. The activity of the hormone resides only in a portion of the molecule
4. Partial hydrolysis of the hormone does not stop its activity
5. The growth hormone preparations from monkey and human are active in both humans and rats

Functions :

1 It increases total growth and causes gigantism in children Its deficiency in children causes dwarfism

2 It stimulates production of *Somatomedins* (*Sulfation factors*) from liver and kidney Somatomedins is similar *to serum insulin like activity*

3 It stimulates protein synthesis causing an increased in nitrogen and phosphorus retention. Blood amino acids and urea are decreased Growth hormone increases synthesis of DNA and RNA in all tissues. It stimulates erythropoiesis

4 It accelerates the mobilization of fat from the adipose tissues and the free fatty acids in the blood. It enhances oxidation of fats in liver and muscle. Increased ketogenesis occurs in the deficiency of insulin

5 In muscle, it functions against the action of insulin causing the decrease in the utilization of glucose and stimulates the secretion of glucagon resulting in the increase in the blood glucose level In liver, it inhibits hexokinase reaction and thus opposes the action of insulin.

6 It increases intestinal absorption of calcium as well as its excretion. As it stimulates the growth of the long bones at the epiphyses as well as the growth of soft tissues, retention of other minerals such as potassium, phosphorus, sodium, magnesium and chloride occurs It causes the formation of sulphate esters for incorporation into cartilage by increasing somatomedin from the liver

Control of secretion of growth hormone :

1 The control of growth hormone is exerted by a specific *growth hormone releasing factor* (*GHRF*) also termed growth hormone releasing hormone (GRH) which is extracted from the hypothalamus

2. Growth hormone release inhibiting hormones (GH RIH, GIH) or Somatostatin release inhibiting hormone (SRIF) is a negative modulator of growth hormone

3 Secretion of growth hormone is influenced by exercise, hypoglycemia, starvation, excitement and exposure to cold.

Prolactin (PL)

Chemistry :

1 It is a protein with a molecular weight of about 23,000

2. It is produced by the pituitary acidophil cells

Functions :

1 It activates the corpus luteum and stimulates progesterone production by the developed corpus luteum.

2. It also stimulates enlargement of crop gland and formation of "crop milk" in pigeons

3 It increases during pregnancy and stimulates mammary development and growth hormone—like metabolic changes.

4 It is inhibited by a hypothalamic factor, *prolactin inhibiting factor*

NEUROHYPOPHYSIS (THE POSTERIOR LOBE OF THE PITUITARY)

OXYTOCIN

Chemistry :

1 It is a cyclic polypeptide containing 8 amino acids.

2. Its molecular weight is about 1000

3 Its structure is quite similar to that of vasopressin The differences are isoleucine of oxytocin is replaced by phenylalanine of vasopressin and leucine of oxytocin is replaced by lysine of vasopressin

Functions .

1 It causes contraction of the smooth muscles in the mammary gland resulting in milk excretion Its level is increased by suckling

2 It is increased during labor It causes uterine contraction and is used in obstetrics when induction of uterine contraction is required

3 It stimulates the contraction of gall bladder, intestines and urinary bladder

VASOPRESSIN

Chemistry

1 It is a cyclic polypeptide containing 8 amino acids

2 Its structure is quite similar to that of oxytocin except isoleucine which is replaced by phenylalanine and leucine which is replaced by lysine

Functions •

1 It has a marked effect on the kidney tubules accelerating the rate of water reabsorption from the distal tubules and thus produces a marked antidiuretic effect Hence, it is termed as *antidiuretic hormone* (ADH)

2 It is an effective inhibitor of the gonadotrophins, particularly LH

3 ADH formation is prevented by the tumor in the hypothalamus or injury to hypothalamus *Diabetes insipidus* occurs in the absence of ADH which is characterized by the large volume of excretion of urine—upto 30 L of urine per day Alcohol also inhibits ADH secretion

4 ADH secretion is increased by emotional and physical stress, electrical stimulation, acetylcholine, nicotine and morphine These stimulations are associated with an increase in RNA synthesis in the neuron indicating an increased protein synthetic activity

THE MIDDLE LOBE OF THE PITUITARY

Intermedin or melanocyte-stimulating hormone (MSH)

Chemistry

1 There are two peptides (α MSH and β-MSH) β MSH is 50 times more than α-MSH

2 α-MSH is smaller containing only 13 amino acids Both have structural similarity with that of *ACTH*

3 α MSH is identical to the first 13 amino acids of *ACTH*

4 Amino acids 11-17 of β-MSH are common to both α MSH and *ACTH*

5 α MSH has some corticotropic activity but not of β MSH

Functions •

1 This hormone increases the deposition of melanin by the melanocytes of the human skin

2 In addison's disease when the prodnetion of the corticosteroids is inadequate, melanocyte stimulating hormone (MSH) is secreted causing the increased synthesis of melanin accompanied by brown pigmentation

3 Both cortisone and hydrocortisone, epinephrine and norepinephrine inhibit the action of MSH

Abnormalities of Pituitary function

A HYPERPITUITARISM

Gigantism

1 It occurs from the hyperactivity of the gland during childhood or adolescence

2 The long bones increase in length so that the individual attains an unusual height

3 The growth of the legs and hands are relatively greater than the trunk.

4 The individuals are mentally subnormal

Acromegaly

1 This condition occurs in adults after the epiphyses have closed and growth has ceased

2 The individual exhibits enlarged nose, growth and enlargement of the hands and feet and thickening of the skin

3 Sexual function is increased Raised BMR, hyperglycemia and glycosuria result.

Excess production of ACTH produces Cushing s disease

B HYPOPITUITARISM

Dwarfism

1 It occurs as a result of hypoactivity of the gland in childhood

2 Cessation of growth and sexual retardation.

Frolich s syndrome

1 This occurs in pituitary destruction in childhood.

2 The children become stunted and quite stupid

3 There are deposits of fats all over the body

4 Obesity and diabetes insipidus occur

5 Disturbances in sleep mechanism and body temperature occur These children spend most of their time asleep

6 The obesity is due to increased apetite and reduced energy output

Simmond's disease (Panhypopituitarism)

1 It occurs due to the deficiency of the function of the hypophysis in the adult.

2. BMR is reduced, subnormal body temperature and the heart rate is low

3 Carbohydrate metabolism is entirely disturbed and the individual develops severe and sometimes fatal hypoglycemic coma

Myxedema is due to lack of thyrotropin.

THE ADRENALS

THE ADRENAL MEDULLA

Epinephrine and norepinephrine

Chemistry

1 These hormones are structurally related to a group of organic compounds

known as catechols The aromatic nucleus of these hormones is that of catechol (1, 2-dihydroxybenzene) but the amino group is attached to an aliphatic side chain Hence, the term catecholamine

2 80 per cent of the catecholamine activity is attributed to epinephrine Naturally occurring epinephrine is the L isomer The unnatural D form is only one-fifteenth as active

3 Epinephrine differs from norepinephrine only in that epinephrine is methylated on the primary amino group of the aliphatic side chain shown in the figure below

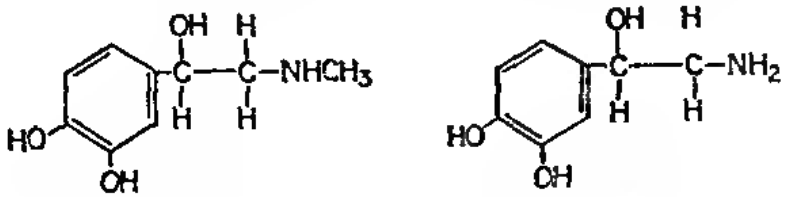


Fig. 30.3.

Functions :

1 Epinephrine gives a rapid physiologic response to emergencies such as cold, fatigue, shock etc In this sense, it mobilizes the term "fight or flight" mechanism

2. These hormones are effective on parenteral administration They are destroyed by oxidation when given orally

3 They increase blood pressure

4 Epinephrine increases cAMP by activating adenylate cyclase cAMP stimulates phosphorylase which causes the breakdown of glycogen in the liver resulting the increased blood sugar Norepinephrine is one-fifth potent in this respect.

5 Epinephrine causes increased breakdown of muscle glycogen to lactic acid thereby increasing blood lactate

6 Epinephrine acts on adipose tissue and releases free fatty acids into the circulation.

7 Epinephrine also increases BMR

8 Epinephrine is an effective stimulant of heart action It increases the irritability and the rate and strength of contraction of cardiac muscle and increases cardiac output It causes vasodilatation of the arterioles of the skeletal muscles and vasoconstriction of the arterioles of the skin and mucous membranes Norepinephrine has less effect on cardiac output

9 Epinephrine causes relaxation of the smooth muscles of the stomach, intestine, bronchioles and urinary bladder This hormone is valuable in the treatment of asthmatic attacks

THE ADRENAL CORTEX

Steroid hormones

Chemistry :

1 All steroid hormones have a parent ring i.e cyclopentanoperhydrophenanthrene ring

2 Most naturally occurring steroids contain alcohol side chains and are referred to as sterols

3 About 50 steroids have been isolated from the adrenal gland, but only a few possess physiologic activity The most important ones are cortisone, hydrocortisone (cortisol, 17 hydroxycorticosterone) aldosterone and the two androgens androstenedione and dehydro epiandrosterone Cortisol is the major free circulating adrenocortical hormone in human plasma.

4 A and B rings of the nucleus are joined in a trans or Cis configuration Estrogens do not show such isomerism since their A ring is aromatic

5 In natural steroids, both the chains attached at C_{17} and various substitutions at C_{11} are in the β-configuration

Functions

A The glucocorticoids

1 These hormones increase glucose, fatty acids and amino acids in the blood

2 In the peripheral tissues (Muscle and adipose tissues) they cause depressed uptake of glucose diminished glycolysis depressed protein synthesis and increased protein degradation

3 In adipose tissue they increase lypolysis and in muscle they cause depletion of protein stores

4 They increase alanine—α ketoglutarate and tyrosine transaminases as well as tryptophan pyrrolase

5 They increase the key enzymes in the regulation of gluconeogenesis (Pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1, 6-diphosphatase and glucose 6-phosphatase)

6 In the liver, they act on the fixation of CO_2 at the level of pyruvate carboxylase which is the key enzyme in gluconeogenesis

7 They are inactive on heart, brain and red cells

8 They have anti inflammatory effects and also immunosuppressive effects

9 They have effects on bone, exocrine secretion, cyclic AMP and stress

B The Mineralocorticoids :

1 They increase the absorption of sodium and chloride by the renal tubules and decrease their absorption by the sweat glands, salivary glands, and the gastrointestinal tract

2 They cause, on administration increased extracellular fluid volume, increased circulating blood volume and urinary output

3 Aldosterone also increases the renal clearance of magnesium

C Sex Hormones (C 19 Corticosteroids) .

1 The adrenocorticosteroids cause retention of nitrogen (a protein anabolic effect), phosphorus, potassium, sodium, and chloride If they are present in excessive amounts, they lead to masculinization in the female

2 Estrogens and progesterones are produced in small amounts

Abnormalities of adrenocortical function .

A. In humans, degeneration of the adrenal cortex due to tuberculous process or in diabetes and hypothyroidism causes *Addison's disease* which shows the following signs

1 Decreased 17 hydroxycorticoid and aldosterone excretion

2 Excessive loss of sodium chloride in the urine

3 Elevated levels of *potassium in the serum*

4 Low blood pressure and low body temperature

5 Muscular weakness, gastrointestinal disturbances, hypoglycemia, and a progressive brownish pigmentation

B Adrenocortical hyperfunction is caused by malignant tumors of the cortex This hyperfunction of the cortex causes *Cushing's disease* which exhibits the following signs

1 Hyperglycemia and glycosuria (Diabetogenic effect)

2 Retention of sodium and water followed by edema, increased blood volume and hypertension

3 Negative nitrogen balance (Protein anti anabolic effect and gluconeogenesis)

4 Potassium depletion and hypokalemic alkalosis

THE ORGANS PRODUCING SEX HORMONES

The testes and ovaries not only provide spermatozoa or ova but also manufacture steroid hormones which control secondary sex characteristics, the reproductive cycle, and the growth and development of the accessory reproductive organs

MALE HORMONES

Testosterone

Chemistry

1 This is formed from cholesterol through pregnenolone, progesterone, and hydroxyprogesterone

2 The active hormone is dihydrotestosterone

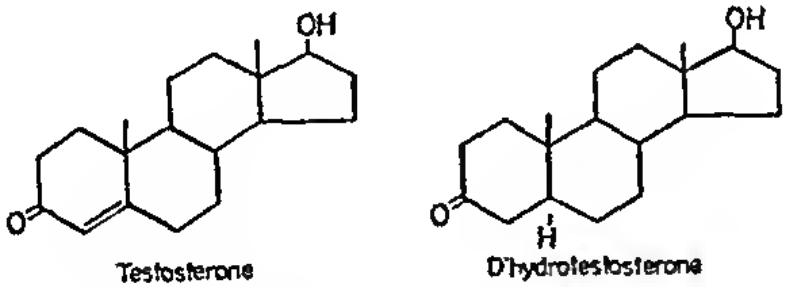


Fig 30 4 Structure of Testosterone and Dihydrotestosterone

Functions :

1 Testosterone promotes the growth and function of the epididymis, vas deferens, prostate, seminal vesicles, and penis

2 It enhances and maintains the motility and fertilizing power of the sperms

3 It stimulates the secretion of sebaceous glands

4 It is largely responsible for the emotional make-up of the male

5 It depresses the estrogenic over activity in women with symptoms of dysmenorrhoea, painful breasts and stops lactation and menstruation

6 It promotes protein synthesis in the body

7 It increases the activity of glycolytic enzymes and decreases the activity of glutamic dehydrogenase and arginase synthetase

8 It increases the rate of synthesis of fatty acids

FEMALE HORMONES

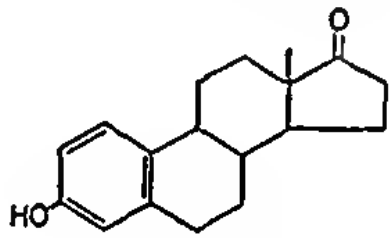
The ovary secretes two hormones 1 Estrogens formed by the follicular tissue 2 Progesterone formed by the corpus luteum

Estrogens

Chemistry

1 The estrogens are C 18 steroids and differ from androgens in lacking the methyl group at C_{10} The ring A is aromatic

2. The androgens testosterone, and androstenedione are precursors for the synthesis of the estrogens in testis ovaries, adrenals and placenta The chemical structure of estrogen is given below



Estrogen

Fig 30 5

Functions

1 Estrogens are responsible for the growth of the uterus vagina, pelvis breasts, pubic and axillary hair

2 They influence the menstrual cycle and are essential for breast development

3 On administration, they promote mitotic activity in the uterine muscle and endometrium

4 The vaginal epithelium is sensitive to the action of estrogen.

5 They influence the secretion of the gonadotrophic hormones in the anterior pituitary

6 They increase the plasma levels of thyroxine and cortisol binding globulins

7 They cause rapid increase in RNA synthesis in uterine tissue

8 They prevent lipid accumulation in liver when administered to animals having diets deficient in lipotropic factors i e methionine and choline

9 They cause decrease in cholesterol level and other lipids in plasma. This is why the incidence of atherosclerosis is low in women as compared to men

10 They regulate normal bone metabolism. Women after menopause develop osteoporosis

Progesterone

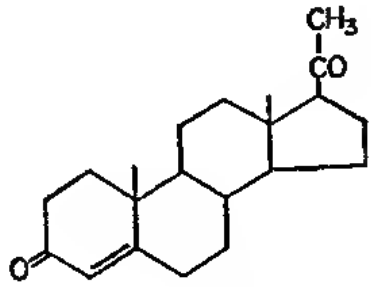
This hormone is formed in the corpus luteum of the ovary It is also formed

in the placenta during the latter part of pregnancy It is secreted 1 or 2 days before ovulation takes place

Chemistry :

1 It is synthesized from cholesterol

2 It is also formed in the adrenal cortex as a precursor of both C-19 and C-21 corticosteroids The chemical structure is given below



Progesterone

Fig 30 6

Functions :

1 Progesterone causes the development of the endometrium preparing it for the implantation of fertilized ovum for conception

2 It suppresses oestrus, ovulation and the production of luteinizing hormone When pregnancy occurs, the ovulation and menstruation are suspended by the action of this hormone

3 It stimulates the mammary glands

4 It increases BMR during the luteal phase of normal menstrual cycle

5 In the normal menstrual cycle, the anti-ovulatory effect of progesterone is the basis for the use of certain synthetic progestins as oral contraceptive agents

THE THYROID

The thyroid gland weighs about 25-30 g and consists of closely packed sacs (follicles) filled with proteinaceous colloid The gland secretes the hormones thyroxine and tri iodothyronine Of a total of 50 mg of iodine in the body, about 10-15 mg are in thyroid

Thyroxine and Triiodothyronine

Chemistry :

1 Iodination of tyrosines in thyroglobulin occurs first in position 3 of the aromatic nucleus and then at position 5 forming mono-iodotyrosine and diiodotyrosine respectively

2 Coupling of two molecules of diiodotyrosine (I_2 Tyr) then occurs within the thyroglobulin molecule to form tetraiodothyronine (Thyroxine)

3 Coupling of monoiodotyrosine (I Tyr) with diiodotyrosine (I_2 Tyr) also occurs to form triiodothyronine (T_3)

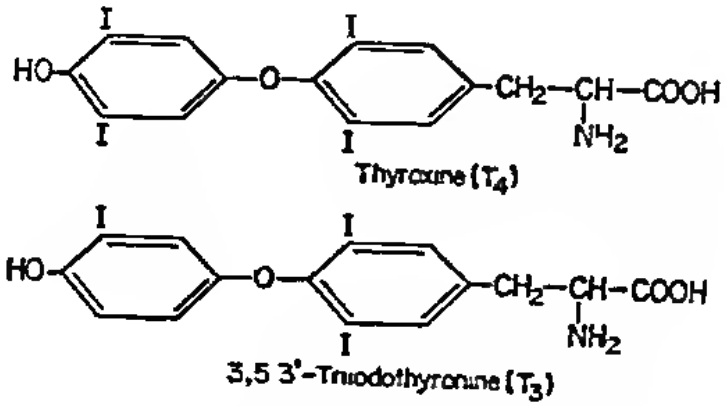


Fig 30 7 Structure of Thyroxine and Triiodothyronine

Protein Bound Iodine (PBI) in blood :

The term PBI in blood represents iodine present in thyroid hormones The PBI values for normal adults is 4 8 μg/100 ml of plasma PBI is a reliable measure of thyroxine content of plasma The values for PBI in hypo and hyperthyroidism are given below

Myxoedema (Hypothyroidism)	0 2 2.5 μg/100 ml
Grave's disease (Hyperthyroidism)	8-18 μg/100 ml

Functions :

1 In hypothyroid animals, the tissues show a low rate of oxygen consumption, the patient has a slow pulse, lowered vigor, obesity, blood cholesterol levels are increased, lipolysis and fatty acid liberation are decreased. In hyperthyroid states, the reverse occurs

2 Thyroxine causes increased intestinal glucose absorption It increases glycogenolysis in liver and muscle It promotes neoglucogenesis

3 It increases RNA, amino acid transport and protein synthesis

4 In hypothyroidism, there is marked increase in serum cholesterol and triglyceride and phospholipid contents in blood are also increased In hyperthyroidism the serum cholesterol level is decreased

5 In high concentration of thyroid hormones, calcium is mobilized from bone, losses of potassium and nitrogen in urine, increased calcium and phospborus in the urine

6 High concentration of thyroid hormone causes uncouple oxidative phosphorylation and increase swelling in the mitochondria. Such action results in the production of heat rather than storage of energy as ATP.

7. Thyroxine is essential for the conversion of β carotene to vitamin A

Abnormalities of thyroid function :

Hypothyroidism causes *Cretinism* in children and *Myxoedema* in adults

Cretinism :

1 Growth in children is retarded

2 The child is mentally defective He has coarse scanty hair and a thick yellowish scaly skin

3 Cretinism occurs in areas where goitre is prevalent

Myxoedema :

1 Puffiness of the face and hands in adults

2 Retention of water and NaCl in the body

3 BMR is low

4 Body temperature and pulse rate are subnormal

5 Body weight is increased due to deposition of fat and retention of water.

6 Mental faculties are retarded

7 Hypochlorhydria or achlorhydria is present

8 Blood cholesterol and lipid levels are increased

Hashimoto's disease

1 Thyroglobulin escapes from the cells of the gland and excites the production of antibodies which produce reactions with thyroid.

2. Fibrosis of the thyroid tissue develops leading to complete loss of thyroid function

Severe *hyperthyroidism* leads to *toxic goiter* This occurs mostly in women

Toxic goiter :

1 The patient complains of nervousness, restlessness, tiredness, undue sweating breathless on exertion, tachycardia and palpitations

2 The subject cannot tolerate warm climate but can tolerate severe cold climate

THE PARATHYROIDS

The parathyroid gland consists of 4 small glands closely associated with the thyroid In humans, the parathyroids are reddish or yellowish brown egg shaped bodies The total weight of 4 glands is 0 05 0 3 g

Parathormone :

Chemistry .

1 It is a polypeptide consisting of 84 amino acids

2 Its molecular weight is 95,00

3 This hormone from different species differ only slightly

4 It is initially synthesized in the chief cells as a preprohormone

5 The secreted hormone is degraded rapidly , it has a half life of about 18 minutes

Functions

1 The parathormone stimulates the membrane bound adenylate cyclase causing increased synthesis of cAMP

2 It mobilizes calcium and phosphorus from bones.

3 It increases serum calcium and lowers the serum phosphorus

4 It increases urinary excretion of phosphate but decreases excretion of calcium

5 It elevates serum alkaline phosphatase activity

6 It increases the absorption of calcium and phosphorus from the intestine

7 It activates vitamin D in renal tissue by increasing the rate of conversion of 25-hydroxycholecalciferol to 1, 25-dihydroxycholecalciferol

8 It increases bone resorption by releasing calcium as well as collagenase, lysosomal enzymes, and hydroxyproline

9 In the kidney, it affects renal tubular reabsorption of calcium and phosphorus

10 It may stimulate protein synthesis in the osteoclasts which effect resorption of bone

Control of release of parathormone :

1 The concentrations of parathormone are decreased abruptly by administration of calcium ion and rise when circulating ionized calcium is lowered by the administration of the chelating agent.

2. Calcium loss associated with uremia results in an increase in circulating parathormone

3 Vitamin A administration decreases parathormone by increasing calcium uptake into the parathyroid gland

Calcitonin (Thyrocalcitonin) :

Chemistry :

1 Calcitonin is a polypeptide consisting of 32 amino acids

2 Its molecular weight is 3600

3 There are large variations in the amino acid composition among different species

4 It is required for biologic activity

5 The half life of the secreted calcitonin is about 4-12 minutes

Functions :

1 It is calcium lowering hormone originated from the C cells of the thyroid gland.

2 It is directly effective on bone It inhibits bone resorption and mobilization of calcium and phosphorus from bone

3 In the kidney, it increases calcium excretion and inhibits synthesis of 1, 25-dihydroxycholecalciferol without affecting cAMP levels

4 It decreases the excretion of urinary hydroxyproline by inhibiting the resorption of the organic phase of bone

Abnormalities of parathyroid function :

Hypoparathyroidism :

1 The symptoms of hypoparathyroidism are muscular weakness, tetany and irritability

2 In case, hypothyroidism begins early in childhood, there may be stunting of growth, defective tooth development, and mental retardation

3 Serum calcium is low, serum phosphate is increased, urinary calcium is low, serum magnesium and hydroxyproline levels are decreased

4 Calcium, parathyroid hormone, and vitamin D precursors are used in the treatment of hypoparathyroidism

Hyperparathyroidism :

1 Hyperparathyroidism occurs due to a tumor of the gland The symptoms of hyperparathyroidism are hypercalcemia, decalcification of bones causing pain and deformities, renal stones

2 Serum phosphorus is low, serum alkaline phosphatase activity is increased, and excretion of calcium in urine is highly increased

3 Removal of tumor brings about prompt relief and injection of calcitonin helps to reduce serum calcium level

THE PANCREAS

Two hormones are secreted by this gland—*insulin* by beta cells of the islet of langerhans and *glucagon* by the alpha cells Delta cells store and secrete *somatostatin*

INSULIN

In 1926, Abel isolated crystalline insulin from pancreas and the amino acid sequence was established by Sanger in 1953

Chemistry

1 Insulin is a protein hormone secreted from the β-cells of the islet of langerhans

2 Traces of zinc are required for the crystallization of insulin

3 It consists of 51 amino acids and contains two polypeptide chains (A and B) linked together by two disulphide bridges [one in 7 7 and another in 20-19 of A & B chains respectively] A third intradisulphide bridge between 6 and 11 amino acids of A chain also occurs

4 It has a molecular weight of 5734

5 Alkali or reducing agents can inactivate insulin by breaking the disulphide bonds

6 Proteolytic enzymes digest the insulin protein and inactivates insulin Hence, it can not be given orally

The structure of insulin is given below

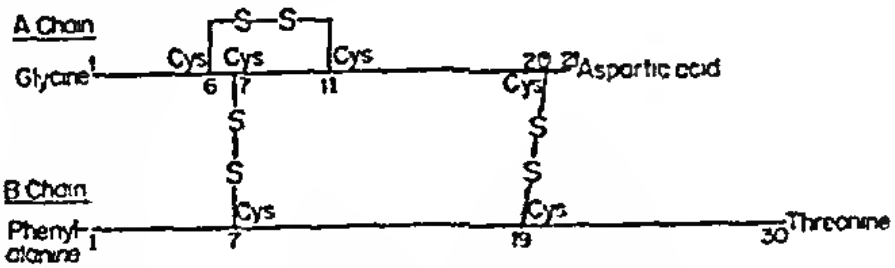


Fig 31 8 Structure of human insulin

Insulin secretion :

About 50 units of insulin are required per day The human pancreas stores about 250 units

Factors stimulating insulin secretion :

1 Increased blood glucose level causes an increase in insulin secretion and decreased blood glucose level depresses insulin secretion

2 The hyperglycemia produced by glucagon enhances insulin production.

3 Since the growth hormone and glucocorticoids cause hyperglycemia they also stimulate insulin secretion.

4 Sugars which are readily metabolized—e g mannose and fructose—can stimulate insulin release But non metabolized sugars such as galactose, L-arabinose and xylose do not stimulate

5 Many agents such as amino acids, fatty acids and some gastro-intestinal products can stimulate insulin release only in presence of glucose

6 Insulin secretion is enhanced by cAMP, ACTH and thyrotropin

7 Amino acids particularly leucine and arginine can stimulate the pancreas to produce insulin in both vivo and vitro Proteins like casein also increases secretion of insulin

8 Central nervous system indirectly influences the release of insulin. Vagal stimulation causes an increase in insulin secretion

9 Sulfonylureas, the hypoglycemic agent, may act on insulin secretion by a different mechanism than that of glucose

Factors inhibiting insulin secretion :

1 Epinephrine is the highly effective inhibitor of insulin secretion

2 Starvation reduces insulin secretion

3 Magnesium also inhibits insulin secretion

4 Vagatomy reduces insulin secretion.

Metabolism of insulin :

1 Insulin is degraded in liver and kidney by the enzyme *glutathione insulin transhydrogenase* which brings about reductive cleavage of the S-S bonds that connect A and B chains of the insulin molecule Reduced glutathione acts as a coenzyme

2. The A and B chains are further degraded by proteolysis But when insulin is bound to antibody, it is much less sensitive to enzymic degradation.

Functions :

1 Insulin is firmly bound to the highly specific receptor site present in the cell membrane The receptor may probably be a glycoprotein. The biologic activities of insulins are proportionate to their binding affinities Insulin, thus, may carry out most of its function without entering the cell. The number of receptors declines where insulin levels are high.

2 Insulin exhibits transport at the membrane site, RNA synthesis at the nuclear site, translation at the ribosome for protein synthesis, and an influence on tissue levels of cAMP It is active in skeletal and heart muscle, adipose tissue, liver, the lens of the eye, and leukocytes It is inactive in renal tissue red blood cells, and the gastrointestinal tract. The most metabolic function is centered in the muscle, adipose tissue, and liver

3 It facilitates the transport of glucose and related monosaccharides, amino acids, potassium ion, nucleosides, inorganic phospbate, and calcium ion in muscle and adipose tissue

4 In muscle or adipose tissue, insulin increases the entry of glucose and tbus leads to increase glycogen deposition stimulation of HMP shunt resulting in increased production of NADPH, increased glycolysis, increased oxidation (increase in oxygen uptake and CO_2 production), and increased fatty acid synthesis

5 In adipose tissue, it increases lipid synthesis by means of fatty acid synthesis and glycerophosphate for triacylglycerol synthesis

6 Insulin increases intracellular concentration of nonmetaholized sugars such as galactose, L-arabinose, and xylose The hormone facilitates the entry of those sugars having the same configuration at carbons 1, 2 and 3 as D glucose Since fructose having a ketone group at position 2 is not transported hy insulin Intracellular transport of glucose is enhanced hy anoxia indicating that glucose transport requires energy

7 It also increases the uptake of nonmetabolizable amino acids such as alpha aminoisohutyrate It maintains muscle protein by decreasing protein degradation

8 In adipose tissue, it quickly depresses the liberation of fatty acids caused by epinephrine or glucagon

9 Insulin directly increases protein synthesis as the bormone promotes the incorporation of labelled intracellular amino acids into protein. At the ribosomal level, it increases the capacity of this organelle to translate information from messenger RNA to the protein synthesizing machinery

10 In the liver, it stimulates glycolysis by increasing tbe synthesis of glucokinase, pbosphofructokinase, and pyruvate kinase It also depresses the enzymes controlling gluconeogenesis sucb as pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1, 6-diphosphatase, and glueose 6-phosphatase Enzymes wbicb are unimportant in the control of gluconeogenesis as well as glycolysis are not affected by insulin

Abnormal metabolism in diabetic states .

1 In diabetes, byperglycemia occurs due to the impaired transport and uptake of glucose into muscle and adipose tissue Transport and uptake of amino acids are also depressed causing the raised level of amino acids into the blood, particularly, alanine, which supply fuel for gluconeogenesis in the liver The amino acid breakdown during gluconeogenesis increases the production of urea nitrogen

2 Lipid and fatty acid synthesis is decreased due to the decrease in acetyl-CoA, ATP, NADPH and glycerophosphate in all tissues Stored lipids are hydrolyzed by increased lipolysis and the liberated fatty acids interfere the carbohydrate phosphorylation in muscle and liver developing hyperglycemia

3 Fatty acids in high concentration reacbing the liver inhibit fatty acid synthesis by a feedback inhibition at the acetyl CoA carboxylase step Increased acetyl CoA from fatty acids activates pyruvate carboxylase, stimulating gluconeogenic pathway for the conversion of amino acid carbon skeletons to glucose Fatty acids also stimulate gluconeogenesis by entering the citric acid cycle and increasing production of citrate which is an inhibitor of glycolysis (at phosphofructokinase) Thus, tbe fatty acids inhibit the citric acid cycle at the level

of citrate synthetase and pyruvate and isocitrate dehydrogenases The acetyl-CoA, which cannot enter the citric acid cycle or cannot be used for fatty acids synthesis, is utilized in the synthesis of cholesterol or ketones or both The rise in ketone concentration in body fluids and tissues leads to acidosis

4 Glycogen synthesis is diminished due to decreased glycogen synthetase activity, increased phosphyrase activity and increased ADP . ATP ratio The phosphorylase activity is stimulated by epinephrine or glucagon

5 The insulin deficiency causes hormonal imbalance and favours the action of corticosteroids, growth hormone and glucagon which enhance gluconeogenesis, lipolysis, and decreased intracellular metabolism of glucose. The excess glucose in the urine requires water to be excreted out causing dehydration

6 In the degradation in insulin, both liver and kidney are required Therefore, in renal or hepatic disease, insulin requirement is decreased This is observed in some diabetics with associated kidney or liver disease

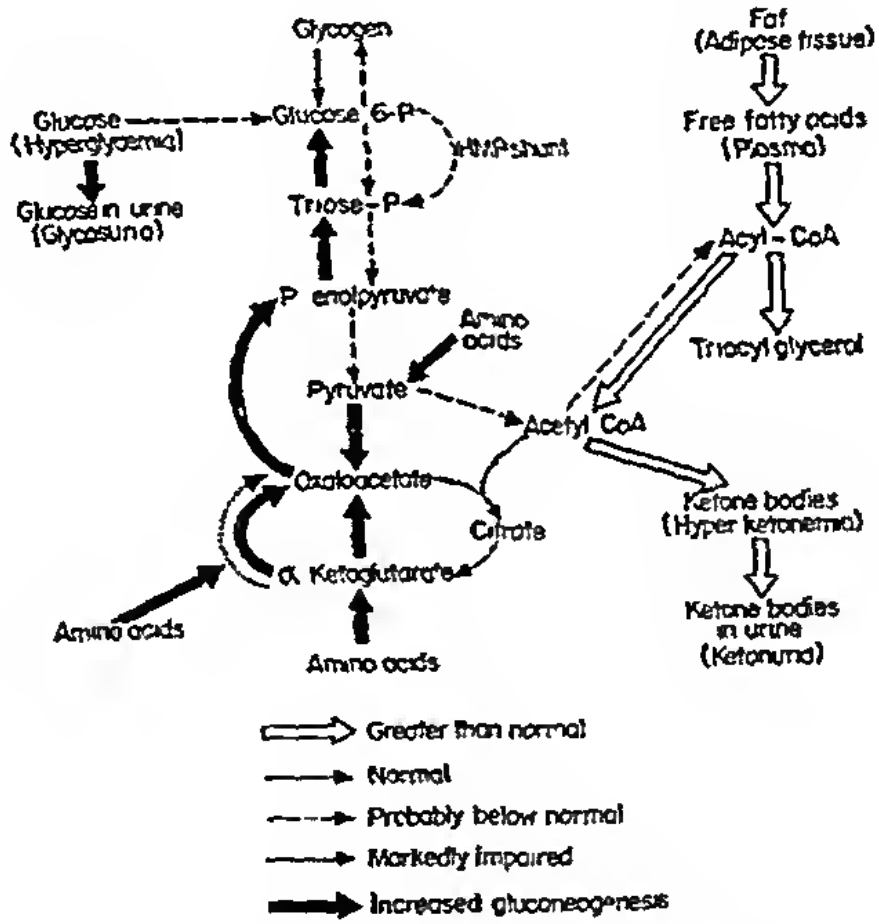


Fig 30 9 Abnormal metabolism in the liver during uncontrolled diabetes.

Antibodies

1 The repeated injection of insulin produces low levels of an antibody to insulin in all subjects after 2 or 3 months of treatment

2. The antibodies can produce lesions in the islet cells and severe diabetes

3 Antibody-bound insulin is only slowly degraded , thus much of the insulin is actually wasted

Experimental Diabetes :

1 Experimental diabetes cao be produced by total pancreatectomy or by a single injection of alloxan, a substance related to the pyrimidioes or with strepto zocin, an N-nitroso derivative of glucosamine

2 Diabetes cao also be produced by injectioo of diazoxide, a sulfooamide derivative whicb inhibits insulin secretion

3 The iojection of large amouots of antibodies to iosulin is also considered to produce experimental diabetes

4 Phlorhizin diabetes can be produced by the iojection of the drug phlorhizin This is actually a reoal diabetes in which glycosuria is only produced by the failure of the reabsorption of glocose by the renal tubules

GLUCAGON

Chemistry ·

1 Glucagon is a polypeptide with a molecular weight of 3485.

2 It consists of 29 amino acids haviog 15 different amino acids The amioo acids are arranged in a straight chain

3 It contains no cystine, proline or isoleucine but contains sufficient amounts of methionine and tryptophan

4 It ean be crystallized in the absence of zine or other metals

5 A proglucagon precursor of about 9000 daltons is also identified

6 It originates primarily in the alpba cells of the pancreas althougb a considerable amount comes from extrahepatie alpha-cells in the stomacb and other portions of the gastrointestinal tract

7 A glucagon like immunoreactive factor (GLI) is also identified in gastric and duodenal mucosa but less active tban pancreatic glucagon

Factors influencing secretions of glocagon :

1 Low blood glucose increases secretion of pancreatic glucagon

2 Most amino acids, particularly arginine, cause a rap d secretion of glucagon from tbe paocreas

3 Fatty acids inhibit glucagon release

4 Exercise stimulates the secretion of glucagon

5 Duriog mixed meals, both insulin and glucagon are secreted, hut the carbohydrate meal causes insulin release High protein meal favours glucagon secretioo

6 In stress, insulio secretioo is inhibited but glucagon secretion is stimulated

Functions :

1 Glucagon is sensitive to the adenylate cyclase receptor sites in the liver and increases cAMP level cAMP activates the enzyme proteio kinase which further activates phosphorylase Phosphorylase causes glycogenolysis making available of glucose in blood cAMP suppresses glycogen synthetase Thus, glucagon increases glycogen hreakdown and inhibits synthesis of glycogen

2 Glucagon can also activate various phosphoenzymes by activating protein kinase and inhibit dephosphoenzymes Thus, glucagon can directly stimulate gluconeogenesis by activation of pyruvate carboxylase and fructose-1, 6-dip hos-phatase.

3 It also inhibits glucose oxidation by inhibiting pyruvate kinase and pyruvate dehydrogenase

4 Elevated glucagon concentration also increases ketogenesis

5 It increases potassium release from the liver

6 In adipose tissue as well as liver, it increases the breakdown of lipids to fatty acids and glycerol

7 Crystalline glucagon polypeptide is used for the treatment of hypoglycemic persons Glucagon is also used as a diagnostic test for glycogen storage disease

PINEAL BODY

Melatonin :

Melatonin is formed from serotonin

Functions

1 It is antagonist to melanocyte stimulating hormone and lightens skin colour

2 Pinealectomy in female rats stimulates estrus and causes ovarian hypertrophy

KIDNEY

Kidney secretes a hormone called *erythropoietin*

Erythropoietin

Chemistry

1 It is a glycoprotein with a molecular weight of about 46,000

2 It contains 30 per cent carbohydrate derivative

Function

It is essential for the formation of red blood cells

Hormones of gastrointestinal tract ·

Described in digestion and absorption chapter

Exercise

1 Discuss the influence of different hormones on carbohydrate metabolism (R. U 72A , M U 73A)

2 Give an account of the chemistry secretion and functions of Insulin (Punj University 68A , P U 70A)

3 Discuss the chemistry and functions of anterior pituitary hormones (M U 69S)

4 Write short notes on

(a) ACTH (P U 71A)

(b) CCPKZ (B U 74A)

(c) Progesterone (R. U 73A)

(d) Thyroxine (M L 74S)

(e) Epinephrine (Bh U 73S)

CHAPTER 31

THE ENERGY REQUIREMENTS OF THE BODY

Atwater of U S A , during the periods 1892 1902, established clearly the relations between food consumed, heat output O_2 consumed and CO_2 output by conducting experiments with human subjects using an improved type of human respiration calorimeter The other scientists H Armsby, F Benedict, and E F Du Bois did extensive study on energy metabolism

The energy of the body is derived from the oxidation of the food A small part of the daily energy output results in the breakdown of the animal s own tissues The potential energy of the food provides the kinetic energy of the body which is mainly in the form of heat and work The potential energy of the food is the heat given out when the food is completely burnt to CO_2 water and nitrogen This is greater than the energy which the animal derives from the food because of the considerable potential energy contained in urea and other nitrogenous compounds being excreted out

The unit of energy contained in foods is expressed as a unit of heat, the calorie

Calorie A calorie is defined as the amount of heat required to raise the temperature of 1 g of water by 1°C

Kilocalorie (C) It is 1000 times the energy for the small calorie

[Since the calorie is very small the kilo calorie (C) is used in nutrition]

The international unit of energy is the joule (10^7 ergs)

1 kilo calorie=4 2 kilo joules (Kj)

Measurement of energy value of foods

The foodstuffs (carbohydrates fats and proteins) on combustion by oxygen produces heat This amount of heat can be measured in a *bomb calorimeter*

Carbohydrate and fat are completely oxidized in the body to CO_2 and water like that of bomb calorimeter But proteins are not completely burned because, urea, the end product of protein metabolism still contains some energy which is not available to the body Therefore the energy value of protein in the body (4 1 KCal/g) is less than that obtained in the bomb calorimeter The energy value of foods in the body is customary to express in round figures The table below shows the energy value of foodstuff

	Kilocalories per gram	
	In bomb calorimeter	*In the body*
Carbohydrate	4 1	4
Fat	9 4	9
Protein	5 6	4

THE ENERGY REQUIREMENT OF MAN

The energy requirement of man can be determined either directly by measuring his output in a calorimeter or indirectly by measuring his oxygen consumption.

A Direct method :

1 This is the most accurate method, but is time-consuming, laborious, and demanding very expensive apparatus

2 The subject is placed in an insulated chamber His heat production is measured directly by recording the total amount of heat transferred to a weighed quantity of water circulating through the calorimeter

3 The oxygen intake, the CO_2 output, and the nitrogen excretion in the urine and feces are also measured during the whole priod of observation

B Indirect method .

1 This method consists of measuring gas exchange and determining the respiratory quotient

2 This method is simple and applicable to field studies and clinical analysis

Respiratory Quotients (RQ) of foodstuffs It is the ratio of the volume of CO_2 eliminated to the volume of oxygen utilized in oxidation

$$\text{i.e. } R\ Q = \frac{\text{Volume of } CO_2 \text{ eliminated}}{\text{Volume of } O_2 \text{ used}}$$

1 Carbohydrates The complete oxidation of glucose is as follows :

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

$$R.\ Q = \frac{6\ CO_2}{6\ H_2O} = 1$$

2. Fats The oxidation of tristearin is as follows

$$2\ C_{57}H_{110}O_6 + 163\ O_2 \rightarrow 114\ CO_2 + 110\ H_2O$$

$$R.\ Q = \frac{114\ CO_2}{163\ O_2} = 0.70$$

Fats have a lower R Q value because the oxygen content of their molecule in relation to carbon content is very low

3 Proteins . Since the chemical structure of proteins is variable their oxidation cannot be so readily expressed The R.Q of proteins by indirect method has been calculated to be about 0 8

4 Mixed diets The R. Q of mixed diets is about 0.85 The R. Q is lowered if the carbohydrate metabolism is impaired

Total heat production

To obtain the heat prodoction the grams of each foodstuff oxidized are multiplied by the caloric value of that food The sum of these caloric values is equal to the total heat production of the diet Therefore,

Carbohydrate	210 7 g. × 4 KCal = 842.8 KCal.
Fat	84 4 g × 9 KCal = 759 6 KCal
Protein	80 0 g × 4 KCal = 320 0 KCal.
Total heat production	= 1922.4 KCal

BASAL METABOLISM

Definition :

The total heat produced or the energy spent by the body under conditions to perform minimum possible work is known as *basal metabolism*. The lowest level of energy production consonant with life is the *basal metabolic rate* (*BMR*).

Conditions for measurement of BMR :

1. Post absorptive state. The patient should not take anything by mouth for the past 12 hours.
2. Complete mental and physical relaxation.
3. Patient should be awake.
4. Recumbent position during the test.
5. Environmental temperature should be between 20°—25°C.

Factors influencing BMR :

1. *Surface area*. The BMR is directly related to the surface area of the subject. Larger the surface area greater would be the BMR.
2. *Age*. The BMR is inversely proportional to age. Children have larger BMR than adults.
3. *Sex*. Males have higher BMR than females. The BMR of females declines more rapidly between the ages of 5 and 17 than that of males.
4. *Climate*. The BMR is lower in warm climates.
5. *Racial variations*. The BMR of oriental women living in the USA is 10 per cent below the standard BMR of American women of the same age.
6. *Habit*. Persons accustomed to heavy exercise or hard physical work have a higher BMR than those involved in sedentary work.
7. *State of Nutrition*. The BMR is decreased in starvation and under-nourishment.
8. *Pregnancy*. The BMR is not changed during pregnancy. The higher values of BMR in late pregnancy is due to the BMR of the foetus.
9. *Disease*. The BMR is increased in infectious and febrile diseases. The increase is usually proportional to the rise of the temperature. The BMR is also increased in increased activity of cells and therefore, it increases in leukemia, cardiac failure, hypertension, polycythemia, dyspnea, and some types of anemia.
10. *Effects of Hormones*. The BMR is increased in hyperthyroidism and decreased in hypothyroidism. In adrenal insufficiency (Addison's disease), the BMR is subnormal.
11. *Drugs*. Caffeine and benzidrine increase the BMR.
12. *Emotion*. The BMR is increased in emotional stress.
13. *Diet*. The BMR of strict vegetarians is 11% lower than that of meat eaters.

Measurement of BMR :

1. The BMR can easily be measured either by the apparatus of Benedict and Roth (closed circuit device) or by Douglas bag method (open circuit device). Benedict-Roth's apparatus is abundently used in hospitals and laboratories.
2. This method provides sufficient accuracy only by measuring the oxygen consumption of the patient for two 6-minute period under basal conditions. The

average of two such readings is multiplied by 10 to obtain the hourly consumption of oxygen and then multiplied by 4 825 KCal, the heat produced by one liter of oxygen This gives the total heat produced in KCal/nour

3 This above value is divided by the surface area of the subject to get the BMR in C/hr /sq m

The simple classical formula of Du Bois is applied which is as follows

$$A = H^{0.725} \times W^{0.425} \times 71.84$$

where, A=Surface area in cm^2
H=Height in cm
W=Weight in Kg

The above value obtained is in sq cm This should be divided by 10,000 to get the surface area in sq meter

A BMR between −15 and +20% is considered normal The BMR may exceed +50 to +75% in hyperthyroidism In hypothyroidism, the BMR may be −30 to −60% The normal BMR is 39 5 $C/m^2/hr$ The BMR increases as much as 600−800% over basal during exercise

Significance of BMR :

1 The determination of BMR is the principal guide for diagnosis and treatment of thyroid disorders

2. The BMR −10% of the normal develops moderate hyperthyroidism In severe hypothyroidism, the BMR may be decreased to 40 to 50 percent below normal

3 It aids to know the BMR to have an idea of the total amount of food or calorie required to maintain body weight or calorie

4 The BMR is below normal in starvation, under nutrition, hypothalamic disorders, Addison's disease, and lipoid nephrosis

5 The BMR is above normal in fever, diabetes insipidus, leukemia and polycythemia

SPECIFIC DYNAMIC ACTION (SDA)

Definition :

It is defined as the extra heat production over and above the calorie value of a given amount of food when used by the body

Example An amount of protein which contains 100 KCal (25 g) when metabolized in the body, the heat production is not 100 KCal but 130 KCal This extra 30 KCal is the product of the SDA of protein Similarly, in the body, a 100 KCal portion of fat produces 113 KCal, and a 100 KCal portion of carbohydrate produces 105 KCal This extra heat is due to the activity of tissues metabolizing these foodstuffs

When these above foods are taken in a mixed diet, the SDA is not the total of the SDA of each foodstuff when fed separately According to *Forbes*, when the glucose and protein are combined, the SDA is 12 5% less than the sum of their individual effects The SDA is 22% less when lard, glucose and protein are combined, the SDA is 35% less in case of glucose—lard combination, and 54% less in case of protein—lard mixture than the sum of their individual SDA

The high SDA of protein can be reduced depending on the quantities of other foodstuffs in the diet It has been found in Forbes's data that fat (lard) has much

influence on SDA than does any other nutrient, i e fat decreases the SDA more than any other nutrient

When different amino acids are fed alanine, glycine and phenylalanine are found to produce high SDA According to *Krebs* the high SDA is due to two main factors

1 The energy required for deamination of amino acids which is again derived by the oxidation of other metabolites

2 The energy required for the synthesis of urea which is obtained by the oxidation of other metabolites present in tissues

There is evidence also that the increment is greater during the process of lipogenesis from glucose than during its oxidation to CO_2 and H_2O

The SDA of carbohydrate is to represent the energy liberated in excess of that required for the conversion of glucose to glycogen

The SDA of fat is due to the increased concentration of fat in the tissue fluids and to its more rapid oxidation

The glands of internal secretion have no direct influence upon the SDA of protein Thyroidectomy in animals reduces the SDA of cabohydrate and fat

A diet very rich in protein is unfavourable to heavy muscular work The SDA of protein is an important factor in the regulation of body temperature With fat and carbohydrate the extra heat is accelerated in the performance of work When new tissue is formed, protein does not exert its usual SDA

CALORIC REQUIREMENTS

The food requirements of a man depend upon his basal metabolism S D A, the work to be done and the climatic conditions Extra calorie is also required for a starved man to build up his tissues, a pregnant woman for the foetus and a child for his growth

A man of 70 kg weight and 180 cm height with a surface area of 1 8 square meter, aged 30 and a BMR of 40 will have a basal metabolism of 1,800 calories per day The allowance for S D A will be 200 calories Extra calories are required to be added to this on the basis of the work done by the individual Total daily energy requirements of different categories of work done by individuals are mentioned below

Recommended Daily Caloric Requirements

	Male	*Female*
Adults*		
Sedentary (Tailors, Shoemakers)	2400	2000
Moderate activity (Farmers Soldiers)	3000	2400
Heavy work (Navies Lumbermen Stonemasons)	4500	3000
Light work (Carpenters, Painters)	2500	2000
Pregnancy (Latter half)		2400
Lactation		3000
Children 16—20 yrs	3800	2400
13—15 yrs	3200	2600
10—12 yrs	2500	2500
7—9 yrs	2000	2000
4—6 yrs	1600	1600
1—3 yrs	1200	1200
under 1 year	110/Kg	110/Kg

*Men 70 Kg, Women 56 Kg

Very heavy work (Lumbermen) entails the expenditure of 8,000 calories.

In long bicycle races, 10 000 calories are required in 24 hours Calories expended per hour at various occupations are given below

Table : Approximate increments in caloric requirements (above basal) per hour for different occupations.

Occupation or *Activity*	*Increased requirement Cal /hour*
Sitting quietly	35
Standing quietly	40
Reading loudly	40
Tailor	70
Typing	75
House work	110
Painter	145
Carpenter	150
Walking (Moderate)	235
Sawing Wood	380
Walking fast	550
Walking up stairs	1000

This above table is according to the recommendations of the Food and Nutrition Board of the National Research Council.

One's food intake varies from day to day depending on his own habit. Sunday is made a high calorie day Sunday s consumption is 36% above the *average for the six days*

Women have a basal metabolic rate 7% 10% lower than that of men, and are smaller in size so that their average caloric requirements are less In a well-to-do home, the adults are not engaged in manual labour and may require less food than adolescents.

The average supplement for muscular work is 600 calories. The nervous and endocrine ' make-up ' or habits of an individual may alter his requirements No certain evidence is yet known that mental work exhibits any significant increase in metabolism

Exercise

1 Define B M R. and describe a method for measuring it. Discuss its significance (R. U 67 , 70A , Luck. 70A)

2. What is B M R ? Discuss the factors affecting the B M R. (M U 73A)

3 What are the methods of determining energy requirement of man ? (Luck. 68A)

4 Write short notes on

(a) B M R. (P U 73A 75A, 76A , M U 72A , Mith 71A R. U 76A)

(b) R. Q (R U 72A)

(c) S D A (P U 71A 74S , Luck 76S)

(d) Daily calorie requirements (Luck 72A , Punj 73S)

CHAPTER 52

PRINCIPLES OF NUTRITION

A correct diet must provide for the maintenance of the body as well as energy requirements, for growth and reproduction The essential elements lost by the body by excretion must be replaced The more important factors are :

1 Energy value
2 Quality and quantity of primary foods, minerals and vitamins
3 Variation in the diet
4 Digestibility
5 Cooking
6 Psychological factors
7 Cost

1. ENERGY VALUE

The average caloric requirement of the adult male is 3000 daily

The figures of the daily caloric requirement of York in 1900 are given below :

Work house	3,702 Calories
Prison (Class B)	3,038 ,,
Prison (hard labour)	4,159 ,,

2. QUALITY AND QUANTITY OF THE CONSTITUENTS OF FOOD

(a) *Primary foods* (Protein, fat and carbohydrate)

1 Protein, fat and carbohydrate are consumed in the ratio of 1 : 1 . 4 in this country

2 3 000 calories are provided by 100 grams of proteins, 100 grams of fat and 400 grams of carbohydrate.

3 Although the 1 1 4 ratio is prevalent in this country still wide variations of carbohydrate and fat may occur without harm

4 It is advisable that 10%-15% of the total calories should be obtained from protein, 20%-35% from fat and 50%-66% from carbohydrate

Proteins :

Although the expected amount of protein is 100 g per day, less amount can also maintain health and nitrogen equilibrium Even 1 g per kg. body weight may be ample Larger amounts are necessary for growth, pregnancy and lactation Increased amounts may be required in wasting diseases The protein requirements vary with the net protein utilization (NPU) of the dietary proteins If the NPU is low, the requirements are high and if the NPU is high, the requirements are low

The requirement of protein in the diet is not only quantitative , the qualitative aspect is also important since the metabolism of protein is connected with that of its constituent amino acids Certain amino acids are called *'essential'* because they must be obtained preformed and cannot be synthesized by the animal organism The remainder, the so-called *non-essential*, are also required by the

organism since they are found in the protein of the tissues, but they can be synthesized from α keto acids by amination Histidine in the diet is also necessary to maintain growth during childhood. Such amino acids are said to be *relatively essential* The nutritive value of a protein depends on its content of essential amino acids

Some proteins, if they are the sole source of nitrogen, will not support life, whereas others are sufficient. Hence, proteins are of two types—*First class* or *Good* protein and *second class* or *poor* protein First class protein can support life in the absence of other forms of protein. Proteins of animal origin (meat, milk and eggs) are almost completely utilized if taken alone , whereas, 10% to 40% of vegetable proteins (peas, beans, potatoes) may remain unabsorbed and excreted in the feces The absorption of vegetable proteins is improved if taken with other foodstuffs Animal proteins are associated with fat, but very little carbohydrate (except milk) whereas vegetable proteins are almost associated with a large amount of carbohydrate

It has been observed that the dietary requirement for protein is influenced markedly by the level in the diet of fat and carbohydrate These latter foodstuffs have a *Protein sparing* ' effect. Fat primarily, functions as a fuel (9 Cal./g), carbohydrate also serves as a fuel (4 Cal /g.), but is required for the synthesis of certain catalytic compounds of metabolic cycles (e g. oxaloacetate in the TCA cycle) and provides the carbon skeletons for the synthesis of the nonessential amino acids At least 5 g of carbohydrate per 100 calories must be supplied if nitrogen equilibrium is to be maintained Protein has a more catalytic function in the form of enzymes In the absence of fat and carbohydrate from the diet, protein is degraded to provide fuel (4 Cal /g) and catalytic compounds of metabolic cycles Essential amino acids may be broken down to supply the materials for the synthesis of nonessential amino acids For these increased burdens, the protein intake is ultimately increased The protein requirement is decreased when fats and carbohydrates are taken alongwith

Proteins differ in "*biologic value*" depending on their content of essential amino acids The proteins of eggs, dairy products, kidney and liver have high biologic value since they contain all of the essential amino acids Good quality proteins include soyabeans, peanuts, potatoes and the muscle tissue of meats, poultry and fish Fair proteins are cereals and most root vegetables Poor biologic value includes nuts and legumes The mixture of poor and fair can provide good biologic value

Proteins are also the important sources of nitrogen, sulphur, and phosphorus for the body Many amino acids have specific functions in metabolism such as methionine as a methyl donor, cystine as a source of —SH groups, the dicarboxylic acids (Aspartic acid, glutamic acid) in transamination, tryptophan as precursor of niacin, arginine in the urea cycle etc

The *protective* substances in a diet are those which are essential for specific purposes other than energy production, i e first class proteins mineral elements, and vitamins

Fats ·

Fat has high fuel value It increases the palatability of foods. It has got high capacity to be stored as energy in the body Fat provides the essential fatty acids such as linoleic, linolenic and arachidonic acids Fats are essential for the absorption of fat soluble vitamins Excess of saturated fat and cholesterol consumption is associated with hypercholesterolemia and atherosclerosis A lack of fat causes a feeling of hunger shortly after a meal due to rapid digestion in its absence Fat reduces the bulk of the food

The consumption of fat usually is over 80 g per day It may increase upto 150 g with an increase of family income

As a source of energy there is little difference between animal and vegetable fats Animal fats have a better biologic value because they contain vitamins A and D in particular Animal fats are most used when the incidence of sunlight is low

Fats

Fats provide fat soluble vitamins and the essential fatty acid *linoleic acid* which is required for the synthesis of arachidonic acid from which prostaglandins are synthesized in the body The major symptoms of essential fatty acid deficiency are a scaly dermatitis, hair loss, and poor wound healing Linoleic acid is widely distributed in the lipid portion of both plant and animal foods, vegetable seed oils are especially rich sources

In societies where fat is the principal food for energy intake, the population tends to develop coronary heart disease, obesity and cancer of the bowel and breast

Blood cholesterol level can be reduced significantly by changing the diet containing less saturated fatty acids or cholesterol The serum cholesterol level is the major factor for the development of atherosclerosis

Carbohydrates

Carbohydrate is the cheapest source of food It can be readily digested absorbed and utilized for producing energy It is the most efficien source of energy for vital processes It can furnish 60 80% of the total calorie intake in the scarcity of proteins and fats It is bulky and liable to undergo fermentation producing acid (e g laetic acid) if digestion is delayed Hence it is not wise entirely to replace fat by carbohydrate

In gluconeogenesis carbohydrate can be supplied from most amino acids as well as from the glycerol moiety of fats A minimum of 5 g of carbohydrate per 100 K Cal of total diet is necessary to prevent the development of ketosis Sufficient carbohydrate should be included in the diet to have complete oxidation of fat The amount is near about 400 g per day

The chief carbohydrates are starch and sugars (from milk, fruits and vegetables as well as sucrose) Glycogen is only consumed in living tissues such as oysters Carbohydrates are almost entirely derived from vegetable sources. Cellulose stimulates peristalsis Some cereals e g oatmeal contain much phytin as to interfere with the absorption of calcium

Galactose is synthesized in the body and lactose is also synthesized from galactose in the body Hence no need of giving lactose to lactating women Pentoses are absorbed and excreted unchanged except ribose or deoxyribose

Sugar is valuable for muscular work in minimizing fatigue

Sucrose (common table sugar) in one of the major etiologic factors in dental caries This sugar is not the cause of diabetes, heart disease, or obesity

Within this century, decrease in fiber consumption in industrialized countries has been accompanied by a high incidence of diverticulosis, colon cancer, cardiovascular disease, and diabetes. Large amounts of dietory fiber decrease bowel transit time, alter the composition of intestinal bacteria, reduce enterohepatic circulation of cholesterol, delay intestinal sugar absorption, and reduce the absorption of certain minerals.

(b) Minerals :

Some minerals are always required since small amounts of inorganic salts are always excreted. Fruits, vegetables and cereals are the chief sources of the mineral elements in the diet. Milk products supply the majority of the calcium and phosphorus in the diet.

Calcium :

1. Increased amounts of calcium are required by children, pregnant and lactating women.

2. Children whose normal intake is low, can absorb a higher percentage of calcium ingested than those with a high intake.

3. In many eastern countries where little milk is taken the average consumption by adults is about 400 mg. daily but this low intake does not show any signs of calcium deficiency.

4. The average intake should be about 800 mg. per day.

5. The best sources are cheese and milk. Other sources are eggs, green vegetables, oranges, nuts, buttermilk, beans and carrots. Meat, fish and fruits are poor sources of calcium.

Hard water is a source of calcium.

Phosphorus :

1 This is taken as organic and inorganic form.

2. Children should intake 1 g. p. per day, adults 1.3 g. and a pregnant or lactating woman 1.9 g.

3. The best sources of phosphorus are animal foods such as meat, fish, milk, cheese and eggs. Considerable proportions of the total phosphorus are provided by the vegetable foods such as beans, rye, oatmeal and lentils. Cereals provides phosphorus in the form of phytic acid which interferes with the absorption of calcium. Hence, animal sources of phosphorus are better.

Iron :

1. It is an important constituent of the diet and its deficiency leads to anemia.

2. The more concentration of iron is from bread, meat and potatoes. Good sources are liver, kidney, egg yolk, green peas, cabbage, carrots and cereals.

3. Only less than 10% of iron is absorbed. In iron-deficient subjects about 20% may be absorbed.

4. Milk is very poor in iron. The infant is born with a store of iron which maintains for six months. After this period, iron is given along with food. Human milk contains 1-2 mg. iron per liter, cow's milk less.

5 Children upto 12 years require 15 to 20 mg daily, Adult requires 40 mg daily aod the daily requirements during pregnancy aod lactatioo are 50 mg

Iodine :

1 The daily requirement of *iodine is 0 05 mg* and Iodine is obtained from water, vegetables and fisb

2 In certain localities where the soil and water lack this element, simple goitre results due to dietary deficiency

3 Fish, cod liver oil and vegetables can supplement iodine-deficient diet

Magnesium :

1 The average daily consumption of about 0 2 g is sufficient

2 *Good sources are meat,* green vegetables and bread

Copper :

1 The daily adult requirement is 2 mg

2 Good sources are liver, oysters, eocoa, nuts etc

3 Human and cow's milk ooly contain about 0 6 mg per liter

Sodium Chloride :

1 Ordinarily 20 g of the salt is consumed per day

2 The intake of the salt is only required if aboormal quantities are lost in sweat during severe muscular exercise at high temperature

3 *Heat* stroke is preveoted by iogestion of salt *solutioos* iostead of water

Vitamins :

1 The chemistry and physiologic functions are already discussed in vitamin chapter 13 Normal persons get sufficient vitamins in their diets and hence, supplementation with vitamin is not required Only in disease state in which digestion and absorption are impaired, the supplementation with vitamin is most essential

2 Many of the vitamins are destroyed by cooking and storage Hence, fresh fruits and vegetables should be takeo daily

3 Cereals when refined loose B vitamins Therefore, food is improved by the addition of vitamins

4 The daily requirements of the vitamins are also discussed in chapter 13

5 Deficiency symptoms of water-soluble vitamin consist of dermatitis, anemia, digestive difficulties, and neurologic disorders

6 Most of the water-soluble vitamins act as coenzymes in metabolism of substrates

7 Vitamin B_{12} is synthesized by *microorganisms* It is present only in meat and dairy foods Therefore, strict vegetarians may be at risk for vitamin B_{12} deficiency

8 The fat soluble vitamins are mainly present in fatty meats, liver, dairy fats, egg-yolks, vegetable seed oil and leafy green vegetables

9 Fat-soluble hypervitaminosis produces *symptom of toxicity*

10 Deficiencies of fat-soluble vitamins occur primarily in young children who lack adequate body stores Deficiencies are rare in adults unless there is malabsorption, biliary obstruction, or other conditions that affect fat metabolism

Water :

Although water is not a food, it is ordinarily consumed in the diet Hence, it is one of the components of food. The water requirements and functions are discussed in chapter 19

3 VARIATION IN THE DIET

1 There is a risk of missing some essential element or vitamin in a varied diet.

2. Eskimos live mainly on fish and meat but the poor orientals chiefly live on rice with small amounts of fish.

4 DIGESTIBILITY OF THE FOOD

1 The food is of no use if it is not digested in the alimentary canal

2 Digestibility is more concerned with absorbability

3 When fats and starch are largely used, vegetable and animal proteins are not absorbed.

4 Absorption is more enhanced with a mixed diet than the substance taken alone

5 COOKING

1 In cooking food is considerably changed. Harmful organisms are destroyed.

2 Cooking breaks down the connective fibres of meat and makes meat easier to masticate and helps digestion. Overcooking shrinks the coagulated protein and decreases the digestibility

3 Cooking increases the water content and digestibility of vegetables Cellulose framework is loosened and starch from starch grains is liberated.

4 Fats are little changed in cooking. Cooking enhances the flavour of the food.

5 When vegetables are cooked, vitamins B_1 and C are destroyed.

6 PSYCHOLOGICAL FACTORS

1 Appetite is reduced by worry and anxiety and digestion is also upset due to imperfect mastication and secretion of digestive juices.

2. Consumption of food is increased while taken in pleasant surroundings and good company with different items

7. COST

1 Dietary food is much influenced by family income.

2 When the income is good, consumption is high with all the protective foods A poor income has poor protective foods

3 The lowest income groups having the low protective foods suffer from rickets and nutritional anemia. The lower groups are less resistant to infectious diseases such as whooping-cough, measles, diphtheria and tuberculosis

BIOLOGIC VALUE OF PROTEINS

The biologic value of a protein is an expression of a number of the nutritional characteristics of the substance Among these are

1 The digestibility

2 The availability of the digested products

3 The presence and amounts of the various essential amino acids

1 The digestibility of proteins It is expressed as the percentage of the food nitrogen which has been absorbed

Absorbed N—Food N—(fecal N—metabolic N)

Proteins of animal origin have the highest digestibility (95 to 100 per cent) with the wastage in digestion only 5% or less The less the digestibility, the less the biologic value

2 The availability of the digested products The greater the proportion of amino acids in the dietary protein which can serve for the construction of tissue protein the greater will be its potential nutritive value A smaller proportion of the amino acids will then be discarded

3 The presence and amounts of the various essential amino acids

An essential amino acid is defined as one that cannot be synthesized by the animal organism at a speed commensurate with the demands for normal growth and which must therefore be supplied in the diet There are eight essential amino acids for man These are methionine threonine tryptophan, valine, leucine isoleucine phenylalanine and lysine

Zein is almost completely free from tryptophan and lysine Growth is continued if zein is given as diet alone with tryptophan and lysine

Gelatin is also lacking tryptophan Casein lacks methionine Normal growth will continue if these amino acids are given along with these diets

A biologically good protein is one which contains all the essential amino acids in proportions not far different from those needed by the body A much smaller intake of such a protein is required to produce nitrogen equilibrium than that of a protein which has a limited amount of one or more of the essential amino acids The most suitable proteins for growth as those of animal origin and especially those which nature has provided for the nourishment of the growing animals namely

Lactalbumin (of milk), ovalbumin (of hen's egg) ovovitellin (of hen's egg)

These support growth when given at a level of about 9 or 10 per cent in the diet

Proteins in the order of biologic value

Proteins of meat glutenin (wheat) casein (milk) glutelin (maize) glycinin (soyabean)

These support growth when given in a higher concentration

The following vegetable proteins are incapable of supporting growth but are suitable for maintenance

Gliadin (wheat) legumin (Pea) legumelin (soyabean) hordein (barley)

The following incomplete proteins are unsuitable for either growth or maintenance

Zein, gelatin

BALANCED DIET

Definition :

A balanced diet is one which contains all the food constituents in proper proportions to meet the energy and nutritional requirements of the individual

The proportions of protein, fat and carbohydrate should approximately be 1 1 4 respectively The balanced diet for an adult male of 70 kg. requiring 3000 calories per day should contain the following :

Protein	70 gms
Fats	50 gms.
Carbohydrate	440 gms
Calcium	0 8 gms
Phosphorus	1 4 gms
Iron	40 mg.
Vitamin A	7,300 I U
Vitamin B_1	1 8 mg
Vitamin C	200 mg

BALANCED DIETS FOR ADOLESCENT BOYS AND GIRLS*

Dietary articles	BOYS				GIRLS	
	13-15 years		16-18 years		13-18 years	
	Vegetarian (gm)	Non vegetarian (gm)	Vegetarian (gm)	Non-vegetarian (gm)	Vegetarian (gm)	Non-vegetarian (gm)
Cereals	430	430	450	450	350	350
Pulses	70	50	70	50	70	50
Green leafy vegetables	100	100	100	100	150	150
Other vegetables	75	75	75	75	75	75
Roots and tubers	75	75	100	100	75	75
Fruits	40	30	50	50	50	30
Milk	500	200	400	150	400	150
Fats and oils	35	40	45	50	35	40
Meat and fish	—	60	—	70	—	80
Egg	—	30	—	30	—	30
Sugar and jaggery	30	30	40	40	30	30
Ground nuts	—	—	50	50	—	—

*Adapted from the Report of the Nutrition Expert Group ICMR (1968)

BALANCED DIETS FOR CHILDREN*

Dietary articles	PRE-SCHOOL CHILDREN				SCHOOL CHILDREN			
	1-3 years		4-6 years		7-9 years		10-12 years	
	Vegetarian (gm)	Non-vegetarian (gm)	Vegetarian (gm)	Non-vegetarian (gm)	Vegetarian (gm)	Non-vegetarian (gm)	Vegetarian (gm)	Non-vegetarian (gm)
Cereals	150	150	200	200	250	250	320	320
Pulses	50	40	60	50	70	60	70	60
Green leafy vegetables	50	50	75	75	75	75	100	100
Other vegetables / Roots and tubers	30	30	50	50	50	50	75	75
Fruits	50	50	50	50	60	60	60	60
Milk	500	200	400	200	500	200	400	200
Fats and oils	20	20	25	25	30	30	35	35
Meat and fish / Egg	—	30	—	30	—	40	—	50
Sugar and jaggery	30	30	40	40	50	50	50	50

*Adapted from the Report of the Nutrition Expert Group, ICMR (1968)

BALANCED DIETS FOR ADULT WOMAN*

Dietary articles	Sedentary work		Moderate work		Heavy work		Additional allowing during	
	Vegetarian (gm)	Non-vegetarian (gm)	Vegetarian (gm)	Non-vegetarian (gm)	Vegetarian (gm)	Non-vegetarian (gm)	Pregnancy (gm)	Lactation (gm)
Cereals	300	300	350	350	475	475	50	100
Pulses	60	45	70	55	70	55	—	10
Green leafy vegetables	125	125	125	125	125	125	25	25
Other vegetables	75	75	75	75	100	100	—	—
Roots and tubers	50	50	75	75	100	100	—	—
Fruits	30	30	30	30	30	30	—	—
Milk	200	100	200	100	200	100	125	125
Fats and oils	35	30	40	35	50	45	—	15
Meat and fish	—	30	—	30	—	50	—	—
Egg	—	30	—	40	—	30	—	—
Sugar and jaggery	30	30	30	30	40	40	10	20
Ground nuts	—	—	—	—	40	40	—	—

*Adapted from the Report of the Nutrition Expert Group, ICMR (1968)

BALANCED DIETS FOR ADULT MAN*

Dietary articles	Sedentary work		Moderate work		Heavy work	
	Vegetarian (gm)	Non vegetarian (gm)	Vegetarian (gm)	Non vegetarian (gm)	Vegetarian (gm)	Non vegetarian (gm)
Cereals	400	400	475	475	650	650
Pulses	70	55	80	65	80	65
Green leafy vegetables	100	100	125	125	125	125
Other vegetables	75	75	75	75	100	100
Roots and tubers	75	75	100	100	100	100
Fruits	30	30	30	30	30	30
Milk	300	100	300	100	300	100
Fats and oils	40	35	45	40	50	45
Meat and fish	—	30	—	50	—	60
Egg	—	30	—	30	—	30
Sugar and jaggery	30	30	40	40	55	55
Ground nuts	—	—	—	—	50	50

*Adapted from the Report of the Nutrition Expert Group, ICMR (1968)

NUTRITIVE VALUE OF FOODS

Milk :

1 Milk is nearest to a perfect and complete food

2 It contains not only protein, fat and carbohydrate but also minerals and vitamins

3 The chief proteins are caseinogen and lactalbumin Caseinogen is a phosphoprotein Caseinogen is associated with calcium as calcium caseinogenate Small amounts of other proteins are also present

4 Milk fat is in the form of a very fine emulsion. It contains all saturated fatty acids as well as unsaturated fatty acids

5 The carbohydrate of milk is lactose which is less sweet than sucrose.

6 It is rich in calcium, potassium, sodium, chlorine and phosphorus The iron content is very low which is insufficient for baby

7 It is rich in vitamin A and vitamin B_2 and good in vitamin B_1 and nicotinic acid but poor in C and D

8 Milk kept on unsterilised becomes sour due to formation of lactic acid from lactose by bacteria present in milk and this lactic acid is sufficient to precipitate (curdle) the caseinogen

9. When milk comes in contact with rennin or proteolytic enzymes, it becomes clotted

10 The proteins of milk neutralise gastric HCl to allow clotting

11 The percentage composition of milk of different species is given below

Species	*Protein*	*Fat*	*Carbohydrate*	*Ash*
Woman	1 0	2 9	6 7	0 2
Cow	3 0	3 6	4 8	0 7
Buffalo	5 0	7 0	2 4	0 6
Goat	4 3	4 8	4 7	0 8

Since iron is less in the milk it is added to the milk

12 *Condensed milk* is prepared by the removal of water by evaporation in vacuo Vitamins A and B are preserved *Dried milk* is prepared by passing a film of milk over hot rollers or spraying milk into hot air to evaporate water

Eggs :

1 The hen's egg contains about 30% yolk, 59% white and 11% shell The chemical composition of duck s egg is similar to that of hen s egg

2 The *white* contains proteins and salts The greater part of the protein is *ovalbumin* The other proteins are *conalbumin*, *ovoglobulin* and *ovomucoid* (a glycoprotein) The pale yellow colour is due to riboflavin The white contains 10 7% protein, 0 1% fat and 1 4% carbohydrate

3 The *yolk* contains 51% water, 15% protein 33% fat of which 4% cholesterol, and 1% mineral The proteins are *vitellin* (a phosphoprotein) and *livetin* (a globulin) The minerals are calcium iron and phosphate The yolk is rich in vitamins A, B_1, B_2 and D but not C Tbe other B group vitamins and E are also present

Meat :

1 Meat contains about 22% protein and B group vitamins but does not contain vitamins A, C or D

2 Nearly all the carbohydrates disappear during hanging and lactic acid as well as *acid phosphates are formed* These acids convert collagen to gelatin when the meat is cooked In boiling there is the loss of salts, gelatin and extractives During roasting this loss is reduced

3 Tbe flavour of meat is due to the organic substances extracted from meat by boiling water

Fish :

1 Fish is free from carbohydrate just like meat The fat content ranges from a trace to 5% It is a fair source of B group vitamins Fatty fish contains some vitamins A and D

2 Large fish is rich in phosphorus but deficient in calcium Small fish eaten with bones are good sources of calcium

3 It contains 22% protein 1 4% fat, 75% water The dried fish may contain about 5% carbohydrate because of the use of flour

VEGETABLE FOODS

A Cereals :

1 The crude cereals (wheat, rice, barley, rye, maize, oats) contain 11% protein, 70% carbohydrate, 0 5 to 8% fat, 11% water and 2% minerals Oatmeal is the richest in protein and fat, rice is the poorest

2 The main *proteins* of cereals are glutelins and gliadins Small amounts of albumins and globulins are generally present

3 The *fats* contain considerable amounts of olein to make them liquid at ordinary temperatures

4 The *carbohydrate* is almost completely stareh in the form of grains covered by a thin membrane of cellulose Small amounts of sugar are also ofte i present in cereals

5 The abundant mineral elements are calcium and phosphate The phosphate is partly in the form of phytic acid (inositol hexaphosphate) wh ch interfers the absorption of calcium Some cereals (wheat and rye) contain the enzyme phytase which hydrolyzes phytic acid and reduces the anticalcifying effect

6 There is a great loss of vitamins B and E, and mineral elements, especially Ca, P and Fe in the roller milled white flour Carotene and riboflavin are lost by chemical blenching

B Pulses :

1 The dried pulses (Peas, beans, lentils etc), contain large amounts of protein The main protein is a globulin called *legumin* The dried pulses contain 20—25% protein, 11% water, less than 2% fat

2 The soyabean contains high protein high fat and low carbohydrate Soyabean oil is used for frying 'fish and chips'

3 The dried pulses are the good sources of many B group vitamins and minerals but deficient in vitamins A, D, B_{12} and C.

C. Nuts :

1 Nuts contain high protein and fat but low carbobydrate The protein content is slightly lower than that of dried pulses

2 They can be used for the preparation of milk substances which can be fed to infants over 6 months of age and to young children of some areas where milk is not available in sufficient amounts

D Roots and Tubers :

1 The most important tuber used in this country is the potato, sweet potato These are the chief sources of starch These can substitute sugar and cereals and are the valuable sources of iron and vitamin C New potatoes contain about 28 mg vit C per 100 g but on storage 5 mg vit C per 100 g is reduced

2 The common roots (carrots, beetroots turnips) are almost free from starch Their caloric value is completely due to sugars (sucrose, fructose and glucose) Carrots, the richest in sugars, are the sources of carotene Roots contain valuable salts but negligible protein and fat Arrowroot contains traces of protein and salts

E. Green Leafy Vegetables :

1 Green leafy vegetables are rich in Carotenes

2 They are good sources of calcium, riboflavin, folic acid and vitamin C

3 They are the cheapest among the protective foods

4 Yellow pumpkin is a fair source of carotene

F. Fruits :

1 Fresh fruits are the protective foods Their energy value is due to sugars and starch The protein and fat are usually less than 0 5% Many fruits contain pentoses and pectins

2 Tartaric acid, malic acid and citric acid are present in fruits

3 Cooking destroys vitamin C and makes fruits more digestible by softening the cellulose

4 Some fruits, notably the *banana* contains starch as well as sugar It has high protein content

5 The carbohydrate of dates is all sugar, not starch The protein content is low (2%)

G Tea, Coffee, Cocoa :

1 The black tea sold consists of the leaves of young shoots of the tea plant which are fermented and dried by heat It has negligible caloric value It has a stimulant and a diuretic effect Strong tea disturbs gastric digestion due to tannic acid

2 Coffee is the roasted seed of coffea arabica The odour is due to an oil, caffeol formed when the beans are roasted It has little caloric value It contains caffeine and tannic acid It can not be regarded as food unless taken with milk and sugar

3 The seeds are obtained from the pods of the cacao tree after fermentation and roasting They contain 50% fat Cocoa as beverage is of little importance It becomes nourishing when taken with milk and sugar *Chocolate* consists of ground cocoa nibs mixed with sugar Starch and flavouring are frequently added

ALCOHOL

1 Alcohol has an energy value of 7 calories per gram Hence, it has been admitted as food Its consumption is restricted due to its pharmacological action. It is consumed in the form of beers, wines, spirits or liquors

2 Beers are the fermented product of malt and contain 4-8% alcohol. It has small amount of protein and some sugar.

3 Wines are the products of fermentation of fruit juices (usually grapes). The alcohol content is 10-20% and sugar 0 1-4% They contain large amount of organic acids (tartaric, malic, succinic etc)

4 Spirits are the distillation product of many fermented products Whisky is a distilled beer and brandy is a distilled wine They are practically free from sugar The alcoholic content is 30 50%

5 Liquors are alcohol sweetened with canesugar and flavoured with essences The sugar content is 30% and alcohol is 35-55%

Principles in planning a balanced diet :

In planning a balanced diet it is to be aimed that the diet must contain various groups of foodstuffs such as energy yielding foods, body building foods and pro

tective fnnds in the correct prnportions The constituents nf balanced diet differ according tn age, sex, physical activity, economic status and the physiological condition

A. Energy yielding foods :

1 This gronp contains high carhnhydrates and also pure fats and carbohydrates They are divided into two groups · (a) cereals, roots and tubers and (b) pure carbohydrates and fats

2 Cereals provide proteins, certain minerals and vitamins in addition to energy in the diets nf the low income groups

3 Roots and tubers provide some amounts nf proteins, minerals and vitamins

4 Pure carhnhydrates and fats provide only energy

B Body building fonds :

These contain high prntein and are divided into two groups : (a) Milk, egg, meat and fish. (b) Pulses, oilseeds and nuts

C. Prntective foods :

The protective foods are rich in proteins, vitamins and minerals. These are classified into two groups

(a) Fnods rich in vitamins, minerals and prnteins nf high biologic value e g. Milk, eggs, fish and liver

(b) Foods rich in certain vitamins and minerals only e g. Green leafy vegetahles and some fruits

Balaneed diets at high cost Large amounts nf costly fnods snch as milk, eggs, meat, fish and fruits and moderate quantities of cereals, pulses, nnts and fats

Bxlanced diets at moderate cost · Moderate amnnnts nf milk, eggs, meat, fish, fruits, fats and large amounts nf cereals, pulses, nuts and green leafy vegetahles.

Balanced diets at low cost Small amounts nf milk, eggs, meat, fish, fats and large amounts of cereals, pulses, nuts and green leafy vegetables

Food Toxins and Additives

1 A good number of potentially harmful compounds are present in foods. Some occur naturally They include harmful substances such as the neurotoxins from shellfish or mushrooms, goitrogens from plants of the cabbage family, bean compounds that interfere with collagen formation.

2 Pesticides and packaging materials are added in food through inadvertent contamination.

3 Many compounds are added to foods in order in preserve them or to add colour, flavour or texture Some of the additives may be harmful

4 Consumption of fresh and unprocessed food are advised in order in minimize intake nf natural toxins and additives

Primary nutritional diseases

1 STARVATION AND ANOREXIA NERVOSA

STARVATION

(a) **Hormone production:**

(i) There is impaired secretion of Pituitary Gonadotrophins and Testosterone as well as 17-oxosteroid concentration in plasma falls

(ii) Plasma Insulin level is reduced because the stimulating substances Glucose and amino acids are not absorbed during fasting

(iii) The secretion of Pituitary growth hormone tends to be increased and this favours fat mobilisation

(iv) In prolonged starvation, the concentration of 3, 5, 3'-*triiodo thyronine* falls which is responsible for the reduced metabolism

(b) **Clinical Features:**

(i) The patients are thin having loss of weight

(ii) The hair is dry and lustreless

(iii) The eyes are dull and sunken

(iv) The skin is thin, dry, and inelastic

(v) Dirty brown splotches of pigmentation may appear over the face and trunk

(vi) Polyuria at night is a frequent troublesome symptom

(vii) Oedema first starts in the face when lying down and the ankle oedema is found when the patient gets up and walks about

(viii) The blood pressure is low, the diastolic pressure may be impossible to estimate, while the systolic pressure may be as low as 70 mm Hg In severe cases the pulse rate is often below 40/min

(c) **Psychological disturbances:**

(i) The mind is never fixed for long on single subject except the desire for food Existence of mental restlessness

(ii) The patient becomes self-centred and indifferent to the troubles of others

(iii) The patient is worried and sensitive to noise and other petty irritations which may make him quarrelsome

(iv) In the last stages of starvation, the personality may completely disintegrate A mother may even steal from her child

(d) **Infections:**

(i) Starvation patients often suffer from infections—malaria, cholera, typhus, Pneumonia, Gastroenteritis

(ii) *Cancrum oris*, an infective gangrene of the mouth eroding the lip and cheeks, is a dreadful catastrophe which occasionally occurs in famines both among children and adults

(iii) The atrophied intestinal glands and the paper-thin walls of the digestive tract are unable to digest and absorb properly even a bland diet

(iv) In almost all famines, there is outbreak of diarrhoea without bacteriological organisms

(e) Treatment

(i) Most famine victims, owing to alimentary dysfunction, cannot consume large quantities of food. The patient's desire for food is immense and no guide to his digestive capacities. Food intake should necessarily be limited.

(ii) The choice of food is vital. Many starving people in the prison camps of World War II died from diarrhoea and collapsed after they had been given bully beef, baked beans which they could not readily digest. Only bland foods can be tolerated by the thin walled intestines lacking essential digestive enzymes.

(iii) Frequent small feeds of skimmed milk, 100 ml or so at a time—as often as the patient is willing and able to take them—is a good way to avert death from starvation. This requires constant personal attention and nursing care.

(iv) A variety of mild flavouring essences may be useful to stimulate the appetite. Slightly sour foods are usually acceptable.

(v) Starving patients may tolerate moderate amounts of fat or edible oils, which provide a larger energy intake. There may be a temporary increase in oedema with refeeding, so the intake of salt should be restricted.

(vi) A time may come in severe starvation when the patient refuses all food. The outlook is then very grave. Nasogastric or parenteral feeding provides the only hope.

(f) Prognosis

(i) Most people with primary undernutrition recover rapidly, once they have a free access to food. Appetite may be sufficient. Over 5000 K. cal/day may be consumed with a weekly gain in weight of 1·5 to 2 kg.

(ii) In some patients, even after careful nursing and a good diet, low blood pressure and diarrhoea may persist. If, after one or two weeks, they show little improvement, this suggests strongly that irreversible changes in the myocardium or small intestine have developed and that the prognosis is poor. After any severe famine there are some who may linger on in this condition for many months if supported by good medical and nursing skill, death rather than recovery is the usual end.

ANOREXIA NERVOSA

This is a psychiatric disease arising from a refusal to eat which often leads to severe emaciation. Patients are usually middle class and often above average intelligence. The disease affects mainly the adolescent girls and young women, but cases are seen in older women and also in men. The patients come from homes where plenty of food is available and often some of the family are obese.

(a) Clinical Features

(i) The weight may be reduced to 30 Kg or less. Patients often deny all normal sense of fatigue.

(ii) The pulse is slow, the blood pressure, peripheral blood flow, and skin temperatures are all low.

(iii) There is usually no anemia, there may be fine downy hair. Amenorrhoea is a characteristic feature but secondary sexual characteristics are present.

(iv) Urinary excretion of gonadotrophins and oestrogens is diminished.

(v) Menstruation starts at puberty when weight rises above 47 kg. and stops when it falls below 47 kg. The plasma potassium may be abnormally low.

(vi) Anorexia is common in patients with an anxiety state, a depressive illness.

(b) **Treatment**

(i) The primary aim is to get the patient to eat Severe cases should be treated by a psychiatrist and under close supervision in hospital until a satisfactory weight has been achieved

(ii) Chlorpromazine in doses upto 150 to 200 mg three times daily makes the patient more amenable, counteracts vomiting and is claimed to increase appetite

(iii) Disturbed relationships within the family need attention Regular interview with relatives conducted by a psychiatric social worker over many months should reduce guilt, misunderstanding and intolerance

(c) **Prognosis**

(i) Restoration of weight and menstruation may occur within several months in favourable cases

(ii) 50 per cent may make a partial recovery continuing to restrict their diet and remaining abnormally thin There is a mortality of about 5 per cent from suicide

2. OBESITY

Is is the condition in which an excess of fat has accumulated In most cases it can be detected by visual inspection

(a) **Clinical features:**

(i) Some of the patients appear to be in good health and leading normal lives, they are likely to have a reduced exercise tolerance with shortness of breath on exertion and to be unduly fatigued by continuing physical activity This is due to the burden of the increased weight that they carry always and to reduced capacity of the circulatory and respiratory systems that work under handicaps imposed by masses of internal fat and fatty infiltration of muscle

(ii) They are also at increased risk when under anaesthesia, surgical operations are more diffieult and post operative complications are more likely

(iii) In obesity grade III, the everyday activities of the patients are seriously restricted by their enormous mass and they are likely to be suffering from Diabetes, hypertension, gall bladder diseases, fatty liver, gout, osteoarthritis, Hernias etc Life expectation is low They have serious psychological distrubances

(b) **Treatment**

(i) If a patient eats a diet providing 500—1000 K cal less than which is needed for the activities of daily life, then and only then the excess reserves of energy in adipose tissue be drawn upon and there will be loss of weight at the rate of 0 5—1 kg each week

(ii) Any curtailment of food intake is liable to reduce intake of essential nutrients All reducing regimes should include ample fruit and vegetables and preferably whole meal bread These should supply ample vitamins and minerals They also supply dietary fibre and this may help to make the inevitably small meals more satisfying It also prevents constipation and there is no need to take supplements of minerals and vitamins To prevent depletion a protein supplement should be given

(iii) Patients should appreciate that there are no slimming foods although advised in magazines and daily newspapers and by radio and television All foods are fattening if taken in excess Foods legitimately advertised as aiding in a slimming diet are modified forms of conventional foods and beverages usually with a lower energy density

(iv) Even if the exercise contributes only a little to a negative energy balance,

it benefits by promoting physical fitness. Exercise prevents atrophy of muscle. Insufficient use of the muscle of the trunk and limbs of the respiratory system and the myocardium increases the risk of patients to orthopaedic, respiratory and circulatory disorders

(v) Obese patients may be advised to walk, to climb stairs, to swim and to gardenings. They are positive and enjoyable.

(vi) Amphetamine and its derivatives have been much used as 'slimming agents' They are psychomotor stimulants and also have an anorectic action. They may also cause insomnia, irritability, increased heart rate, raised blood pressure and severe psychotic reaction. Serious withdrawal symptoms may occur on discontinuing the drug So amphetamine should not be prescribed.

Diethylpropion. phenmetrazine and fenfluramine are the drugs which have some chemical resemblance to amphetamine. They are also psychomotor drugs and they may be prescribed for cases refractory obesity for periods of upto six weeks After this period their appetite suppressing effect usually wears off. Patients with a history of depression or other psychological disturbance should not be treated by these drugs

Thyroxine stimulates metabolism and for this reason it has had an extensive trial in the treatment of obesity In euthyroid people thyroxine produces no increase in metabolism unless given in doses which cause tremor, diarrhoea, palpitation and tachycardia. Hence, the administration of thyroxine to obese euthyroid patients is not only useless but potentially dangerous

Methyl cellulose is indigestible and adds bulk to the diet. It has little effect in promoting weight loss However, it is quite harmless.

Sedatives and tranquillisers can play no part in the treatment of obesity but they may be useful for some obese patients who suffer from an anxiety state.

Diuretics are potentially dangerous and are of no value in promoting weight loss unless the patient has oedema

(vii) Diets providing only 500 Kcal daily or a period of total starvation are justifiable in some patients who are not responding well to less vigorous restrictions. In selected patients with no orthopaedic or cardiovascular complication, it is possible to increase physical activities. Patients have been kept for upto six weeks on diets providing only 400 Kcal, whilst they walk 10 miles daily Negative energy balances of upto 3000 Kcal/day and weight losses of upto 3 Kg a week followed. If dietary intake is below 1000 Kcal/day, multivitamin and mineral supplements may be necessary Excessive losses of potassium and nitrogen may occur and urinary output of these should be checked regularly

(viii) Surgical treatment to reduce food intake may be considered in patients in whom medical treatment has failed and whose life has been made miserable by severe obesity Various forms of jejunoileostomy which by creating a bypass of the small intestine, lead to malabsorption have been carried out. The operation is unphysiological. Complications with features of the malabsorption syndrome and the blind loop syndrome frequently arise.

Gastroplasty is an operation in which the stomach is reduced to a small reservior, about 60 ml. in capacity, in the fundus which drains through a narrow channel, about 12 mm. in diameter, along the greater curvature and into the duodenum. This is much more safe

Surgical removal of large masses of fat from the abdomen, thighs or arms is contraindicated. There may be appearance of irregular ugly lumps of fat at the operation sites.

MARASMUS

Marasmus is due to a continued restriction of both dietary energy and protein as well as other nutrients The marasmic form of the syndrome occurs in infants

under one year and more frequently in towns During the nineteeth century in the industrial towns in Europe and North America marasmus, resulting from the poor diets and numerous infections, took a toll of infant lives like many Asian, African and South American towns today It is due to rapid succession of pregnancies and early and abrupt weaning followed by dirty and unsound artificial feeding of the infants with very dilute milk products given in inadequate amounts to avoid expense Thus the diet is low in both energy and proteins Poor houses due to lack of equipments make the preparation of clean food almost impossible Repeated infections develop especially of the gastrointestinal tract They are deprived of mother's breast milk

Clinical Features:

(i) Diarrhoea is frequent Many infants are hungry, but some are anorexic

(ii) The child is wizened and shrunken and there is little or no subcutaneous fat

(iii) There is often dehydration The weight is much below the standard for age

(iv) The temperature may be subnormal

(v) If the disease is of long duration, the length of the child is also below the standard, but less so than the weight

(vi) There is usually watery diarrhoea with acid stools

(vii) The abdomen may be shrunken or distended with gas

(viii) Peristalsis may be easily visible because of the thinness of the abdominal wall

(ix) The muscles are weak and atrophic and this together with the lack of subcutaneous fat makes the limbs appear as skin and bone

(x) The skin and mucous membranes may be dry and atrophic.

(xi) Psychological disturbances, resulting from a lack of mother's love and care, can depress the appetite

KWASHIORKOR

Kwashiorkor is due to a quantitative and qualitative deficiency of protein, but in which energy intake may be adequate It is mainly a disease of rural areas occurring in the second year of life This disease occurs when the child is weaned into the traditional family diet, this may be low in protein because of poverty, insufficient land and poor agricultural practice There is no supplement of milk. The disease is frequently precipitated by outbreaks of febrile illnesses such as malaria, measles or gastroenteritis It arises as a result of poverty and ignorance Many mothers have received no satisfactory instruction in infant feeding although there is money to buy food In Africa, the disease is commoner

(a) **Clinical Features:**

(i) There are oedema, anorexia, diarrhoea and a generalised unhappiness or apathy An infection often precipitates at the onset for which the child is brought to the doctor

(ii) Failure of growth is an early sign Oedema is more marked in the lower limbs

(iii) The characteristic *dermatosis* consists of areas of both hypo- and hyperpigmentation. The skin first becomes thickened as if varnished. This then peels and appears like "flaky paint" leaving cracks or denuded areas of shallow ulceration In moderate cases, the dermatosis resembles crazy paving, when severe, the desquamated part looks as if there has been a burn The lower limbs, buttocks

and perineum are usually most affected but ulcers can occur over pressure points and deep cracks in skinfolds

(iv) The hair is sparse, soft and thin. Negro children lose their characteristic curl. There may be changes in pigmentation with diffuse patches or streaks which may be red or grey in colour

(v) Angular stomatitus, cheilosis and a smooth atrophic tongue are commonly seen, as in ulceration around the anus

(vi) Watery diarrhoea or large semi-solid, acid stools are usual. The liver can generally be palpated and is firm not tender

(vii) The muscles are always wasted and as a result many children may no longer be able to walk or crawl

(viii) Some degree of anemia is always present and may be severe

(ix) Apathy is a characteristic feature and the child appears constantly unhappy. Neurological features are unusual but some children during recovery have tremors resembling parkinsonism.

(x) Many patients show a mixture of some of the features of both marasmus and kwashiorkor. These children are said to have marasmic kwashiorkor

(xi) Some children adapt to prolonged insufficiency of food by a marked retardation of growth. They resemble children a year or more younger

(b) Biochemical and metabolic disorders

(i) A high body water content, loss of the fat stores, and loss of protein from the wasted muscles and other tissues greatly alter the chemical composition of the child.

(ii) Plasma concentrations of essential amino acids, especially branched chain amino acids and tyrosine are low, but those of some non-essential amino acids may be higher than normal. Sooner the treatment starts with protein, the concentrations of amino acids in the plasma rise and there may be an overflow aminoaciduria

The plasma albumin level is low owing to a failure of synthesis in the liver. In severe cases, it is usually below 20 and sometimes below 10 g/l. Plasma IgG is often raised if infections are present but other immunoglobulins are usually normal. Plasma transferrin is lowered, especially in severe cases, and may be a better guide to prognosis than plasma albumin.

The plasma concentrations of some enzymes such as cholinesterase, alkaline phosphatase, amylase, and lipase are lowered

The blood urea is usually low and may fall to 6 mg/100 ml. This indicates a reduced protein intake rather than a lowered rate of protein catabolism. Urinary creatinine is also reduced reflecting decreased muscle mass

(iii) There is fatty liver and the excess of fat in the liver is triglyceride. Plasma triglyceride and cholesterol are low due to a decreased ability of the liver cells to mobilise lipid in the form of lipoproteins.

(iv) Blood glucose is usually normal. Hypoglycemia may occur and is a complicated matter to be kept in mind

(v) Plasma potassium level is below normal due to diarrhoea. Plasma magnesium level is also low due to increased losses in the stool.

(vi) Plasma $[H^+]$ may be either raised or lowered. Acidosis is probably due to poor circulation and consequent tissue hypoxia. Alkalosis may be associated with potassium depletion and a failure of the kidneys to excrete bicarbonate

(vii) The total body water may increase from 60 per cent to 80 per cent. The severity of clinical oedema is not closely associated with the size of the increase in total body water or with the level of plasma albumin. The cause and nature

of the oedema still remains in part a mystery The oedema may shift from one part of the body to another Clinical dehydration and shock may be found in a child, associated with gross oedema in parts of the body

(viii) Malnourished children are likely to have infections and may have complications requiring drug treatment Some antibiotics and antimalarias act by interfering with nutrition more in the micro organism than in the human host, and they need to be used with care in Protein Energy Malnutrition (PEM) The antibiotics streptomycin, chloramphenicol and the tetracyclines inhibit protein synthesis by interfering with the action of messenger or transfer RNA The antimalarial trimethoprim is folate antagonist

Some drugs are carried in the circulation bound to plasma proteins Kwashiorkor plasma with a low albumin content has a reduced binding capacity with the drugs Higher concentrations of the free form of the drug increase the risk of toxic effects Many drugs are detoxicated in the liver by the microsomal enzyme oxidising system and its function may be impaired in PEM Therefore, all drugs should be used with caution

(c) Changes in the organs and systems of the body·

(i) The atrophy of the cells of the pancreas and intestinal mucosa cannot produce digestive enzymes in normal amounts Duodenal contents contain reduced amounts of amylase, trypsin, and lipase The activity of lactase, sucrase, and maltase are greatly reduced in the atrophic mucosa which is also associated with impaired absorption of nutrients

(ii) The fat first accumulates in small droplets within liver cells, situated at the periphery of the lobules The droplets increase in size and extend from the periphery to the centre of the lobules In severe cases, all the liver cells may be filled with big fat droplets, pushing aside the cell nucleus and reducing the cytoplasm to a narrow rim Yet the liver function is well maintained and severe liver failure is unusual Plasma bilirubin is normal, prothrombin concentrations are often reduced, but return to normal on treatment with vitamin K In case, the plasma concentrations of alanine amino transferase and isocitrate dehydrogenase are found raised, suggest the presence of damage from a bacterial or viral infection With proper treatment the lipid accumulated in the liver cells is all cleared with return to a normal structure

(iii) Plasma concentration of growth hormone may be raised as the pituitary responds effectively to the stimulus of protein depletion Plasma concentrations of cortisol and other adrenocortico steroids are normal or raised Plasma thyroxine is often low, but free T_4 is usually normal or raised

(iv) Atrophy of the heart leads to a reduced cardiac output and a poor circulation In many severe cases the extremities are cold and cyanosed and the pulse small or impalpable The electrocardiogram (ECG) shows low-voltage changes in the QRS complex and the T-wave may be depressed or inverted Some of the changes are rapidly reversed by potassium therapy

(v) There is no specific structural or functional abnormality of the kidneys The glomerular filtration rate may be low, but this is probably due to dehydration or reduced cardiac output The concentrating power of the kidneys is often poor, but this may be due to the depression of tubular function by electrolyte deficiencies They are all reversible by treatment

(vi) The immune responses of the body are produced by cells arising in the thymus, lymph nodes and spleen, the lymphoreticular organs These are very immature at birth and develop rapidly in the first two years of life In kwashiorkor, the thymus, tonsils, spleen, and other lymphoid tissues are atrophied These changes are accompanied by a delayed or absent tuberculin response and other skin hypersensitivity reactions, reduced complement activity in the serum, especially the C3 component, reduced numbers of thymus-dependent lymphocytes (T cells)

in the blood, reduced lymphocytes transformation in response to phytohaemoglutinin The bactericidal action of neutrophil leucocytes is also impaired These signs of reduced cell mediated immunity are in contrast to a usually unimpaired humoral immunity, the response to injected antigens is often normal and plasma IgG may be higher than normal

The depression of cell mediated immunity is mainly due to protein deficiency Once the immunological system has reached maturity it is much less susceptible to malnutrition

(d) **Treatment**

(i) Children who are seriously ill require treatment in hospital as their recovery depends on high standards of clinical skill and nursing care, with some laboratory support When the acute phase of the illness is over, they need several weeks of special feeding and some medical supervision before recovery is complete

(ii) Most children admitted to hospital have had repeated attacks of diarrhoea and often of vomiting and as a result are dehydrated Some of these respond to oral rehydration therapy but if the response is not prompt or the dehydration is severe, intravenous fluid should be given at once

(iii) A small infusion of plasma is beneficial when there is severe peripheral circulatory failure and of whole blood or red cells when there is severe anemia Under these conditions the myocardium may be damaged by hypoxia and there may be acute heart failure, infusion should be low and a fast diuretic (frusemide) given at the start

(iv) Many patients have malaria, pneumonia, dysentery and other infections and infections do not cause fever in the malnourished It is better to give a short course of procaine benzylpenicillin and ampicillin If ampicillin is not available, chloramphenicol and tetracycline may also be given

(v) Hypothermia is often present and needs urgent treatment. It is, therefore, wise to let their mothers sleep with them in hospital

(vi) The child should be given a dilute milk feed with added sugar from the first or second day Thus the strength can be increased and a vegetable oil added to give extra energy

(vii) Infants who are seriously ill will improve when given 1 gm protein per kg body weight daily Good recovery can be obtained with 2 gms and recovery is not accelerated by giving more than 3 5 gms

(viii) A high energy diet is required and this can only be made by including large amounts of fat Some infants can tolerate at first the amount of fat in whole cow s milk. Feeds therefore be made up from skimmed milk powder

(ix) As soon as children are able to take normal food and infection or other complications are controlled, they should be discharged to a centre where their nutritional rehabilitation can be supervised

(e) **Prognosis**

(i) A child may suffer for a short period from one of the forms of PEM and make a complete recovery If growth is retarded slightly, the child may reach the normal size for its age quickly, provided the dietary supply is satisfactory If growth is retarded for a long period the child may be stunted and develop into a small size but healthy adult

(ii) If the disease is so severe as to demand treatment in hospital the prognosis is uncertain and often bad

(iii) Children who survive severe PEM in early childhood perform less well in intelligence tests than controls A poor performance can be due to their growing up in an unfavourable psychological environment rather than to a short period of malnutrion in early life

(iv) It is proper to request government that failure to provide adequate nutritional services for mothers and young children may well lead to a school population with a diminished capacity for learning On the other hand, a mother whose child has suffered a period of severe malnutrition and made a good recovery may be reassured that subsequent mental development is not likely to be impaired seriously, if at all

GOITRE

The term "Goitre" is used to denote enlargement of the thyroid gland of whatever kind Simple Goitre is said to be present when the gland is visible and palpable, but the subject has no symptoms either of hypothyroidism or hyperthyroidism Such goitres do not usually affect health, but sometimes they may be complicated with serious consequences Environmental factors determine the prevalence of most simple goitres—especially dietary factors, of which iodine deficiency is the major one

(a) Clinical symptoms:

The classification of goitre is recommended as

Grade Oa	Thyroid not palpable or if palpable not larger than normal
Grade Ob	Thyroid distinctly palpable but usually not visible with the head in a normal or raised position, considered to be definitely larger than normal
Grade I	Thyroid easily palpable and visible with the head in either a normal or a raised position
Grade II	Thyroid easily visible with the head in a normal position
Grade III	Goitre visible at a distance
Grade IV	Monstrous Goitres

(b) Clinical effects and complications:

In the majority of cases of simple goitre there are not clinical manifestations due to hypofunction or hyperfunction of the thyroid gland Simple colloid goitre may require surgical treatment because of pressure effects on the adjacent structures The following complications occur rarely (i) hypothyroidism, (ii) hyperthyroidism, (iii) cretinism, (iv) deaf mutism Such complications are more likely encountered in regions where endemic goitre is prevalent

(c) Treatment:

(i) A simple goitre in a non-endemic area rarely requires treatment It happens so that in time it gets smaller without causing any harm If this does not occur and the goitre becomes disfiguring, iodine therapy is seldom effective, thyroxine 0 2 to 0 3 mg/day may be given This inhibits production of TSH by the pituitary gland and so reduces the size of the thyroid gland If there is no response to thyroxine and the goitre continues to be disfiguring, thyroidectomy should be considered, and this is indicated if the size of the goitre leads to obstruction of the trachea

(ii) Cretinism may affect up to 5 per cent of the population in places where endemic goitre is severe There are two types endemic cretinism In *nervous cretinism* there is mental deficiency, deaf mutism, spasticity and ataxia but features of hypothyroidism are rare In *myxoedematous cretinism* there is dwarfism, signs of myxoedema and no goitre Nervous cretinism can be prevented by giving a single injection of iodised poppy-seed oil to the women of childbearing age but it must be given before pregnancy starts It thus appears that iodine is required for the early development of the nervous system before the fetal thyroid appears in the third month of gestation In myxoedematous cretins the nervous system develops

normally in the critical early months but the thyroid gland fails to adapt adequately by hypertrophy to severe iodine deficiency The thyroid glands are small and uptake of radioiodine very low Consequently, such cretins have low plasma T_3 and T_4 with clinical signs of hypothyroidism, including dwarfism Both types of cretinism are seen in endemic areas but the proportion of the two varies from region to region

(d) **Prevention**

(i) *Iodisation of table salt* greatly reduce the prevalance of goitre in many countries The iodised salt must contain 76 kg of iodine/g salt and daily consumption of sal is between 2 and 6 g Lower levels of iodisation are used in most other countries and are effective. Potassium iodide is used for the iodisation of the crude moist salt consumed in many countries

(ii) Where goitre is endemic daily intakes of dietary iodine are likely to be less than 50 μg This should be increased to the normal range of from 100 to 300 μg The amount of iodine added to the salt should be related to the usual consumption of salt in the community so that individual iodine intakes fall within this range Iodine given for medical purposes may cause allergic skin rashes and induce hyperthyroidism, but the risk of these adverse effects from iodised salt is minimal

(iii) *Iodised oil injections* provide an alternative means of prevention in parts of the world where the use of iodised salt is impossible and where endemic goitre is most severe and accompanied by cretinism. Goitres are not merely a cosmetic problem, but impair the vitality of many of the people by causing hypothyroidism, and cretinism retards the intellectual development of children A single injection has been found to correct the deficiency for a period of two to three years Iodised oil has now been used successfully in many countries

(iv) *Iodisation of the water supply* in a remote village, where distribution of iodised salt and injection of iodised oil are impractical, reduce the prevalance of goitre Iodinators consisting of cannisters containing iodine crystals are connected to main water pipes and a fraction of the water diverted through them.

XEROPHTHALMIA

Xerophthalmia (Greek xeros, dry, ophthalmos, eye) is a condition caused by vitamin A deficiency In its milk form it is confined to the conjunctiva which is very common in many countries There is danger of corneal ulceration when it spreads to the cornea and a permanent defect in vision In severe cases there is softening of the cornea, keratomalacia, which, if not immediately treated, soon leads to permanent blindness Keratomalacia is associated in young children with protein-energy malnutrition

Xerophthalmia arises when the diet contains practically no whole milk and butter and very limited amounts of fresh vegetables and fruit and so lacks both retinol and carotenes Xerophthalmia and keratomalacia both occur in the first year of life amongst artificially fed infants but are rare amongst the breast fed. Children in poorly nourished communities are born to mothers who have had small intakes of vitamin A and consequently their liver stores are small at birth

(a) **Clinical Features**

(i) The bulbar conjunctiva is dry, thickened, wrinkled and pigmented, due to a failure to shed the epithalial cells, and consequent keratinisation The pigmentation gives the conjunctiva a peculiar 'smoky' appearance. The pigment is diffuse and especially marked in the interpalpebral fissure Dryness, thickening and pigmentation, characteristic of the condition, are also caused by long periods of exposure to glare, dust and infections This is common in older children and adults in the tropics

(ii) When dryness spreads to the cornea, this takes on a dull, hazy, lacklustre appearance This is due to the keratinisation which is the results of Vitamin A deficiency on all epithalial surface The cornea often becomes insensitive to touch with a wisp of cotton wool Corneal ulceration may occur from many causes and be unrelated to Vitamin A deficiency The characteristic feature is a loss of substance (erosion) of a part or the whole of the corneal thickness Unless there is secondary infection, there are no signs of inflammation The lesion only heals by scarring Corneal xerosis may progress suddenly and rapidly to keratomalacia

(iii) Softening and dissolution of the cornea follow and are known as colliquative necrosis This presents a grave emergency When the process involves only part of the cornea, there is ulceration but the inflammatory reaction is mild If the process is not stopped by treatment, perforation of the cornrea leads to prolapse of the iris, extrusion of the lens and infection of the whole eyeball The chances of saving any useful vision are slight Healing results in scarring of the whole eye and frequently in total blindness The retinol content of the plasma is below 200 $\mu g/l$, the lower limit of the range

(iv) Night blindness is an early symptom of Vitamin A deficiency and is often present without any signs of xerophthalmia

(v) The prolonged Vitamin A deficiency in school children or young adults may show lesions appearing as spots, either white or yellow, scattered along the sides of the blood vessels The spots may fuse and the lesions are most numerous on the periphery of the fundus and never appear on the macula

(vi) Corneal scars are white, opaque patches on the cornea and the result of healing of an older ulcer Vision may be seriously affected, depending on the size of the scars There are other causes of corneal scars but Vitamin A deficiency is the most effective

(b) Treatment:

(i) Vitamin A in a dise of 30 mg of retinol (100,000 I U) should be administered daily for three days immediately the diagnosis is made or strongly suspected It is recommended that half the dose should be given orally in the form of halibut oil and half intramuscularly as water-miscible retinol palmitate An oil solution should not be injected as the retinol is then absorbed very slowly from the injection site

(ii) Cod liver should not be instilled directly into the eye During convalescence 9 mg of retinol in the form of a fish liver oil orally is adequate It is also most essential that the diet is satisfactory in regard to other nutrients

(iii) Secondary bacterial infection should be treated by the use of antibiotics which are of great value Local treatment of the eye will only be required provided the diagnosis is present

(c) Prevention:

(i) Most cases occur in urban poor and rural peasants in under doctored areas, and keratomalacia develops without the children being seen by a doctor Therefore to prevent this, doctors, nurses and midwives, and other paramedical staffs associated with maternal and child health clinics should be trained.

(ii) Pregnant women should be advised to eat dark supplements rich in Vitamin A in prophylactic doses This helps to build up stores of retinol in the fetal liver and should be continued during lactation Mothers should be advised to include in the weaning foods dark green leafy vegetables or yellow and orange fruits, which are locally available, cheap and known to be good sources of β-carotene

(iii) In blindness from keratomalacia single large prophylactic doses of retinol in oily solution are recommended This is given as a capsule to be taken by mouth The dose is safe and adverse effects are rare All prophylactic pro-

grammes should be evaluated by periodic field surveys of the prevalance of xerophthalmia

(iv) Nutritional disorders of the eye are only one of several causes of blindness There are more than ten million blind people in the world. Most of them are blind before the age of 5 Trachoma, a virus infection, is the most important eye disease in the world It is common in children and, if untreated, often causes progressive loss of vision and blindness Small pox often affect the eyes and is responsible for $\frac{1}{3}$th of the blindness in India Onchocercosis is caused by a filarial worm which is transferred from man to man by flies It causes nodules in the skin and thence microfilaria invade the eye which leads to blindness The disease commonly affects people who live besides river In some villages, all the adult population are blind Veneral diseases, congenital syphilis and gonococcal opbthalmia neonatorum are important causes of blindness in parts of the world where the maternity services are inadequate or totally lacking Accidents in the home and at work are common causes of blindness Many young children lose their sight in this way Diabetes, cataract and glucoma are important causes of blindness in elderly people

Vitamin A deficiency is thus one of the important causes of blindness and the most easily preventable

RICKETS AND OSTEOMALACIA

Rickets is a disease of children in which the bones are softened and deformed. It arises as a result of deficiency of Vitamin D and a failure to absorb calcium from the small intestine Osteomalacia, which means softening of bone, arises when there is Vitamin D deficiency in adults The resultant calcium deficiency leads to demineralisation of the bones The severe forms of both diseases with gross deformities of the skeleton are now rare but cases may be seen occasionally in bospitals in most countries. Only a few foods are good sources of Vitamin D and about 90 per cent of the vitamin in our bodies comes from photosynthesis in the skin

(a) Risk factors·

(i) In northern latitudes during long winters with only a few hours of daylight greatly reduce exposure to ultraviolet radiation. Vitamin D deficiency is then a risk for all children and adolescents, as they have greater need for the vitamin than adults, and also for all elderly people and others with disabilities restricting outdoor activity

(ii) Puppies developed rickets when white bread in their diet was replaced by unrefined oatmeal Asian communities whose staple food is chapattis made from high extraction wheat flour appear to be at increased risk of both rickets and osteomalacia

(iii) Strict Hindus and other who eat no animal food provide an increased proportion of cases of osteomalacia seen in Britain. This complete exclusion of Vitamin D from the diet does not normally lead to rickets or osteomalacia but increases the risk.

(iv) When an infant is fed exclusively on milk from a Vitamin D deficient mother for more than three months, the risk of infantile rickets rises.

(v) Heavy pigmentation reduces synthesis of Vitamin D in the skin. This can only be a minor risk factor since in Britain rickets is much less common in the darker West Indian than in the lighter Asian Communities

(b) Secondary osteomalacia and rickets:

(i) Osteomalacia may arise in patients with the malabsorption syndrome after many months This is due to impaired calcium absorption.

(ii) Osteomalacia and other bone disorders arise in patients with chronic renal failure Impaired formation of 1,25 $(OH)_2$ D in the kidney may be responsible

(iii) Osteomalacia is sometimes found in patients with cirrhosis of the liver due to failure to form 25 (OH) D

(iv) Five different genetic defects that lead to rickets are known All are uncommon

(c) **Clinical Features**

Rickets:

(i) The child is restless, fretful and pale, with flabby and toneless muscles which allow the limbs to assume unnatural postures (acrobatic rickets)

(ii) Excessive sweating on the head is common

(iii) The abdomen is distended as a result of the weak abdominal muscles, the atony of the intestinal musculature and the intestinal fermentation that may arise from excessive carbohydrate in the diet

(iv) *Gastrointestinal upsets with diarrhoea are common*

(v) The infant or child is prone to respiratory infections

(vi) Development is delayed so that the teeth often erupt late and there is failure to sit up, stand, crawl and walk at the normal ages

(vii) There is extension and widening of the epiphyses at the growing points, where cartilage meets bone The earliest bony lesions are usually enlargement of the epiphyses at the lower end of the radius and at the costochondral junctions of the ribs or 'rickety rosary', an early and important diagnostic feature Later features are 'bossing' of the frontal and parietal bones and delayed closure of the anterior fontanelle

(viii) There may be deformities of the chest such as undue prominence of the sternum (Pigeon chest) and a transverse depression, passing towards from the costal cartilages towards the axillae which deepens with inspiration In unusually severe cases, respiratory functions can be seriously impaired by the combination of respiratory infection and a rachitic chest

(ix) If rickets continues into the second and third year of life, these signs may persist or be magnified Deformities such as kyphosis of the spine develop as a result of the new gravitational and muscular strains, caused by sitting up and crawling At the same time there may be enlargement of the lower ends of the femur, tibia and fibula When the rachitic child begins to walk, deformities of the shafts of the leg bones develop, so that 'knock knees' or 'bow legs' are added to the clinical picture Anterolateral bowing of the tibiae at the junction of the middle and lower third is frequently noted in young children with rickets

(x) The spinal kyphosis is often replaced by lordosis Pelvic deformities may follow and lead years later to serious difficulties at child birth

(xi) When ionised calcium in the plasma is reduced, infantile tetany may result, with spasm of the hands and feet and of the vocal cords The latter causes a high pitched, distressing cry and great difficulty in breathing

(d) **Diagnosis:**

A flabby baby towards the end of its first year, unable to pull itself up, fretful and easily irritated, with too few teeth showing and liable to profuse sweats, should always be suspected by having rickets Early evidence of rickets may be overlooked in a child ill with bronchopneumonia or diarrhoea, especially in the first year of life If there is any doubt, a radiograph of the wrist may show characteristic changes at the epiphyses, the outline of the joint is blurred and hazy, and the epiphyseal line becomes broadened Later, in older children, as a result of decalcification of the metaphysis and the effects of movements and stresses the classical

metaphysis and the effects of movements and stresses the classical concave 'saucer' deformity is clearly shown radiographically The opinion of an experienced radiologist may be needed to distinguish the picture from that of scurvy The diagnosis is supported by a raised plasma alkaline phosphatase and confirmed if plasma 25 (OH) D is low

It is sometimes necessary to distinguish rickets from other rare disorders involving the bones, such as congenital syphilis, achondroplasia and osteogenesis imperfecta Radiographs of the bone are helpful in differentiating these disorders

Osteomalacia

(a) **Clinical Features:**

(i) Deformities of the spine, pelvis and legs are now rarely seen

(ii) The common features are pain and muscular weakness Pain ranges from a dull ache to severe pain The affected sites are the ribs, sacrum, lower lumber vertebrae, pelvis and legs

(iii) Bone tenderness on pressure is common

(iv) Muscular weakness is often present and the patient may find difficulty in climbing stairs or getting out of a chair

(v) Spontaneous fractures may occur

(vi) Tetany may be manifested by carpopedal spasm and facial twitching

(b) **Diagnosis·**

The early symptoms may resemble those present in osteoporosis and rheumatic disorders The Measurement of plasma 25 (OH) D should clear the diagnosis The distinction between osteomalacia and Osteroporosis is given below

	Osteomalacia	Osteoporosis
Clinical features		
Skeletal pain	A major complaint usually persistent	Episodic and usually associated with a fracture
Muscle weakness	Usually present and producing disability and a characteristic gait when severe	Absent
Fractures	Relatively uncommon, healing delayed	The usual presenting feature heals normally
Skeletal deformity	Common, especially kyphosis	Only occurs where there is a fracture
Radiographic features:		
Loss of density of bones	Widespread	Irregular and often most marked in the spine
Loss of bone detail	Characteristic.	Not a feature
Looser's Zones	Diagnostic.	Absent
Biopsy		
Histological changes	Excess osteoid tissue with bone present in normal quantity	Bone reduced in quantity but fully mineralised
Biochemical changes		
Plasma Ca and P	Often low	Normal
Plasma alkaline phosphatase	Often high	Normal
Urinary calcium	Often low	Normal or high.
Response to treatment:		
Vitamin D	Dramatic.	None

(d) Treatment:

(i) A daily oral dose of 25—125 μg (1000—5000 I U) of Vitamin D cures rickets and osteomalacia This should be reduced to 10 μg, the prophylactic dose, when plasma alkaline phosphatase has returned to normal and radiographs show that healing is established.

(ii) Children can be given halibut liver oil in a very small dose (1 ml) since it contains 30 to 40 times the concentration of Vitamin D of cod liver oil For severe cases, synthetic calciferol is useful

(iii) When an infant or young child may be seen once by an emergency medical service and perhaps not again for months, a single massive dose of Vitamin D (150,000 I U) (three strong calciferol tablets) can be given by mouth with reasonable safety and curative effects. The single dose can be given by injection but this has no proved advantage over the oral route A daily small dose is recommended to avoid danger of overdosage

(iv) If there is evidence of malabsorption in osteomalacia, the dose of Vitamin D should be upto 1 25 mg (50,000 I.U) daily and it may have to be given intramuscularly at weekly or monthly intervals If the disease is secondary to kidney or liver disease large doses and either 1,25 $(OH)_2$ D or 25 (OH) D are indicated

(v) An adequate intake of calcium is essential The best source is milk and at least 500 ml should be drunk daily When this is not practical and in severe cases, calcium lactate, taken by mouth, should be prescribed

(vi) An egg and butter daily is required to increase the dietary intake of Vitamin D

(vii) Mothers of young children require tactful education in feeding and general care, as do elderly patients

(viii) Unnecessary clothing should be removed and these should be every opportunity to go out to enjoy the sunshine

(E) Prognosis

Rickets is not a fatal disease but the untreated rachitic child is a weakling with an increased risk of infections notably Broncho Pneumonia The skeletal changes usually tend to heal spontaneously as the child gets older The bony deformities, if mild, usually right themselves as growth proceeds, but in severe cases pigeon chest, contracted pelvis, knock knees or bow legs may persist With early and sufficient treatment these changes are entirely avoided

In osteomalacia, Vitamin D quickly relieves the pain and muscular weakness but it takes many weeks or months to restore the bones to their normal strength

(F) Prevention:

(i) All people can be protected from rickets by a supplement of 10 μg of Vitamin D daily The dose is effective and safe Supplements are necessary in all countries with long dark winters for all children up to 5 years of age, and for their mothers during pregnancy and lactation All children on anticonvulsive drugs should continue to receive a supplement In some communities there is now evidence that large numbers of the elderly require extra Vitamin D to protect them against osteomalacia

(ii) Rickets occurs predominantly in families which are poor and where the mother lacks education An intensive programme of health education is needed to ensure that the supplements are taken regularly, this should also provide advice on diet, clothing and general hygiene

(iii) Children in countries with abundant sunlight should not normally need a Vitamin D supplement Where rickets is present, teaching of mothercraft,

emphasising that young children must not be excessively protected from the sun, is necessary, protein-energy malnutrition is sometimes associated with rickets and routine dietary supplement may be needed.

(iv) In tropical countries, fish liver oils are more easily obtained They contain useful amounts of Vitamin A. Sometimes it may be advantageous to give children a single massive dose (1—2 m) of Vitamin D This is stored in the liver, liberated slowly and protects a child for several months

(v) Smoke abatement, slum clearances, and provision of open-air play grounds were mainly responsible for the marked fall in the prevalance of rickets in the early part of the present century The great improvement in these matters have to be maintained and extended where necessary These are public health measures of major importance

BERIBERI AND THE WERNICKE—KORSAKOFF SYNDROME

BERIBERI

Beriberi is a nutritional disorder formerly widespread in the rice-eating people of the East 'Beri' means weak The main features of beriberi are due to deficiency of thiamin Three forms of the disease occur

(1) Wet beriberi, characterised by oedema often associated with high-output cardiac failure

(2) Dry beriberi, a polyneuropathy

(3) The infantile form.

Raw fermented fish which contains thiaminase and fermented tea leaves both reduce the availability of the small amount of thiamine in the diet.

(a) **Clinical Features.**

(i) At first there is anorexia and ill-defined malaise, associated with heaviness and weakness of the legs This may cause some difficulty in walking

(ii) There may be a little oedema of the legs or face and the patient may complain of precordial pain and palpitation

(iii) The pulse is usually full and moderately increased in rate

(iv) There may be tenderness of the calf muscles on pressure and complains of 'pins and needles' and numbness in the legs

(v) The tendon jerks are usually sluggish, but occasionally slightly exaggerate.

(vi) Anaesthesia of the skin, especially over the tibiae, are common

Such conditions may persist for months or even years with only minor alterations in the symptoms Patients can work to earn their living with low efficiency At any time this chronic malady may develop into severe forms

Wet beriberi

(i) Oedema is the most prominent feature and may develop rapidly in the legs as well as in the face, in the trunk and also in the serous cavities

(ii) Palpitations are marked and these may be breathlessness

(iii) Anorexia and dyspepsia are commonly present

(iv) There may be pains in the legs after walking

(v) The calf muscles are frequently tense, slightly swollen and tender on pressure

(vi) The neck veins become distended and show visible pulsations

(vii) The apex beat of the heart is displaced outwards

(viii) In the arteries there is often a lowered diastolic pressure and systolic pressure is disproportionately higher, hence on auscultation over the femoral and other large arteries, a curious 'pistol shot' sound may be heard

(ix) The pulse is generally fast

(x) If the circulation is well maintained, the skin is warm to the touch owing to the associated vasodilatation

(xi) When the heart begins to fail, the skin becomes cold and cyanotic, particularly on the face

(xii) ECG often shows no changes but in some cases there are low voltage of the QRS complex, inverted T waves or evidence of disturbed conduction

(xiii) The urinary volume is diminished, but there is no albuminuria

(xiv) The mind is usually clear

(xv) The patient is in danger of sudden increase in the oedema, acute circulatory failure, extreme dyspnoea and death

Dry beriberi

(i) The essential feature is a polyneuropathy

(ii) The muscles become progressively more wasted and weak, and walking becomes increasingly difficult

(iii) The thin patients need at first one stick, then two and finally become bedridden

(iv) The disease is a chronic malady, which may be arrested at any stage by improving the diet

(v) Bedridden patients are very susceptible to infections

(vi) Dysentery or tuberculosis are often fatal unless prompt and efficient treatment is given

Infantile beriberi

(i) This occurs in breast-fed infants, usually between the second and fifth months

(ii) The mother may have no clinical signs of beriberi although they must have been eating a diet and secreting milk with a low thiamin content

(iii) Frank beriberi can develop in late pregnancy and the puerperium

(iv) It exists in an acute and chronic form

(v) In acute form cardiac failure may develop abruptly, the mother may have noticed that the infant is restless, cries a lot, is passing less urine than normal and shows signs of puffiness The infant then may suddenly become cyanosed with dyspnoea and tachycardia and die within 24 to 48 hours Other serious signs are convulsions and coma

(vi) In the chronic form, which is much less common, the main symptoms are due to gastrointestinal disturbances There is constipation and vomiting The child is fretful and sleeps poorly. The muscles are soft and toneless, but not markedly wasted There is often intense pallor of the skin with cyanosis round the mouth Cardiac failure and sudden death are common

(vii) Infantile beriberi is the chief cause of death between the ages of 2 and 5 months in rice eating rural areas

(b) **Differential diagnosis**

(i) In mild and chronic cases of beriberi, these may be few or no physical signs and the diagnosis fully depends on the interpretation of symptoms and the dietary history

(ii) The oedema of wet beriberi has to be distinguished from that associated with hepatic and renal disease and heart failure The warm extremities in cardiac beriberi and the absence of protein in the urine are useful diagnostic points Famine oedema should seldom be a diagnostic difficulty if a proper dietary history is taken

(iii) Cardiovascular beriberi has to be distinguished from other causes of high output cardiac failure, notably hyperthyroidism and severe anemia

(iv) In all doubtful cases of wet beriberi the therapeutic response to thiamin usually settles the diagnosis

(v) The diagnosis of dry beriberi is mainly based on the dietary history In endemic areas the disease may be confused with neuritic leprosy, but this is characterised by palpable, cord like superficial nerves and areas of skin anaesthesia These two diseases not infrequently occur together and when they do, dry beriberi, if mild, may be overlooked

(vi) The diagnosis of infantile beriberi may be difficult Neither oedema nor paralysis is an early sign and sudden death may occur before either is present A history of sudden death of a previous child between the ages of 2 and 5 months is suggestive Infantile beriberi may be confused with PEM and the two may occur together

(c) Treatment

Wet beriberi

(i) Treatment must be started as soon as the diagnosis is made because of the sudden fatal heart failure

(ii) Complete rest is essential and thiamin should be given at once intramuscularly 25 mg twice daily for three days Thereafter an oral dose of 10 mg two or three times a day should be continued until convalescence is established

(iii) Thiamin treatment to a patient with cardiovascular beriberi is the most effective in medical science Within a few hours the breathing is easier, the pulse-rate slower, the extremities cooler and a rapid diuresis begins to dispose of the oedema Within a few days the size of the heart is restored to normal Muscular pain and tenderness are also dramatically improved.

(iv) During convalescence and rehabilitation, a good mixed diet with less rice is needed Another cereal should be substituted for part of the rice in the diet Pulses have a well-deserved reputation for curing and preventing beriberi

Dry beriberi

(i) Patients are generally undernourished and, if they take sufficient of a good mixed diet to enable them to gain weight, slow improvement may be expected

(ii) If the dietary intake is adequate, there is no need to continue with supplementary thiamin

(iii) Infections and intercurrent disease should be treated and appropriate physiotherapy given

Infantile beriberi

(i) The mother's milk is the simplest way of treatment The mother should receive 10 mg thiamin twice daily—in severe cases this should be by injection

(ii) The infant should be given thiamin in doses of upto 10 to 20 mg intramuscularly once a day for three days This should be followed by 5 to 10 mg orally twice a day

(iii) In severe heart failure or convulsions and coma the initial dose may be increased to 25 to 50 mg given intravenously very slowly.

(d) **Prevention:**

(i) Beriberi can be prevented by the use of undermilled, home pounded or parboiled rice or by increased use of pulses and other foods containing thiamin. Medicinal preparations of thiamin are also available and cheap.

(ii) Changed milling practices are probably the most important and in many areas rice is not so highly polished as to remove all the bran.

(iii) Changed milling practice of rice is the most important without removing all the bran.

(iv) Improvement in social and economic conditions and the consumption of a better diet with more thiamin-containing foods are essential.

(v) The establishment of maternal and child Health Centres has led to many pregnant and lactating women getting good advice on diet as well as vitamin supplements; infants may also receive them or extracts of rice bran.

Thiamin deficiency in those whose staple diet is not rice

Thiamin deficiency in countries where rice is not the staple food arises in the great majority of cases in persons whose diet has been greatly restricted usually as a result of chronic alcoholism. It also arises, but rarely, secondary to carcinoma of the stomach and other conditions associated with prolonged partial starvation. Hence there is always a lack of other essential nutrients.

(a) **Alcoholic Neuropathy:**

Alcoholics who have restricted their food intake for many weeks often develop a disorder of peripheral nerves sometimes indistinguishable from dry beriberi. The administration of thiamin leads to no dramatic improvement, but if the patient takes a good diet and gives up alcohol completely a slow diminution of the symptoms may be expected. The nerves of the lower limbs are affected more severely than those in the upper limbs. There is dysfunction of both sensory and motor fibres. The effects on sensory nerves may be paraesthesiae (pins and needles) or sometimes severe nerves pains, as in the burning feet syndrome; there may be loss of sensation, either numbness of the extremities or loss of position sense. Signs of motor nerve involvement are foot drop, muscle wasting and impaired knee and ankle jerks.

Other causes of polyneuropathy are as follows:

(i) Deficiency diseases—Pellagra, subacute combined degeneration, burning feet syndrome and pyridoxine deficiency.

(ii) Metabolic diseases—Diabetes Mellitus, Uremia, Porphyria etc.

(iii) Chemical poisoning—Heavy metals (lead, Arsenic, mercury) and some drugs.

(iv) Infective—Diphtheria, Leprosy etc.

(v) In association with carcinoma.

(vi) Rare genetic types—Refsum's disease.

(b) **Occidental beriberi heart disease:**

Cardiac failure with generalised oedema, pulmonary congestion and dyspnoea sometimes develops in chronic alcoholics. Damage to the myocardium may be due to direction action of ethanol or thiamin deficiency.

Less frequently there is a sudden circulatory collapse with lactic acidosis. It is wise to give all alcoholics with evidence of heart failure sufficient thiamin to replenish their tissues; in most cases no benefit is likely but it will do no harm and in few may be life-saving.

WERNICKE-KORSAKOFF SYNDROME

Wernicke in 1881 described a neurological disorder in three patients, two of them alcoholics and the third a seamstress who had persistent vomiting after ingestion of sulphuric acid It is characterised by weakness of eye muscles, so that the patient cannot look upwards or sideways and a state of disorientation and apathy Sometimes there is jerky, rhythmical movements of the eyes (nystagmus) and if the patient can stand he is unsteady (ataxia) It was found that many of the cases recovered dramatically after large doses

Korsakoff in 1887 described a psychosis, also occurring in alcoholics, characterised by a severe defect in memory and learning Confabulation is a characteristic feature, though not always present The patient can remember past events with verifiable accuracy He cannot remember what he did earlier in the same day but tends to provide a superficially convincing tale rather than say he has forgotten

Wernicke's disease and Korsakoff's psychosis are manifestations of thiamin deficiency After carrying out 82 postmortem cases it had been found out that there were symmetrical lesions in various parts of the brain stem, diencephalon and cerebellum, the areas commonly affected being the mamillary bodies, the nuclei of the thalamus and the periaqueductal grey matter In the most advanced lesions there was virtually complete tissue necrosis, the less severe lesions were characterised by destruction of myelin with less damage to neurones Small haemorrhages are characteristic but not always present.

The same histological features were seen in cases of Wernicke's disease and of Korsakoff's psychosis Most of the patients who recovered from the acute confusional state subsequently developed some memory defect

Treatment with thiamin is effective in these cases when given promptly Large doses are also needed

Diagnosis is confirmed by the RBC transketolase test Although the syndromes occur in alcoholics, it may arise secondary to any disorder which seriously impairs nutrition

(E) Comment:

Thiamin deficiency can thus lead to an encephalopathy or a cardiomyopathy or a peripheral neuropathy Two or rarely all three diseases can occur together in a patient but it is surprising how often they do not We can not yet explain why the brain is affected in one person, the heart in another and the peripheral nerves in a third. Alcoholism is widespread and body stores of thiamin in alcoholics are likely to be small, but thiamin deficiency should be registered There are still many unsolved problems in the relation between ethanol, thiamin and brain damage

PELLAGRA

Pellagra is a nutritional disease endemic among poor peasants who subsist chiefly on maize It has been called the disease of the three Ds dermatitis, diarrhoea and dementia But diarrhoea and mental changes are not always present in mild and early cases and the mental symptom is usually depression and not dementia The clinical features are loss of weight, increasing debility, an erythematous dermatitis characteristically affecting parts of the skin exposed to sunlight, gastro-intestinal disturbance especially diarrhoea and glossitis, and mental changes

(a) Clinical Features:

Skin

(i) There is an erythema resembling severe sunburn, especially the backs of the hands, the wrists, the forearms, face and neck.

(ii) Exposure to trauma or mechanical irritation of the skin, especially over bony prominences, may also determine the site of the lesion.

(iii) The skin in the affected areas is at first red and slightly swollen, it itches and burns

(iv) In acute cases the skin lesions may progress to vesiculation, cracking, exudation and crusting with ulceration and sometimes secondary infection, but in chronic cases the dermatitis occurs as a roughening and thickening of the skin with dryness, scaling and brown pigmentation

Digestive system:

(i) There is usual digestive upset, and diarrhoea is not always present.

(ii) There may be nausea, a burning sensation in the epigastrium, and sometimes constipation in chronic cases

(iii) The digestive symptoms may be aggravated by the presence of intestinal parasites

(iv) The mouth is sore and often shows angular stomatitis and cheilosis

(v) The tongue characteristically has a 'raw beef' appearance—red, swollen and painful, though usually without loss of papillae

(vi) Secondary infection of the mouth with Vincent's organisms is common

(vii) A non-infective inflammation followed by mucosal atrophy may involve the gastrointestinal tract and account for the diarrhoea which is characteristically profuse and watery, sometimes with blood and mucus in the stools

(viii) The rectum and anus are frequently affected and chronic gastritis with reduction or absence of acid secretion is a common finding

(ix) Vaginitis and amenorrhoea may occur

Nervous System:

(i) In mild cases the symptoms consist of weakness, tremor, anxiety, depression and irritability, in severe acute cases delirium is common and dementia occurs in the chronic form

(ii) In chronic cases there may be decreased sensation in the feet to touch and loss of vibration and position sense The loss of position sense may give rise to ataxia

(iii) Spasticity and exaggerated tendon reflexes give evidence of involvement of the pyramidal tracts Then features are those of subacute combined degeneration of the cord and may be due to associated Vitamin B_{12} deficiency

(b) **Diagnosis:**

(i) The skin lesions are of diagnostic importance since they are only found in pellagra, whereas the gastrointestinal and mental features may be present in many other diseases A variety of erythemas and exfoliative skin lesions may mimic pellagra The two characteristic features of cutaneous pellagra are its symmetrical distribution, determined by the clothes of the patient and exposure to sunlight, and the therapeutic response to nicotinic acid.

(ii) A nutritional glossitis identical with the tongue changes seen in pellagra may occur without the other signs of the disease in people who have been all the times indoors, out of sunlight.

(iii) Pellagra is a disease affecting poor people on bad diets Hence it is often accompanied by signs of protein energy malnutrition by anaemia and by deficiencies of thiamin and other vitamins These together with chronic infections may complicate the clinical picture

(c) Laboratory Findings:

The fasting plasma tryptophan ranges from 1 to 4 8 mg/l in pellagrins and from 6 5 yo 8 8 mg/l in healthy adults Plasma tryptophan may prove to be a convenient test for confirming a diagnosis of pellagra

(d) Prognosis:

Mental symptoms, especially dementia, are the most serious feature and may be permanent Occasionally a fulminating form develops, with fever and severe prostration which can be fatal In the past many deaths were due to secondary infections (notably tuberculosis and dysentery) or to emaciation due to general dietary failure, intensified by the diarrhoea

(e) Treatment:

(i) Nicotinic acid or nicotinamide are the standard treatment for quick relief of symptoms Nicotinamide is to be preferred because it does not cause the unpleasant flushing and burning sensations that often result from taking nicotinic acid These are transitory and harmless, but may alarm the patient The vitamin is rapidly absorbed from the stomach, despite severe digestive disorders There is no need to give intravenous or intramuscular injections The immediate response to nicotinamide is usually dramatic, within 24 hours the erythema diminishes, the tongue becomes paler and less painful and the diarrhoea ceases Often there is striking improvement in the patient's behaviour and mental attitude. But nicotinamide alone is usually insufficient to restore health due to other associated deficiencies, notably of protein and other components of Vitamin B complex. Therefore, B complex should be given as a routine and if there are signs of peripheral neuropathy or subacute combined degeneration of the cord larger doses of thiamin or Vitamin B_{12} are indicated

(ii) To restore the patient to normal weight, the diet should provide ample energy and good quality protein, as is present in milk, eggs, meat or fish. In severely ill patients it is necessary to climb the dietetic ladder cautiously The food should be low in bulk to avoid further diarrhoea The diet may be poorly tolerated because of the mental state of the patient and the sore mouth which may make eating difficult Alcohol should be forbidden

(iii) Rest in bed and sedation are necessary for severely ill pellagrins, especially those with marked mental symptoms If the dermatitis is associated with much crusting or secondary infection, gentle washing with a bland solution is indicated.

(F) Prevention

(i) Enrichment of maize meal with vitamins is technically simple and inexpensive but is difficult to implement for subsistence farmers who grow their own maize It is wise to avoid dependence on a single cereal crop, such as maize.

(ii) Animal husbandry should be encouraged in all areas where pellagra is endemic so that the production of milk and milk products, and meat is increased Encouraging the planting of opaque 2 maize may help It contains about three times as much tryptophan and twice as much lysine as conventional maize

SCURVY

. Scurvy is a nutritional disease which results from prolonged subsistence on diets practically devoid of fresh fruit and vegetables Lack of ascorbic acid causes a disturbance in the structure of connective tissue, leading to swollen, bleeding gums, and haemorrhages into the skin and elsewhere

(a) Clinical Features:

(i) The gums are swollen, particularly in the region of the papillae between the teeth, sometimes producing the appearance of 'scurvy buds' These may be

so extensive that they project beyond the biting surface of the teeth and almost completely conceal them The spongy gums are livid in colour and bleed on the slightest touch There is always some infection

(ii) The first sign of cutaneous bleeding is often to be found on the lower thighs, just above the knees These haemorrhages are perifollicular—tiny points of bleeding around the orifice of a hair follicle The condition in scurvy can be distinguished by its appearance from the follicular keratosis sometimes associated with Vitamin A deficiency In the latter condition there is usually a horny plug of keratin projecting from the orifice of the hair follicle In scurvy there is a heaping up of keratin like material on the surface around the mouth of the follicle, through which a deformed 'cockscrew' hair characteristically projects Perifollicular haemorrhages may appear on the buttocks, abdomen, legs and arms African patients often present with pain in a leg due to haemorrhage into intermuscular septa in the thigh or calf

(iii) Anaemia is present in most patients In patients the bone marrow may be normoblastic or megaloblastic and associated deficiency of iron or folate is often responsible, but there may be other unidentified factors Destruction of erythrocytes in muscle haematomata may lead to bilirubinaemia and mild jaundice

(iv) Osteoporosis may occur in scurvy, since ascorbic acid is necessary for the synthesis of collagen in all parts of the body, including the bones

(v) Haemorrhages into any of the internal organs may occur and a patient die suddenly and without warning, apparently from cardiac failure

(vi) There is scurvy in infants Until the teeth have erupted, scorbutic infants do not develop gingivitis When this occurs the gums have the classical appearance of 'scurvy buds' The first sign of bleeding is usually a large subperiosteal haemorrhage immediately overlying one of the long bones—frequently the femur—producing the characteristic 'frog legs' position This gives rise to intense pain, especially on movement The infant may cry continuously and agonisingly, and scream even louder when lifted

(b) Diagnosis :

(i) The inflamed rim of the gums is bright red in colour, in contrast to the cyanotic appearance in scurvy, and there is usually much less swelling In vincent's angina the gums are acutely inflamed, ulcerated and painful, but the bright red appearance of the lesions is distinctive Poisoning with heavy metals, particularly lead and mercury, produces a gingivitis in which the gum margin is stained blue, but there is usually little swelling and the appearance is easily distinguished from scurvy Phenytoin, a drug used in epilepsy, may cause marked swelling the gums, but they preserve their normal colour and do not bleed

(ii) Scurvy in infants and children may sometimes be mistaken for rheumatic fever or osteomyelitis, because of the pain caused by a subperiosteal haemorrhage The refusal of the child to use one leg may cause the disease to be mistaken for poliomyelitis

(iii) Blood ascorbic acid can easily be determined If the concentration of ascorbic acid in blood is low, it is a sure case of scurvy

(c) Treatment:

(i) Adequate amounts of synthetic ascorbic acid should be given at once because of the danger of sudden death The vitamin is very soluble and quickly absorbed from the digestive tract It can be given intravenously, but a large part of it is immediately lost in the urine It is the aim to saturate the body with ascorbic acid The fully saturated body contains about 5 grams of the vitamin Therefore, a dose of 250 mg by mouth four times daily should achieve this within a week, despite some loss in the urine

(ii) Scurvy arises among people for removed from supplies of synthetic ascorbic acid (e g , among prisoners of war), in such conditions valuable therapeutic effects are obtained by the use of natural sources of the vitamin such as fresh fruit and vegetables

(iii) In case of anaemic patient ferrous sulphate and folate tablets should be given by mouth

(iv) With adequate and immediate treatment no patient dies of scurvy, but if it is delayed he may die

(d) **Prevention**

(i) Scurvy generally occurs at the two extremes of age The prevention of scurvy in infants has been accomplished by the better education of mothers and helped by the distribution of cheap, concentrated orange juice of standard ascorbic acid content For old people living alone, the provision of proper meals is the best means of preventing scurvy This should be the responsibility of their family If there are no relations, responsibility falls on the social services In cases where an old person is unwilling to eat foods containing the vitamin, he should be given ascorbic acid tablets

(ii) In cases of persons who travel in barren lands or make long sea voyages, they should be given ascorbic acid tablets to carry along with them.

(iii) In times of drought and famine, when fresh vegetables are not available, ascorbic acid can be obtained by the germination of pulses or cereals 30 grams of dried pulse on germination yield 9 to 15 mg ascorbic acid which is sufficient to prevent scurvy

OTHER NUTRITIONAL DISORDERS OF THE NERVOUS SYSTEM

A. Burning feet syndrome

(i) The earliest symptom is aching, burning or throbbing in the feet. This becomes more intense and is followed by sharp, stabbing, shooting pains, which may spread up as far as the knee like an electric shock, causing excruciating agony They come on in paroxysms and are usually worse at night.

(ii) Most patients get some relief by walking about, and sufferers may spend the night limping up and down outside their quarters

(iii) Some patients manage to get relief by wrapping their feet in cold wet cloth or sitting with their feet in a pail of cold water Continuous pain and loss of sleep produce a thin, exhausted, irritable patient

(iv) The tendon jerks may be normal but may be exaggerated.

(v) The syndrome has been associated with the prolonged consumption of a diet deficient in protein and the B group of vitamins

(vi) Patients who suffer from it may also develop the orogenital syndrome or nutritional amblyopia, but rarely beriberi

(vii) The syndrome can be seen sometimes in chronic alcoholics and patients with diabetic and other neuropathies, and rarely in other disorders.

B Spinal ataxia

(i) Patients living on unbalanced diets for long periods develop neurological signs which indicate that the principal lesion is in the dorsal columns of the spinal cord involving particularly proprioceptive sensation The gait is unsteady and the patient is unable to stand upright without swaying when the eyes are closed Vibration sense in the legs is often lost.

(ii) In tropical ataxic neuropathy, Vitamin B_{12} plays only a secondary role In the fully developed syndrome there is sensory spinal ataxia, retrobulbar

neuropathy or optic atrophy and sometimes bilateral nerves deafness Signs indicating mild involvement of the lateral or pyramidal tracts may be found Epidemiologically the condition is found in people who regularly consume large amounts of cassava Cassava contains a cyanogenic glycoside, Linamarin, which can be broken down to yield free hydrogen cyanide by enzymes in the plant tissue if it is crushed or left standing in water

(iii) The cyanide is detoxified by sulphur containing amino acids, which convert it to thiocyanate, and by hydroxocobalamin which forms cyanocobalamin Patients with tropical ataxic neuropathy have increased plasma concentration and urinary excretion of thiocyanate with increased Vitamin B_{12} and reduced cystine in the plasma The condition can be prevented in part by cooking methods which wash out the glycoside or boil off the HNC

C Cerebellar cortical degeneration

This condition in which the characteristic clinical feature is ataxia of the legs arises from degenerative changes limited to the anterior superior part of the vermis of the cerebellum It is associated with alcoholism and poor nutrition but the response to vitamin thereby and nutritional rehabilitation is less consistent than in the Wernicke Korsakoff syndrome

D Vitamin B_{12} neuropathy

(i) Early symptoms are tingling, coldness and numbness in the extremities due to peripheral neuropathy

(ii) Motor weakness and ataxia appear later and become increasingly severe as the cord is involved

(iii) The physical signs depend on the relative involvement of the peripheral nerves and the dorsal and lateral columns of the cord

(iv) In severe cases ataxia is the outstanding feature with loss of reflexes especially in the lower limbs Sometimes the pyramidal tracts are involved and spasticity, increased reflexes and an extensor plantar response are present

(v) If the brain is affected there may be an organic psychosis and this may be the first evidence of Vitamin B_{12} deficiency

E Spastic paraplegia

(i) If kesari dhal eaten in excessive amounts and for a long period it gives rise to lathyrism

(ii) The onset of lathyrism is sudden and is often preceded by exertion or exposure to cold A patient may go to bed well and wake up paralysed, or he may fall down at the plough Sometimes backache and stiffness of the legs precede the onset of the paralysis by a few days The condition is a spastic paralysis of the lower limbs, due presumably to precisely localised lesion of the lower parts of the pyramidal tracts The motor nerves to the muscles of the trunk, upper limbs and sphincters are spared The sensory nervous system is not involved In mild cases there is only stiffness and weakness of the legs and exaggerated knee and ankle jerks In more severe cases, the patients walk with bent knees on tiptoe The legs are often crossed, a 'scissors gait' develops and walking is only possible with the aid of sticks In severe cases paraplegia in flexion follows and walking becomes impossible The patient can only move about by pushing himself along, supporting his body on his hands, buttocks and heels The paraplegia is typically spastic with greatly increased ankle and knee jerks and with clonus The final stage of the disease is completely incapacitating and the sufferers may move to the cities where they are easily recognised amongst the beggars

(iii) Epidemic lathyrism is mainly a disease of famine When the price of wheat rises, many of the poor increase their consumption of the pulse and cases of

lathyrism arise Prevention requires change in agricultural and economic policies The toxin can be extracted from kesari dhal by heating it in four volumes of water for an hour

(iv) There is no specific treatment All patients need a good diet

LESS NUTRITIONAL DISORDERS

Disorders of the skin

A Follicular hyperkeratosis

(i) The follicles become blocked with plugs of keratin derived from their epithelial lining which has undergone squamous metaplasia This is due to Vitamin A deficiency Halibut liver oil red palm oil or other oils rich in Vitamin A or carotene may produce a striking clinical improvement, vegetables oils are also likely to be rich in essential fatty acids and Vitamin E, and the condition has responded to Vitamin E therapy Other factors may contribute to its development, such as exposure to sunlight and lack of cleanliness

(ii) Slight follicular keratosis may be found in people who are adequately nourished in respect of Vitamin A Thus the condition is not a specific or constant feature of Vitamin A deficiency

(iii) The typical distribution is over the backs of the upper arms and the fronts of the thighs, but it may extend over the buttocks and indeed over the whole trunk. Only the feet, hands, and face may be spared. Some degree of xeroderma is commonly associated The horny plugs that project from the follicular orifices can often be pulled out with a fine pair of forceps, they give the skin a characteristic feeling of roughness Because of its appearance, the condition has been called 'toad skin' or phrynoderma

(iv) Folliculosis is sometimes mistaken for follicular hyperkeratosis The follicles are raised above the surface, but no horny plug projects from the follicular orifices

B Xeroderma

It means dryness of the skin The skin feels dry and often rough On uncovering the legs, a cloud of fine branny dandruff is often seen. Xeroderma is commonly but not constantly associated with follicular keratosis and 'cracked skin'

C Crazy-paving skin

In this condition the appearance indicates a layer of lacquer painted on the surface, which on drying has broken up into individual islands of varying size There is often some desquamation from the borders of each island, while the intervening gaps may become fissured The commonest site for this lesion is the skins, and it seems probably that exposure to dirt and alternate heat and moisture is often responsible

D Pachyderma (elephant skin)

(i) The affected skin areas are thick, rough and thrown into folds like the skin of an elephant

(ii) It starts as a roughness of the skin on the back of the hands and feets, and the skin of the whole body may be affected The changes are most marked at the back of the elbows and front of the knees

(iii) Fissures may occur round the heels The condition is seen most often in boys and in the dry season

E Pigmentary changes and colour

Nutritional failure can affect the colour of the skin in many different ways In pellagra there is typically an erythema with subsequent desquamation and

pigmentation The areas of skin especially involved are those exposed to sunlight or affected by friction In anaemia the skin may be unduly pale. The hands of underfed children are often cyanosed, even in warm weather, while in cold, damp climates they may be affected by chilblains

F Tropical Ulcer

(i) They are chronic ulcers, affecting chiefly the lower limbs, occurring in hot, damp climates among people whose tissues are vitiated by malnutrition

(ii) They are often caused by minor injuries in people living in poor hygienic surroundings, infested by diseases such as dysentery and malaria

(iii) They are not attributable to lack of single nutrient, but their presence in any community or labour force is an indication that the diet and hygienic conditions are unsatisfactory

G Angular stomatitis

(i) This is an affection of the skin at the angles of the mouth, characterised by heaping up of greyish white sodden epithelium into ridges, giving the appearance of fissures radiating outwards from the mouth

(ii) Secondary infection and staining by food may give the lesion a yellowish colour It may extend across the mucocutaneous boundary and produce whitish patches on the mucus membrane lining the cheeks

(iii) If often responds rapidly to large doses of riboflavin and sometimes to pyridoxine

(iv) It occurs in association with iron deficiency anaemia and other diseases

H. Cheilosis

(i) This is a zone of red, denuded epithelium at the line of closure of the lips

(ii) It is frequently seen in pellagrins and is often associated with angular stomatitis

(iii) It only appears during periods of drought and lack of fresh foods, but it is unlikely that lack of any one specific vitamin or nutrient is the sole cause The condition overlaps with chapped lips, seen in healthy people who have been exposed to cold winds or excessive sunlight

I Orogenital syndrome

(i) There is angular stomatitis, but in addition there are changes in the epithelium of the mouth, tongue and lips, and other mucocutaneous junctions are affected

(ii) The earliest sign is oedema and milky opacity of the buccal mucosa which goes onto patchy or diffuse desquamation of the lips, tongue and sometimes soft palate These areas are red and sensitive Secondary infection with superficial ulceration may occur

(iii) Soggy, whitish patches at the outer angles of the eyes, within the ears, at the vulva or prepuce of the penis, and around the anus are often present Along with these changes there is often corneal vascularisation and a scaly, greasy eczema at the angles of the nose, on the lips, chin and behind the ears

(iv) A dry, intensely itching, erythematous dermatitis, with a well-defined edge, may appear on the genitalia—the scrotum or mons pubis, over the perineum and down the inner sides of the thighs There is often secondary infection

(v) In health the hair is sleek and glossy, often with a natural wave or curl In malnourished or undernourished people the hair frequently becomes dull

and lustreless, it is not easily brushed and tends to stand up straight (staring hair) At the same time the colour of the hair may change In fair people it may turn to a dirty brown while in black haired people there may be loss of pigment, with a change of colour ranging from brown, rusty red to almost white This occurs in kwashiorkor

(vi) Dietary factors such as deficiency of pantothenic acid or biotin can change the colour of the hair of black rats to grey, but the white or grey hair of human middle age has no nutritional significance Nor is baldness a manifestation of nutritional failure

(vii) In chronic iron deficiency anaemia the fingernails may be spoon shaped (Koilonychia)

(viii) In other forms of malnutrition the nails may be brittle or thickened or lined on the surface, either transversely or longitudinally, but these changes may also be seen in well nourished people

(iv) Severe protein deficiency may result in transverse white bands in the nails, occurring symmetrically on both hands

Disorder of the eye

A Night blindness

(i) Night blindness is a frequent complaint in underdeveloped communities who have no night lights and where the diet is grossly lacking in retinol and β-carotene Children who stray from home after dark may get lost, or fall down a well or injure themselves in other ways

(ii) Many factors besides retinol deficiency may contribute to complaints of night blindness These include fatigue, emotional disturbances associated with acute danger and also chronic anxiety states

(iii) There are organic causes such as retinitis pigmentosa

(iv) Night blindness arising from Vitamin A deficiency always respond to suitable vitamin therapy and it is unwise to make the diagnosis before adequate therapeutic trials have been carried out

B Bitot s spot

(i) Greyish or glistening white plaques formed of desquamated thickened conjunctival epithelium, usually triangular in shape and firmly adherent to the underlying conjunctiva

(ii) Sometimes the spots are covered with material resembling dried foam which can be scraped away but forms again. It consists of epithelial debris, fatty globules and often masses of xerosis bacilli The spots are generally bilateral, on the temporal sides of the cornea, and in coloured races are often surrounded by dense brown pigmentation

(iii) Pigmentation of the conjunctiva is frequently associated with xeroph thalmia Pigment may be deposited round the cornea (Pigmented ring), in the lower eyelid (pigmented gutter), and over the sclera equatorially in the area commonly occupied by Bitot s spots Various forms of irritation appear to play a major role in its causation

C Corneal vascularisation

(i) The essential lesion in this condition is an invasion of the normally avascular cornea by capillary blood vessels These vessels cannot be seen with the naked eye, nor with an ordinary hand lens

(ii) Small greyish white opacities may also be seen on the surface of the cornea.

(ii) The patient often complains of a burning sensation in the eyes, misty vision, lachrymation and photophobia—the latter symptom may make slit lamp examination difficult

(iv) There is often injection of the conjunctiva with diluted blood vessel which are easily visible on simple inspection

(v) The presence of an injected conjunctiva should not allow the assumption that a vascular cornea is also present

(vi) Corneal vascularisation may be associated with the orogenital syndrome, with keratomalacia and with ariboflavinosis

(vii) Nutritional amblyopia is a major nutritional disorder of the eyes

Disorders of the mouth

A Nutritional glossitis

(i) Deficiencies of nicotinic acid, riboflavin, Vitamin B_{12} folic acid and iron may all give rise to glossitis

(ii) It is a feature of pellagra, sprue and the various types of nutritional anaemias

(iii) The tongue seems to be particularly susceptible to metabolic disorder of all kinds

(iv) If the deficiency is partial and extends over months or years, chronic atrophic glossitis is more often seen

(v) In acute glossitis the tongue is swollen, sometimes to such an extent that it is continually pressed against the lower jaw and well marked dental impressions are visible

(vi) The papillae are usually very prominent

(vii) The colour of the tongue is characteristically red, but in some cases it may have a purplish blue

(viii) The mucous membrane sometimes desquamates in pitches leaving areas of red raw surface

(ix) Deep irregular fissuring is common and shallow ulcers may occur, especially on the sides or tip

(x) The tongue may be extremely painful, so much so that fear of pain may prevent the patient from eating

(xi) In chronic atrophic glossitis the tongue is small, with an atrophic mucous membrane and small or absent papillae so that its surface appears smooth, moist and abnormally clean Fine fissuring may be present It is usually not painful

B Parotid gland enlargement

(i) The condition may be confused with mumps Histological examination of the swollen gland shows hypertrophy of the acini In the final state, fibrosis develops with cystic dilatation of the ducts—a parotid cirrhosis

(ii) The parotic glands are sometimes enlarged temporarily during the refeeding of people who have been severely undernourished

DIET AND OTHER DISEASES

INBORN ERRORS OF METABOLISM

Phenylketonuria (PKU)

PKU occurs due to a defect in the metabolism of amino acid phenylalanine The affected children show normal physical development but impaired mental development to a varying degree The untreated children become mentally defective adults

1. The biochemical and genetic defects

(i) PKU had been named by the finding of a ketone, phenylpyruvic acid, in the urine The primary defect is in the phenylalanine hydroxylating system which converts the amino acid into tyrosine

(ii) This system when cannot convert all of the phenylalanine derived from the protein in a mother's milk, the level in the blood rises and this causes the impaired development of the nervous system Some of the excess phenylalanine is deaminated to phenylpyruvic acid which is excreted in the urine

(iii) Hydroxylation is effected by phenylalanine hydroxylase (PH) and a coenzyme 5, 6, 7, 8 tetrahydrobiopterin (BH_4) The coenzyme is oxidized to BH_2 from which it is reformed by another enzyme, dihydrobiopterin reductase (DHPR) Hydroxylation may be impaired by genetic defects in production of PH of DHPR and of an enzyme responsible for the formation of BH_2

(iv) The genes responsible for the control of the hydroxylation are not closely linked and may be on different chromosomes The defects are transmitted by autosomal recessive inheritance Unaffected heterozygous individuals act as carriers

2. Classical phenylketonuria

(i) When the baby is at the age of 8 to 10 months, the parents may become anxious because their child is slow in learning to sit and handle things and is generally unresponsive

(ii) About 25 p c of the affected children develop eczema

(iii) The retarded development becomes obvious and there may be signs of severe birth damage, such as myoclonic epilepsy and marked hyperactivity

(iv) Most affected children grow up to become physically sound but are mentally defective

3 Variant forms

Besides classical PKU, other forms of hyperphenylalanemia are known and at least nine types have been recorded

4 Dietary Management

(i) Clinical symptoms do not arise in case the affected infant is put on a low phenylalanine diet soon after birth and kept on it for a long period

(ii) A severe emotional strain is imposed on a young child on an entirely artificial diet

(iii) As soon as the diagnosis is made, breast feeding should be stopped and the infant bottle fed with a low phenylalanine milk substitute

(iv) Greater difficulty arises when the baby has to be weaned A mother then has to prepare a low phenylalanine diet for her child from five lists of foods Therefore, she needs continuing help from a dietitian The lists are

(a) Basic foods containing negligible phenylalanine which can be used freely (these include sugar, sweets, jams, solid vegetable oils and cooking oils)

(b) Fruits and vegetables which can be taken freely since they provide negligible phenylalanine and protein in a normal helping

(c) A basic list of 59 mg phenylalanine exchanges of foods

(d) Manufacturer's foods of negligible phenylalanine content.

(e) Exchanges of foods containing 50 mg of phenylalanine (by calculation taking one gram of protein as 50 mg phenylalanine)

GALACTOSEMIA

This hereditary defect due to reduced activity of glucose-1-phosphate uridyl transferase impairs the metabolism of galactose ra sing its concentration in the blood Toxic signs appear soon after birth when an infant begins to take milk and are due to accumulation of galactose-1-phosphate within the cells This defect is less common than phenylketonuria

1 Clinical Features

(i) The disorder is found in infants in its severe form within two or three weeks after birth with the manifestation of vomiting difficulty in feeding, loss of weight and the onset of jaundice

(ii) The spleen may be palpable and the liver greatly enlarged and very firm and ascites may be present

(iii) Sugar (galactose) and protein are found in urine

(iv) If not treated properly, cataracts may develop which may lead to blindness

(v) Mental and physical retardation are likely to occur

(vi) Without immediate dietetic treatment of such severe cases death rapidly occurs

(vii) Lack of galactokinase also causes rarer form of this disorder in which mental development is normal and the liver is not damaged but severe cataracts leading to blindness occur early in childhood

2 Diagnosis

The raised blood galactose and the reduced glucose I phosphate uridyl transferase activity in erythrocytes confirm the disorder

3 Treatment

(i) Breast feeding should be stopped immediately and the infant should be given a milk powder in which lactose has been replaced by dextrin, dextrose and maltose

(ii) Milk milk products and food preparations containing these should be excluded from the diet It is necessary to continue these restrictions throughout life

(iii) The intake of galactosides, present in small quantities in most foods, and widely used in the food industry as a filler or flavouring agent, should be reduced

(iv) Lactose is used in the pharmaceutical industry in the formulation of many drug products Such preparations can normally be taken with safety but when a patient requires large doses for a long time it may be wise to use an alternative drug

REFSUM'S DISEASE

This is due to a defect in the enzyme systems responsible for the metabolism of phytanic acid (3, 7, 1, 15 tetramethyl hexadecanoic acid) which accumulates in the plasma and tissues Phytanic acid is derived from phytol, a product of the hydrolysis of chlorophyl

1 Clinical Features

(i) The main features are peripheral neuropathy, cerebellar ataxia, nerve deafness and retinitis pigmentosa

(ii) Symptoms usually first appear in childhood and progress slowly, patients become severely disabled between the age of 20 to 30 years

2 Treatment

(i) Great improvement is possible by removing phytanic acid by plasma exchange and by a diet low in chlorophyll

(ii) There should be restriction of many fruits and vegetables, butter and ruminant fat

MAPLE-SYRUP URINE DISEASE

A defect in the oxidative deamination of the branched-chain amino acids, leucine, isoleucine and valine, leads to accumulation of their oxoacids in the blood and these are excreted in the urine imparting an odour of maple syrup

1 Clinical Features

(i) Soon after birth the infant has difficulty in feeding, loss of reflexes, convulsions and coma follow and in severe cases death within a month

(ii) Long surviver are mentally defective unless fed with a formula diet low in leucine, isoleucine and valine

(iii) A few children has been reared successfully and when they are older require a low protein diet based on gelatin, gluten free flour, butter, sugar and fruits.

FRUCTOSE INTOLERANCE

The lack of the enzyme aldolase which converts fructose-1-phosphate to dihydroxyacetone phosphate and glyceraldehyde cause this disorder When fructose is ingested, fructose-1-phosphate accumulates in the liver This inteferes with release of glucose from the liver and leads to severe hypoglycemia

1 Clinical Features

(i) There may be vomiting and hypoglycemic fits

(ii) A series of episodes may lead to jaundice and enlargement of the liver

(ii) The teeth of the patient do not show caries

2 Treatment

Sucrose and fruit should be excluded from the diet

VON GIERKE'S DISEASE (GLYCOGEN STORAGE DISEASE, TYPE 1)

This is due to a defect in the low activity of glucose-6-phosphatase for which glycogen is not mobilised and large amounts accumulate in the liver Most patients survive into adult life in this rare disorder

1 Clinical Features

(i) Growth is retarded and there is marked enlargement of the liver so that the abdomen is protruded.

(ii) Hypoglycemia and ketoacidosis may occur in the new born and attacks may continue throughout life, often brought on by an infection or temporary starvation.

(iii) Mental development is retarded only if episodes of hypoglycemia have been frequent and severe.

(iv) As the child grows up attacks of hypoglycemia become less severe

2 Treatment

(iv) A diet high in protein accelerates glyconeogenesis from amino acids and so helps to maintain blood sugar

(ii) In severe cases, frequent feeds every 3 to 4 hours may be required

(iii) A moderate amount of carbohydrate is necessary, but this should be in the form of glucose or starch

(iv) Both sucrose and lactose should be avoided, because fructose and galactose are readily converted to glycogen in the liver

HYPERLIPIDEMIA

Hyperlipidemia may arise from an increased concentration of either cholesterol or triglycerides and frequently both are raised

There is slow rise of cholesterol with age starting at 20 years but ceasing at about 60 years when it may be followed by a slight fall The rise is less steep in women until the menopause when it may increase abruptly After 60 years there is little or no difference between the sexes

A high fasting plasma triglyceride is often associated with a high plasma cholesterol, but is not by itself a risk factor for CHD

Dietary cholesterol has little effect on plasma cholesterol over the range of intake since increasing intake inhibits endogenous production Very high intakes, only obtainable by eating two or more eggs daily, raise plasma concentration and this can be lowered by eliminating the diets—eggs, meat etc Dietary cholesterol is much less important than dietary fat in determining plasma cholesterol

People who subsists on diets high in starchy foods, like rice or maize, do not have high plasma triglycerides unless they are obese Sucrose in large amounts may have a greater effect than starch in raising plasma triglycerides Plasma cholesterol usually falls with increasing carbohydrate in the diet

Alcohol favours hepatic lipogenesis and this stimulates the synthesis of VLDL as well as leading to a fatty liver

Familial hypercholesterolaemia

This disorder consists of an increase in low density lipoproteins (LDL), the main carrier of cholesterol in the blood, and is due to genetic failure of LDL receptors

1. Clinical Features

(i) There is a greatly increased risk of coronary heart disease A heart attack due to myocardial infarction before the age of 30 is not uncommon and 50 p c of patients present evidence of CHD before they are 50 years old Thus it greatly reduces life expectancy

(ii) Xanthomata, swellings or small tumours containing cholesterol and sometimes triglycerides, commonly occur Common sites are tendons, especially the Achilles tendon and tendons on the back of the hands and skin, and around the eye where they are known as Xanthelasmata

(iii) Corneal arcus occurs early and when seen in someone under 40 years is probably due to the disorder

2 Management

(i) The coronary heart disease (CHD) may be prevented by lowering plasma LDL cholesterol level throughout life and beginning in early childhood This needs lifelong dietary restriction and the use of drugs

(ii) Since clinical symptoms rarely arise before the patient is an adult, early diagnosis depends on the examination of the blood Whenever the diagnosis is made in a new adult patient, a biochemical examination of the blood should be made on as many of the near relatives as possible, especially the children All affected members of the family should then be advised to begin lifelong dietary restrictions to reduce plasma cholesterol and often prolonged periods on a drug

(iii) The diet should be low in saturated fats and cholesterol, but polyunsaturated fats from vegetable oils and in other foods are permitted

(iv) Two drugs lower plasma cholesterol effectively *Cholestyramine* is an anion exchange resin that absorbs bile salts and increases their excretion in the stools Although this leads to increased synthesis of cholesterol, plasma cholesterol falls Gastrointestinal side effect may be severe *Nicotinic acid* in pharmacological doses (3—6 g/day) inhibits lipolysis and mobilisation of free fatty acids from adipose tissue and also reduces plasma VLDL Both drugs have been shown to be very safe in the short term Nicotinic acid has vasodilator effects which cause hot flushings in some individuals but these are not serious Reduction of plasma cholesterol for long periods increases the risk of stone formation in the bile and the incidence of gall bladder disease A third drug, clofibrate, that lowers plasma cholesterol is no longer prescribed since its continued use has been associated with an increased number of deaths from a variety of causes

DISEASES OF THE CARDIOVASCULAR SYSTEM
ATHEROSCLEROSIS

It is the most important of the degenerative diseases of arteries It consists of accumulation in the intimal lining of a variable combination of lipids, complex carbohydrates, blood and blood products, fibrous tissue and calcium deposits There are associated changes in the media of the arteries Arterioles are relatively unaffected

The three major clinical forms arise from narrowing of the coronary arteries, the cerebral arteries and the femoral artery and its branches, but other arterries can be affected, such as the renal or mesenteric

Coronary atherosclerosis is almost invariably associated with aortic atherosclerosis

Types of lesion

It is customary to separate the lesions of atherosclerosis into fatty streaks, plaques and complicated lesions

Fatty streaks are short, thin, slightly raised yellow lines running longitudinally along the internal surface of arteries and consist of an intracellular accumulation of lipids within the intima

Plaques are the lesions of atherosclerosis They are raised, focal, circumscribed lesions upto 1 cm in diamter, consisting of various amounts of fibrous tissue and lipid The lipid accumulates mostly in extracellular amorphous masses, plaques in which this process is prominent are called soft or atheromatous plaques In others, fibrous tissue is prominent and lipid is widely scattered or localised to the deeper portions of the lesion; these are called hard or fibrous plaques

Four other processes may complicate the lesions (i) the endothelium may be lost so that the surface ulcerates and the fatty contents may be exposed to the blood stream (ii) Fibrin is commonly deposited and thrombosis occurs on the plaque surface (iii) Free blood can be found in plaque (iv) Calcification may occur

Development with ageing

A few small lesions are seen in most adolescents and they increase in number and size throughout life It does not usually lead to clinical disease until middle age

Thrombogenesis

An arterial thrombus almost always forms at the site of an atheromatous plaque, these are present in most of us from an early age Small thrombi form frequently in the circulation These microthrombi are then attached to the arterial wall where they are rapidly lysed A failure of the fibrolytic mechanism could in this way allow a thrombus to grow Hence one of the primary faults responsible

for atherosclerosis might lie in an increased tendency to thrombosis or in an efficient fibrinolytic mechanism.

CORONARY HEART DISEASE (CHD)

Coronary heart disease (CHD) or ischaemic heart disease (IHD) are synonymous terms for a group of syndromes arising from failure of the coronary arties to supply sufficient blood to the myocardium These syndromes are in most case associated with atherosclerosis of the coronary arteries They include myocardial infarction, angina pectoris and sudden death without infarction

Myocardial infarction

(i) This is necrosis or destruction of part of the heart muscle due to failure of the blood supply (ischaemia)

(ii) It may lead to sudden death or heal leaving a scar

(iii) Patients with healed lesions may be severely disabled or may be able to return to their normal life with little or no restriction of their physical activities, but they carry an increased risk of a second infarct

(iv) The infarction is usually due to a thrombus forming in an atherosclerotic coronary artery and blocking the lumen

(v) Sometimes there is no thrombus and the infarct arises becuase the lumen of a coronary artery has been so narrowed by atherosclerosis that the blood flow is insufficient to supply the oxygen needed to maintain the cardiac muscle

(vi) Occasional cases of myocardial infarction are seen in which neither thrombosis nor significant narrowing of the lumen can be recognised

Angina pectoris (Pain in the chest)

(i) In this condition exercise or excitement causes severe chest pain

(ii) Patients may live for many years and remain free of further disability, so long as they keep within the limits of their exercise tolerance

(iii) The patients carry an increased risk of sudden death or myocardial infarction, especially if they undertake any unusual exertion

(iv) Emotional stress may also bring on angina

Sudden death

(i) A proportion of sudden death results from angina pectoris or myocardial infarction

(ii) The death is presumed to be due to CHD

(iii) The most death is unexpected but autopsy shows evidence of old myocardial infarction or extensive atheroma of the coronary arteries

1 Clinical Features

(i) The prominent symptom is a severe, pressing or constricting pain, poorly localised deep in the centre of the chest and radiating down the left or both arms

(ii) In angina pectoris this pain comes on with exertion or excitement, it forces the patient to stop and when he does so that pain passes off in a few minutes

(iii) Patients with angina can suffer several attacks of pain in a day and they can go on like this for long periods of time

(iv) In myocardial infarction the pain often comes on at rest, it is very severe, lasts for hours and is only relieved by strong opiates like morphine It is accompanied by general symptoms such as weakness, collapse, cardiac arrhythmias and circulatory shock

(v) Some patients have a mild myocardial infarction without noticing pain (silent coronary).

(vi) There are also several other causes of pain in and around the chest which can mimic CHD, such as Pericarditis, Pulmonary embolism, Oesophageal and respiratory disease.

(vii) The E.C.G. often changes temporarily during an attack of angina, it is usually normal between attacks. But soon after the onset of myocardial infarction the ECG may undergo a permanent change and show the infarct pattern.

(viii) Patients with angina or who have had a myocardial infarct are liable to develop cardiac failure or arrhythmias.

2. Risk factors

A. Sex and age:

(i) Men are more prone to CHD than women and incidence rises with age in both sexes.

(ii) Greatly increased incidence are found in women after the menopause and by the age of 70 there is no difference between the sexes.

(iii) The secretion of the ovarian hormones during the reproductive life of women aids in the relative immunity against the rise in cholesterol level.

(iv) The concentration of plasma total cholesterol is lower in women aged 20 to 45 years than men of the same age group but HDL cholesterol is higher.

(v) If both ovaries of women are removed before the age of 35, the plasma total cholesterol will increase with the increase in CHD.

B. Family history:

(i) Some families are more susceptible to CHD than others.

(ii) Family clustering of any disease may be due either to inherited susceptibility or family sharing of environmental experience, e.g., an atherogenic diet. More than 50 per cent of plasma cholesterol is genetically determined.

(iii) In families susceptible to CHD there is often a high incidence of other diseases which have multiple aetiology, e.g., hypertension, diabetes mellitus, gout and hyperlipidemia.

C. Behaviour patterns and personality traits

People with behaviour pattern A above an increased incidence and prevalence of CHD. Such people show an excessive sense of time urgency, a preoccupation with vocational deadlines and enhanced aggressiveness and competitive drive. This behaviour pattern is probably constitutional.

D. Hyperlipidemia

A patient with a very high plasma cholesterol is at high risk of developing the disease at an early age but for the majority with values not greatly raised the increased risk is small.

E. Hypertension

The incidence of CHD in men aged 45 to 65 years with blood pressures exceeding 160/95 was more than five times that in normotensive men (blood pressure 140/90 or less). Elevations in both diastolic and systolic pressures correlate positively with CHD, the diastolic pressure perhaps being more important in younger people.

F Obesity

(i) Obesity is associated with an increased risk of death from heart disease

(ii) A moderately increased plasma cholesterol or mild hypertension or Grade I obesity (plumpness), each by itself, carries only a small risk of CHD, a combination of any two of them increases it much more, and in a patient with all three the prognosis is very uncertain unless they are reduced by appropriate treatment

G Diabetes Mellitus

This is an important risk factor both in its clinically recognised and latent forms This association is not due solely to blood lipid disturbances Diabetics are particularly prone to hypertension and proliferative lesions of the small blood vessels

H Hyperuricaemia and gout

There is a positive association between these abnormalities and CHD, although not all hyperuricaemia represents pre gout

I Electrocardiographic abnormalities

(i) Persons with an abnormal ECG or with ECG changes or left ventricular hypertrophy have an increased risk of developing CHD

(ii) If the changes are present when the patient is at rest, they may represent early CHD

(iii) Abnormalities which appear on exercise usually indicate relatively advanced ischaemia

J Cigarette smoking

(i) Peripheral vascular disease and CHD, particularly myocardial infarction, occur much more frequently in heavy cigarette smokers than in those who do not smoke

(ii) There is a clear relationship between CHD and the amount of smoking

(iii) The mechanism by which nicotine or some other constituent of tobacco causes this adverse effect is not clear However, it may be due to the vasoconstrictor action of nicotine, to inhalation of carbon monoxide, or to some undesirable effect on the coagulability of the blood

(iv) Heavy smoking may be a manifestation of a susceptible personality and a reaction to stress and strain

(v) Patients with CHD should give up smoking

K Diet

(i) A high intake of saturated fatty acids may raise plasma cholesterol and so promote the formation of atheroma

(ii) A low intake of polyunsaturated fatty acids may modify platelet function and so promote the formation of thrombi

(iii) An intake of energy above requirements leads to obesity and high intake of salt may lead to hypertension in susceptible individuals

(iv) It has been found that some individuals suffer a heart attack early in life In that case, diet is less important than genetic hypercholesterolaemia or cigarette smoking in the aetiology of coronary artery disease

(v) Excessive intake of alcohol should be avoided

(vi) A higher proportion of energy should come from bread, potatoes, and other vegetables and lower proportion from separated fats and fatty meats

L Lack of exercise

(i) Physical activity protects against CHD Bus conductors have a lower incidence than bus drivers, and postmen who deliver letters have a lower incidence than telephonists and post office clerks

(ii) Active recreations are also important In middle aged Harvard alumni the incidence of heart attacks was lower in those who have been physically active

M Emotional stress and tension

(i) The modern prosperous communities suffer from more stress and strain than their less wealthy predecessors Increased risk of myocardial infarction could be due to acute or chronic adrenergic overdrive caused by stress Possible mediating factors include plasma noradrenaline and FFA.

(ii) The stress of racing driving raise plasma triglycerides and FFA but not plasma cholesterol The same reaction probably occurs in some urban drivers

N Drinking water

The harder the drinking water the lower the death rate from cardiovascular disease Calcium and magnesium in hard waters may have a protective action and there are several trace elements in hard water that may be beneficial

Soft waters, being more acidic, are more likely to dissolve potentially toxic trace elements like lead and cadmium from pipes or rocks

Since there is no concrete evidence to be based on its will be premature to advise modification of water supplies in the hope of p eventing cardiovascular diseases

O Coffe and alcohol

(i) Myocardial infarction is associated with high consumption of coffee has not yet been confirmed

(ii) Moderate alcohol consumption is not a risk factor, and in patients with disorders directly attributable to alcohol the incidence of CHD is no higher than normal

3 Prevention and management

(i) Smoking must be stopped.

(ii) The diet should be moderate so as to prevent being overweight.

(iii) Physical exercise should be taken as much as possible without causing undue breathlessness or fatigue

CEREBROVASCULAR DISEASE

Cerebrovascular disease is second only to heart disease as a cause of premature deaths Atherosclerosis is common to both conditions, the overwhelming risk factor is a high blood pressure Cerebrovascular disease has little association with hypercholesterolaemia, but much more with obesity than does CHD

HYPERTENSION

Hypertension is an important cause of several major diseases and it is amenable to treatment Hence measurement of blood pressure is a part of all routine clinical examinations of adults

The pathological changes accompanied by hypertension are a thickening of the arterioles with hyaline material and, later, hypertrophy of the myocardium of the left ventricle If untreated, moderate hypertension eventually leads to cardiac failure, with dilatation of the left ventricle and congestion of the pulmonary or systemic veins It also causes ischaemic changes in the kidney, nephrosclerosis

The blood pressure if very high is said to be *malignant hypertension.* The heart, Kidneys, retinal and other arteries are quickly affected and it shows immediate risk of a dangerous vascular accident. Therefore, vigorous treatment is most essential.

80 per cent of cases suffer from *essential hypertension.* Treatment has to be symptomatic. In the remaining cases the hypertension is usually secondary to renal disease, e.g. glomerulonephritis or pyelonephritis, and less commonly, to an endocrine disorder, e.g. Cushing's syndrome, acromegaly, pregnancy also shows hypertension.

I. Criteria for diagnosis:

(i) In healthy young adults the systolic blood pressure is about 120 mm Hg and the diastolic pressure about 80.

(ii) There is usually a gradual rise of blood pressure as age advances and at 65 years the mean figure is about 160/90.

(iii) The rise with age is very variable. In some people it is more rapid and reaches a higher level than the above figure. It is difficult to state at what point the level ceases to be normal and hypertension begins.

(iv) The diastolic pressure is a more reliable guide to the presence or absence of hypertension than the systolic pressure.

(v) A diagnosis of hypertension is usually made in a patient under 25 years of age when the diastolic pressure is 90 or above, but in a patient aged 70 years or more not unless it is over 100. Unless diastolic pressure is above 105, hypertension may be said to be mild or moderate and when it exceeds 120 to be severe.

A. Heredity:

(i) Hypertension frequently affects several members of a family and there is a high correlation between the blood pressures of identical twins.

(ii) Individual susceptible to hypertension is related to decreased activity of (Na^+, K^+) ATPase, the enzyme that through the sodium pump maintains intracellular concentrations of Na^+ and K^+. The activity of the enzyme is genetically determined.

B. Obesity:

(i) Hypertension commonly accompanies obesity though it may occur in thin people.

(ii) Obese patients with hypertension, if reduce their weight to normal value, their blood pressure frequently returns to normal without any other treatment. This indicates that in susceptible individuals obesity is a direct cause of hypertension.

C. High salt intake:

High salt intake in same countries causes hypertension; but in some other countries high salt intake shows no effect. It is, therefore, assumed that there is a threshold for salt intake, as yet undefined, above which hypertension commonly arises in susceptible individuals and that susceptibility is genetically determined.

D. Other possible risk factors:

(i) Stress, either mental or physical, raises the blood pressure immediately but this is only temporarily.

(ii) Environmental stresses may contribute to hypertension and of the theory that individuals with a poor capacity for relaxation are more prove to bypertension indicate that stress is not a major risk factor.

(iii) Inactivity may be a risk factor but is only a minor one.

2 Treatment:

A Drugs:

(i) Hypotensive agents lower blood pressure by differing actions on the cardiovascular system They relieve the symptoms in most patients and their widespread use is mainly responsible for falling death rates from hypertension. However, all have adverse effects and it is sometimes difficult to find a drug which is effective in a dose that a patient can tolerate

(ii) Diuretics are given along with hypotensive agents and act by increasing sodium output in the urine They also increase potassium output, and their long-term use tends to cause potassium depletion unless a supplement is given.

B Diet:

(i) If a patient is overweight, dietary restriction lowers the blood pressure This is the only treatment for patients with mild hypertension. When weight and blood pressure have both returned to normal, if weight is kept down, blood pressure is unlikely to rise again.

(ii) In the manifestations of hypertension, there is a strong psychological component Many patients need much support to enable them to change old habits and eat a sensible diet and lead a sensible life Daily periods of relaxation and regular holidays are good for hypertensives

(iii) Moderate reduction of sodium intake is of benefit to patients This regime involves no hardship and can be prescribed for all patients in addition to any drugs that are needed.

3 Prevention·

(i) The dietary restriction is the reduced intake of salt

(ii) It is advised widely not to use table salt and to use a minimum salt in home cooking It is more important for the public to know that prepared meats contain about 20 times as much salt as is present in the original fresh meat Chips and crisp potatoes are very rich sources of salts Salted and canned fish are fine for an occasion but if eaten frequently may increase salt intake greatly Many snack foods are rich in salt.

(iii) As a preservative salt is added to manufactured foods. But it should be such as to be ineffective Manufacturers are not unresponsive to medical opinion and have greatly reduced the previously high salt content of infant foods when it was shown that this was a cause of hyperosmolar dehydration, a serious disorder in infants A significant amount of salt is added to bread, butter and cheese. It is to be looked upon as medicinal products that low salt bread, butter and cheese are obtained easily If there is a popular demand for low salt foods of all sorts, manufacturers will produce and advertise them.

(iv) Legislation to restrict the addition of salt to foods will be difficult to enforce A more practical approach will be to provide more money and support for appropriate health education

HEART FAILURE

In severe CHD the heart fails because its muscle is weakened, in hypertension it fails because the muscle is working against an increased load In mild degrees of heart failure the only manifestation is an inability to increase the cardiac output in response to strenuous exertion. In moderate grades there is venous congestion Acute and severe cardiac failure causes pulmonary oedema and cardiogenic shock

1 Treatment.

(i) Complete rest.

(ii) The administration of diuretics.

(iii) A diet low in sodium, and also in energy if the patient is obese

(iv) In special circumstances a digitalis preparation

Heart failure is usually precipitated by a respiratory infection, arrhythmia or some other complication If these are diagnosed and effectively treated, the patient may well get over the episode and be able to lead a normal life

2 **Dietary management:**

(i) Since there is oedema developed sodium intake should be reduced Because oedema is always associated with sodium retention

(ii) Sufficient fluid must be allowed to mitigate thirst and to make the patients comfortable

(iii) Owing to the congestion of the digestive organs, each feed must be small in quantity and easily digestible In the initial treatment, fluid or semifluid food is advisable for patients who are seriously ill

(iv) Patients who are seriously ill after a severe myocardial infarction, it is important to prevent ketosis arising and for this purpose they should be given ample glucose or other carbohydrate

DIABETES MELLITUS

Diabetes mellitus is a syndrome due to different diseases characterized by a raised glucose concentration in the blood, due to diminished effectiveness of insulin The disorder is chronic and also affects the metabolism of fat and protein It has an increased risk of atherosclerotic diseases and of certain obstetrical difficulties

It is the commonest endocrine disorder There are two major types—Type I insulin dependent diabetes which was formerly known as juvenile onset diabetes occurring between 10 and 12 years of age Type 2 non insulin diabetes occurring in middle age or later

Genetic and dietary factors, infections and possibly stress may increase the risk of developing diabetes

Primary diabetes:

1 **Genetic factors :** Many separate genetic mechanisms increase the risk of diabetes and its various manifestations, and these differ in type 1 and type 2 diabetes

2 ***Obesity:*** *Although most type 2 diabetics are obese, only a minority of obese* patients develop diabetes In simple obesity there is insulin resistance, particularly in muscle, and hyperinsulinemia There is impaired insulin uptake by receptors in target tissues In general, the more carbohydrate tolerance is impaired in obese diabetics, the more deficient the insulin secretory response to various stimuli Obese people in general are less physically active than those whose weight is normal It is possible that physical exercise may reduce the risk of diabetes in susceptible individuals

3 **Dietary restrictions :** Restrictions on the food supply of a community, affect diabetes *Rationing* is beneficial to individuals susceptible to diabetes

4 **Sugar intake :** A high intake of sugar is definitely associated with a high prevalence of obesity Sucrose has a specific diabetogenic effect, though the very high intake may contribute to the high prevalence of diabetes

5 **Dietary fibre:** The high fibre content of the diet causes the reduced prevalence of diabetes Most diets now recommended for diabetics are high in fibre

6 **Infections :** Diabetes is frequently diagnosed by finding glucose in the urine with an acute staphylococcal or other infection Infections cause a non specific outpouring of catabolic hormones which antagonise insulin action and this may trigger the onset of the disorder More evidences show that type I diabetes speci-

ally in younger patients in caused by virus infections The virus may trigger an autoimmune reaction in the pancreatic islets and this impairs insulin secretion and ultimately destroys the beta cells

7 **Stress :** Stress causes a sudden increase in secretion of catabolic hormones which may precipitate the disorder It probably does not cause diabetes in people who would never have developed it The impaired secretion by pancreatic islet cells may cause the disorder Many environmental factors may lead to such impairment Genetic factors appear as the main determinant of susceptibility to such environmental factors, those leading to overweight and obesity being the most important in type 2 diabetes and viral infections in type I

Secondary diabetes:

A minor cases of diabetes occur as a result of pancreatitis, haemochromatosis, carcinoma of the pancreas and pancreatectomy

Diabetes may also accompany endocrine disorders which increase concentrations of catabolic hormones or modify the regulation of insulin receptors

CLINICAL FEATURES·

Type 1 diabetes : This usually appears in early age (between 10 to 12 years of age) in patients of normal or less than normal weight Symptoms are usually severe and develop rapidly Severe Ketoacidosis occurs and is often fatal without insulin treatment Since insulin is required for their survival an alternative name for this patient is *insulin dependent*

Type 2 diabetes· This usually appears in middle age or later in patients who are often obese and their hyperglycemia is controlled by dietary means alone or by an oral hypoglycemic drug The patient are less prone to develop Ketosis. Therefore, type 2 in less severe disease the type 1

SYMPTOMS

(a) Some patients complain of some or all of the classical symptoms which are thirst, polyuria, nocturia, tiredness, loss of weight, reduced visual activity, white marks on clothing, polydipsia.

(b) Many patients are first found to have glycosuria in the course of some routine examination, for insurance, for employment purposes They may have had few or no symptoms

(c) Ketoacidosis may occur in diabetics in acute infection. Epigastric pain and vomiting may be the main complaints These are usually type 1 diabetes.

(d) Patients may complain one of the symptoms like failing vision, parasthesia in the limbs or pain in the legs, impotence, infection of the skin, lungs or urinary tract

Physical signs There is dehydration, loose dry skin, dry furred tongue with cracked lips The pulse is rapid and the blood pressure is low Breathing may be deep and rapid The sweet small of acetone may be noticeable in the breath. There may be coma

Early signs of diabetic neuropathy are depression of the ankle jerks and impaired vibration sense in the legs The presence of neuropathy may be indicated by proteinuria

DIAGNOSIS

Urine testing : (a) Glycosuria (b) Ketonuria.
Random blood sugar : The oral glucose tolerance test.

MANAGEMENT:

Diabetic patients no longer die in Ketoacidosis in any number as they once did, The increased death rate of treated diabetic patients is due to coronary heart

disease Many of those whose duration of life has been extended are chronic invalids They may live for many years with cerebral, coronary, or peripheral vascular disease, or with renal disease or serious visual impairment

TREATMENT:

1 Diet alone

2 Diet and oral hypoglycemic drugs

3 Diet and insulin

About 40 per cent of new cases of diabetes can be controlled adequately by diet alone, about 30 per cent require insulin and another 30 per cent need an oral hypoglycemic drug Insulin is needed for juvenile diabetes older patients do not require insulin except when control of their diabetes is disturbed by an illness, infection or operation

1 **Diet·**

(a) In all diabetics the amount and time of food intake, especially the carbohydrate, should be controlled to prevent the fluctuations of blood glucose beyond the normal range

(b) Intake of refined sugars should be low because their consumptions is followed by absorption and a high peak of blood glucose

(c) Patients should avoid fasting or feasting, their intake from day to day should be maintained with adjustments for exercise and appetite, they should not miss a meal or over-indulge

(d) Type 1 patients require insulin and their food, particularly carbobydrate, should be adjusted to match the time of action of their insulin This depends on the type of insulin being used and whether the patient is having a single injection or more than one each day The balance between insulin and meals has to be adjusted from time to time They usually want to take moderate and sometimes strenuous exercise They therefore require a generous amount of dietary energy

(e) Type 2 patients are usually obese Being middle aged or elderly they may not take much exercise For these reasons the daily energy intake should be restricted to about 1000 K cal The flying career of Airline Pilots depends on avoiding insulin or drugs

(f) The nature of carbohydrate is important Sucrose should be eliminated or greatly reduced Starchy foods rich in dietary fibre and beneficial for diabetics There are two essential points about a diabetic diet First, energy intake should be adjusted to maintain ideal body weight Secondly, all patients taking insulin should follow a regular pattern of meals, matched to the injected insulin

(i) **Carbohydrate:**

(a) A minimum of 100 grams is needed to prevent ketonuria

(b) Foods rich in sucrose and other sugars should be kept to a minimum

(c) 60 grams of fructose should be taken a day obese diabetics should not use fructose and sorbitol as they have the same energy value as other sugars , Although fructose may not raise blood glucose as much as sucrose or glucose, it may raise plasma triglycerides more

(ii) **Protein:**

Amino acids stimulate insulin secretion in both normal subjects and in those with type 2 diabetes A smaller rise in blood glucose also occurs when carbohydrate is consumed along with protein A minimum amount about 50 grams of should be specified in all diabetic diets unless the patient is

(iii) Fat:

As diabetic patients have an increased risk of death from coronary heart disease and as this may be related to the amount of saturated fat in the diet, the total amount of fat should be restricted even in those who are not obese

2 Types of diet:

There are two types of diet

(a) **Measured diet :** The amount of food to be eaten at each time of the day is specified

(b) **Unmeasured diet:** The patient is supplied with a list of foods grouped three categories—foods with a high concentrated in carbohydrate content which are to be avoided altogether, foods with a relatively stable unconcentrated carbohydrate content which are to be eaten in moderation only, and non-carbohydrate foods which may be eaten as desired

Alcohol : Patients may take alcohol if they need to have energy value and carbohydrate content Beer may contain 10 to 30 grams of carbohydrate per half litre and this provides 150 to 400 K cal depending on the strength of the beer

Sweetening agents : Saccharin and aspartame may be used but have no energy value.

Drugs : A good number of compounds reduce hyperglycemia in patients who would require insulin The sulphonylurea compounds, tolbutamide, chlorpropamide, glibenclamide, glipizide, gliquidone, metformin have a place in the management of 30 per cent of diabetic patients It is dangerous to attempt to control juvenile—onset diabetes with these compounds

Insulin • Most diabetics now manage with two injections daily, one before breakfast and one before the evening meal, each containing soluble and depot insulin

(a) **Soluble insulin :** This is a clear solution whereas depot insulins are cloudy It begins to lower the blood glucose in 30 minutes, the effect is maximal in 4 to 6 hours and ends after 6 to 10 hours Soluble insulin is essential in the following circumstances

(i) For new cases with severe dehydration or Ketoacidosis

(ii) For emergencies associated with ketosis, such as acute infection, gastroenteritis or some surgical operations

(iii) For the treatment of nearly all young patients

(b) Depot insulins : Depot insulin do not lower blood sugar before 5 to 6 hours, the effect is maximal at 8 to 14 hours and ends only after 20 to 30 hours.

Choice of therapeutics:

(a) All young patients who develop diabetes before the age of 40 years require treatment with insulin The majority are best controlled by a combination of short-acting and intermediate-acting (depot) insulin injected twice daily, before breakfast and before the evening meal

(b) Most patients developing the disease over the age of 40 years can be controlled by diet alone Obese patients should be treated by dietary restriction and weight reduction, but others may do well on dietary therapy alone Insulin and the sulphonylureas increase the appetite, and thus may increase weight and intensify disability

(c) Those over the age of 40 who are not controlled by dietary measures alone usually respond well to sulphonylureas If adequate control is not achieved by one drug, a combination of sulphonylurea and biguanide may be tried. If this fails, insulin is needed

(d) Elderly patients who require insulin often do well with a small dose (20 units) of a depot insulin alone But those who require more than 40 units a day, should be given soluble insulin in addition

Clinical features of Ketoacidosis :

The most common cause of Ketoacidosis is neglect of treatment due to carelessness, misunderstanding or illness.

There is intense thirst and polyuria Constipation, muscle cramps and altered visions are common Sometimes, there is abdominal pain with or without vomiting Weakness and drowsiness are commonly present

The signs include dry tongue, soft eyeballs due to dehydration, hyperventilation indicated by rapid, deep, sighing respirations and a rapid, weak pulse, with low blood pressure and acetone may be smelt in the breath Sometimes there is abdominal rigidity and tenderness Ultimately, coma appears

Laboratory tests show heavy glycosuria and ketonuria, blood glucos• usually between (360 and 720 mg/100 ml), and low plasma bicarbonate and blood pH

Treatment:

This condition should be treated with the utmost urgency in hospital Intravenous therapy is required although the patient is able to swallow Extracellular fluid is repleted first with sodium chloride infusions It is better to give low dose insulin starting with 6 to 9 units per hour and halving the dose when the blood glucose has returned to normal To the majority of cases potassium therapy should be started from the outset Intracellular fluid is replaced once the blood glucose has fallen below 250 mg/100 ml by infusing glucose solution Intensive medical care is needed and the blood glucose, pH, electrolytes and ketones have to be monitored, hourly at first

Vascular disorders•

Atherosclerosis occurs commonly and extensively in diabetics Diabetics are more prone to myocardial infarction and gangrene of the toes and feet at an earlier age than other people

The peripheral pulses in the legs are often diminished, ischaemic changes in the feet are frequently apparent Defective circulation in the legs results in the dangerous complication of gangrene Diabetic gangrene usually starts in one foot Toxic absorption from necrotic tissue and secondary infection may kill the patient unless the limb is amputated Amputation of a toe, a foot or even a whole leg is sometimes necessary to save life

Cataract:

Cataract is more prevalent in old people having diabetes Rarely a specific type of opacity of the lens occurs in diabetic children whose disease has not been adequately controlled

Infections:

Poor control of diabetes has lowered resistance to infection The following forms are especially important

(a) **Carbuncle :** The development of a carbuncle may unmask diabetes and may even precipitate ketosis and coma cleanliness is very important in the prevention of skin infection in diabetes Once infection has occurred a suitable antibiotic is needed

(b) **Urinary tract infections :** The presence of glucose in the urine provides a favourable medium for the growth of bacteria Intractable infections of the urinary tract frequently occur and for this reason catheterisation should be avoided Once infection has been started treatment consists of controlling the glycosuria and the administration of suitable antibiotics

(c) **Pulmonary tuberculosis :** In countries where this is prevalent all new diabetic patients should have a chest radiograph

(d) **Vulvitis :** Candida albicans is nearly always present in diabetic women In the majority, the treatment is abolition of glycosuria which brings rapid relief

PROBLEMS IN MANAGEMENT:

1 Children:

(a) Diabetes is not common in childhood, but when it occurs it is relatively severe and always requires treatment with insulin The problem of matching the dose of insulin to the food intake raises practical difficulties

(b) As the children should be growing, their energy requirements are large and difficulties arise in meeting them It is important to make sure that the child does not become too fat because too much insulin can lead to excessive appetite and hence to obesity It is better to encourage them to take responsibility of their own care They should be trained to swim under supervised pools

(c) Children are not expected to lead a steady life and their activities fluctuate unexpectedly Excessive activity may result in hypoglycemia, and lethargy in hyperglycemia Hyperglycemia may be caused by infectious disease A combination of one of the depot insulins and soluble insulin before breakfast and a second dose of soluble insulin before supper

2 Pregnancy:

(a) Pregnancy in a diabetic woman carries certain risks In the later stages, she may develop an excessive accumulation of amniotic fluid, in addition the fetus is sometimes unusually large leading to difficulty in labour There is also an increased risk of her baby having a neural tube defect or other error in development A planned pregnancy reduces the risk

(b) A pregnant diabetic patient requires close supervision by a team consisting of physician, obstetrician, anesthesist, nurse and dietitian After the diagnosis of pregnancy, the patient should be seen at first fortnightly and later at, weekly intervals In the later stages of pregnancy, lactosuria occurs and may lead to confusion Therefore, blood glucose estimation should be done by finger prick

(c) Now a days many pregnancies are allowed to go on to full term with improved glycemic control and caesarian section is less used

3 Diabetes and surgery:

Any surgical operation causes a metabolic stress which the diabetic is less able to meet The position is worse if there is tissue wasting with much breakdown of fat and protein It is to be kept in mind that there is the need to provide an adequate supply of energy for the tissues and the need to be on the alert for acidosis Diabetes is to be first diagnosed before operation

PROGNOSIS:

The prognosis of diabetes has improved steadily since the introduction of insulin It is difficult to estimate the prognosis of an individual patient because so many variable factors have to be considered The incidence of the complications of diabetes is mainly related to the duration of the disease but probably also to the precision with which it has been controlled

PREVENTION:

Diabetes is a disease of the prosperous and in wealthy countries It is one of the major health problems Sufficient exercise and avoidence of excess diet

have repeatedly been stated Diabetes, like obesity and atherosclerosis, is likely to occur in persons who eat too much and exercise too little

GOUT AND HYPERURICAEMIA

Gout is a characteristic arthritis which affects single joints, often the big toe giving pains that last only a few days but are liable to recur Middle aged men are chiefly affected It is caused by the deposition of urate crystals in the joint The increased concentration of urate in the plasma causes hyperuricaemia

Hyperuricaemia may be asymptomatic Such individuals carry a greatly increased chance of the clinical complications, gouty arthritis or uric acid stones in the urinary tract Those who have recurrent gout and hyperuricaemia over a long time are liable to develop tophi, accumulations of urate in tendons or cartilage

CLINICAL FEATURES

(a) The patient has suddenly stabbing pain in one joint The big toe is easily stubbed or injured by an ill fitting shoe The wrists, ankles and knees are much less common sites. The spine is practically immune

(b) The joint is swollen and exquisitely tender, pain is aggravated by the least movement

(c) The overlying skin is tense, red and shiny with distended veins and may later show oedema

(d) Fever, malaise, loss of appetite, gastrointestinal upset and scanty highly coloured urine are common

(e) The patient may be very irritable

(f) Blood E S R rate is raised

(g) As the inflammation subsides the skin over the joint becomes scaly and itches The joints recovers completely

(h) If the hyperuricaemia is high and not regulated, attacks tend to occur with increasing frequency and to last longer Eventually a stage may be reached where there is chronic persistent though generally less painful arthritis of several joints

Complications

People with hyperuricaemia have a greatly increased liability to form *uric acid stone* in the urinary tract These can present with renal colic and are one cause of the chronic renal disease that is an important late complication in gout In gout the kidneys may receive decreased amounts of glutamine because more has been diverted into purine synthesis

Degenerative renal disease occurs in patients with chronic gout, and hypertension itself appears to reduce uric acid clearance Raised plasma urates have also been found in patients with cerebral vascular disease

DIAGNOSIS

(a) When the big toe is affected, clinical diagnosis is confirmed by a raised plasma urate

(b) There is another type of arthritis caused by deposition of crystals of calcium pyrophosphate in joints, sometimes called *pseudogout*

(c) Large joints are usually involved, especially the knees, which show calcification of articular cartilage It does not respond to colchicine and the plasma urate is *normal*

TREATMENT

1 Drug

(a) There are drugs which relieve the acute arthritis, but do not affect plasma urate concentrations. The oldest effective remedy for acute gout is colchicine. It acts by inhibiting the polymorph response to urate crystals and thus breaks the vicious cycle The dose is 1 mg two hourly until relief is obtained or diarrhoea supervenes If the response with colchicine is too slow or diarrhoea too troublesome, it may be replaced by a short course of phenylbutazone or of corticotrophin or one of the adrenocorticosteroids During the acute attack the patient gets little rest from pain which is always worse at night. He should be in bed and the joint made as comfortable as possible by supporting it on pillows and protecting it from pressure and knocks

(b) The second group of drug is *Probenecid* which impairs the reabsorption of uric acid by the renal tubules and so increases uric acid excretion in the urine by 30 to 50 per cent. As its administration is continued, plasma urate falls and later the frequency of arthritis decreases and tophi regress

(c) When there is gross overproduction of uric acid, the newer drug *allopurinol* is the best for treatment It inhibits *xanthine oxidase* so that uric acid production is reduced and the patient excretes instead xanthine and hypoxanthine which are more soluble Allopurinol has no direct effect on gouty arthritis and prophylactic doses of colchicine 0 5 mg three times a day should be given for the first two or three months Other adverse effects are unusual with these drugs

2. Diet

(a) If the patient is overweight, he should be advised to bring his weight down by a gentle dietary regimen

(b) Fasting should be avoided Heavy, rich meals high in purines or fat are likely to raise the plasma urate and may be followed by an acute attack.

(c) Excessive alcohol precipitates gout The patient should be advised not to drink alcohol at all

(d) It is always better to avoid foods rich in purine, e g , liver, kidneys, sweetbreads, fish and meat extracts

(e) The patient should be advised to drink water before going to bed Coffee and tea although contain methyl xanthines such as caffeine can be drunk because caffeine is not converted into uric acid in the body

(f) Lastly, it is wise to check fasting plasma lipids and treat hyperlipemia if present and to give some advise to gouty patients on diets which may reduce the risk of coronary heart disease

DISEASES OF THE KIDNEYS AND URINARY TRACT

Principles of dietetics in renal disorders

(a) The major task of the kidneys is the elimination of surplus nutrients from the body When renal function is greatly reduced, homeostasis may be maintained by reducing oral consumption of water, sodium, potassium and magnesium. Urea production is reduced by minimising the intake of protein

(b) In other renal disorders the capacity of the kidney to excrete sodium is impaired. This can be treated by reducing the dietary intake of sodium.

(c) The nephrotic syndrome damages the glomerular capillaries and result in massive loss of albumin and other proteins from the plasma into the urine Increased dietary intake of protein allows increased hepatic synthesis of albumin which compensates the urinary loss

(d) Diseases involved in the renal tubules can result in excessive urinary losses of water, sodium, potassium, phospbate and other substances These losses can easily be replaced by increasing the oral intake

1 **Aente glomerulonephritis**

This is characterized by acute inflammation of the glomeruli with congestion Renal blood flow and glomerular filtration rate are reduced by 50 per cent The urine volume falls and sodium excretion is greatly reduced The urine contains moderate amounts of protein, red and white blood cells in abundance and casts of the renal tubules formed by precipitation of protein and red cells in the tubular system

When the patient continues to ingest normal quantities of sodium and water, oedema develops and the blood pressure rises leading to headaches and swelling of the face and hands in the morning and of the ankles at night

Treatment:

Fluid intake should be restricted to 500 ml daily During the first few days of treatment, the fluid given should be less Sodium intake can be relaxed when oedema resolves and the blood pressure falls Protein is restricted only when the bloed urea is raised

2 **Nephrotie syndrome**

This is characterised by heavy proteinuria, hypoalbuminaemia and peripheral oedema It occurs when glomerular capillaries are damaged resulting in the increased losses of plasma proteins from the body into the urine This syndrome can arise in diabetes mellitus, amyloidosis, multiple myeloma.

Treatment

Salt should not be added at table and only small quantities should be added during cooking Fresh meat and fish can be used to supplement the protein intake Eggs can be used freely but cheese should be reserved for special occasions

3 **Acute renal failure**

This is a catastrophie event The causes are

(a) Loss of blood from any cause including complications of pregnancy, trauma or gastrointestinal bleeding

(b) Loss of plasma as in burns and crush injuries

(c) Loss of fluid from severe vomiting, diarrhoea, acute intestinal obstruction

(d) Serious infections especially septicaemia

(e) Acute haemolytic disorders

Treatment:

Protein intake should be reduced A daily intake of 100 grams of sugar has a marked protein sparing effect If the patient is vomiting, dextrose in water has to be given intravenously The diet should contain potassium chloride by mouth

4 **Chronic renal failure (Uremia)**

Uremia is a term used to describe general renal failure from any cause. As a result, many complex biochemical changes occur which are more responsible for the clinical features than the elevation of blood urea. These changes include disturbances in hydrogen ion concentration and abnormalities in water and electrolyte baiance

Clinical features

(a) The failing kidney is unable to compensate for large fluctuations in salt intake and for other increased metabolic demands.

(b) Uncompensated losses of water and sodium result in dehydration and salt depletion, with a fall in plasma volume, arterial blood pressure, renal blood flow and glomerular filtration rate

(c) Renal function is lost and mild renal failure progresses to severe uremia.

(d) Tiredness, breathlessness on exertion may arise from anemia. A tendency to bleed due to abnormal platelet function

(e) Anorexia, nausea and vomiting may result from the accumulation of urea, creatinine or an unknown uremic toxin. When GFR falls below 5 ml/min the kidneys may be unable to excrete even normal quantities of sodium and water Many patients at this stage develop hypertension, oedema and features of water intoxication

(f) The excretion of hydrogen ions is impaired, the plasma bicarbonate concentration falls and an observer may notice compensatory hyperventilation of which the patient is often unaware.

(g) In the final stages death can result from hypertension, uremic coma, pulmonary oedema, gastrointestinal haemorrhage, hyperkalemia or severe infection.

Treatment

(a) In mild cases, active steps should be taken to control hypertension, to correct salt and water imbalance and to treat active urinary tract infection. Fluids and electrolytes should be given intravenously A high protein diet is usually indicated in nephrotic patients with excessive losses of protein in the urine. Sodium restriction may be necessary in some patients, but others may need extra salt to compensate for urinary losses Sodium bicarbonate may be needed for treatment of acidosis

(b) Vitamin D can be used for the treatment and prevention of metabolic bone disease Increase in plasma calcium may damage the kidney and accelerate loss of renal function.

(c) As renal failure progresses and the patient develops symptoms of uremia, more active measures are necessary to compensate for the loss of renal function. Patients may be treated by dietetic measures alone, by regular hemodialysis or by renal transplantation.

Stones in the urinary tract

Stones may form in the bladder (vesical calculi) or the kidney (renal calculi) 95 per cent of stones are made up of calcium salts About 3 per cent are uric acid salts and about 1 per cent are cystine Most stones are a mixture of calcium oxalate, calcium phosphate and magnesium ammonium phosphate, but about one third are pure calcium oxalate Stones form more readily infected urine in which bacteria have converted urea into ammonia so making the urine more alkaline

Vesical calculus

Blood stones usually occur either in boys, in young men or in old men (in whom it is generally associated with prostatic obstruction or other cause of urinary stagnation)

Renal calculus

Renal colic, the severe pain caused by the passage of stones down the urinary tract. It stops when the stone is passed naturally or removed by a surgeon. Most

stones remain in the kidney and they produce no symptoms (silent stones). They may grow these sometimes to a very large size. Infection may lead to pyelonephrosis, the commonest cause of chronic renal failure.

Prevention

(a) *Fluid intake*: A good flow of urine washes out particles of gravel. So water should be drunk before going to bed as urine flow is lowest at night. All patients who have suffered from stones should drink sufficient water to produce 2.5 litres of urine daily.

(b) *Diet*: The patient should drink only moderate amounts of milk and tea and eat only moderate amounts of milk products, meat and fish. Vegetables and fruits may be of benefit by increasing fibre intake.

(c) *Drugs*: Bendrofluazide reduces urinary calcium by about 30 per cent. Pyridoxine may be given to patients who form oxalate stones in the hope of diverting glycine metabolism towards serine and away from oxalate. Penicillamine given by mouth is useful in cystinuria, as it combines with cystine which is then excreted in a more soluble form.

DISEASE OF THE GASTROINTESTINAL TRACT

Diseases of the mouth

Lesions primarily nutritional in origin are angular stomatitis, cancrum oris, nutritional parotitis and nutritional glossitis Glossitis is often a presenting feature in pellegra, the sprue syndrome, pernicious anemia and iron deficiency anemia of long standing.

1. The tongue

(a) The tongue may be dry in mouth breathers and coated with whitish yellow fur in those persons who smoke excessively.

(b) A clean red tongue which is inflamed and painful (acute glossitis) suggests an acute primary deficiency of some members of the Vitamin B complex.

(c) A clean pale and smooth tongue (chronic atrophic glossitis) suggests pernicious anemia or a long-standing iron-deficiency anemia.

(d) A local ulcer may be due to an ill-fitting denture or malignant disease, but rarely now a days syphilis or tuberculosis.

2. The teeth, gums and mouth

(a) A bad taste in the mouth may be due to pyorrhoea.

(b) Inflammatory and hemorrhagic lesions in the mouth and gums can result from many causes, e.g., infections, hemolytic streptococci, drug reactions and blood diseases (acute leukemia, aplastic anemia).

(c) Any inflammatory condition of the mouth may contribute to a nutritional disorder.

Treatment: If lesions are causing pain on chewing or swallowing, a fluid or semiliquid diet must be given until the condition is brought under control.

Diseases of the oesophagus

(a) Difficulty in swallowing (dysphagia) is the main feature and may lead to choking and even inhalation of food causing pneumonia or death. Dysphagia results from a functional defect with failure of onward movement of the peristaltic waves; alternatively the wave may be adequate but a block caused by spasm, inflammation or malignant disease prevents the food from getting through the affected area.

allow food to enter the stomach and prevent regurgitation of stomach contents into the oesophagus If this neuromuscular mechanism is disturbed, dysphagia or heartburn may ensue

(c) Dysphagia may be produced by an neurological disorder which damages the motor pathway between the cerebral cortex and peripheral muscle Common causes are a stroke and achalasia

Dyspepsia

(a) It means indigestion or difficulty in digestion Any gastrointestinal symptom associated with the taking of food is called dyspepsia, e.g , nausea, heartburn, epigastric pain, discomfort or distension

(b) Dyspepsia may be a symptom of any organic disorder of the alimentary canal It may also be caused by disease or disorder of structure outside the alimentary tract, e g , the gall bladder, pancreas etc

(c) It may be a symptom of a general disease, e g , chronic nephritis and cardiac failure

Dyspepsia and acid secretion

(a) When dyspepsia occurs with achlorhydria, it is probably due to chronic gastritis, cancer of the stomach or disease of the gall bladder or to emotional states

(b) Hyperchlorhydria may be found in people who have never suffered from dyspepsia It is frequently found in patients with duodenal ulcer

(c) Hydrochloric acid may be partially responsible for the pain and dyspepsia of the acute stage of peptic ulcer

Management of dyspepsia

(a) Dyspepsia in young people may be due to overworking or overworrying or eating his meals when excessively tired or has been smoking excessively or taking too much alcohol The patient should be advised to give up his such habits His symptoms will clear up rapidly

(b) Dyspepsia occurring in middle age for the first time accompanied by weight loss should be carefully investigated without delay

(c) Patients with functional dyspepsia need dietary advice Patient may find that certain foods bring on their symptoms whereas other can be taken with impunity Hence bland diets have been prescribed

Peptic ulcer

The term peptic ulcer is used because it appears to develop from a loss of ability of the mucosa to withstand the digestive action of pepsin and HCl A balance exists between acid pepsin secretion and mucosal resistance In patients with gastric ulcer the secretion of acid is often within normal limits, but patients with duodenal ulcer nearly always have a high output of acid The great importance of gastric hypersecretion is supported by the intractable peptic ulceration of the Zollinger Ellison syndrome in which gastrin produced by a tumour of the non β islet cells of the pancreas stimulates excessive gastric secretion by day and by night. Mucin is a protective agent It adheres to the stomach wall as a thin but resistant coating It is secreted in response to local, nervous and hormonal influences

Caffeine, ethanol, aspirin, and nicotine promote peptic ulcers Peptic ulcers occur more frequently in persons with blood group ' O" than in those in other groups, and possibly with those with HLA-B5 antigens

1 **Clinical features and diagnosis**

(a) The commonest symptom is pain or discomfort in the upper central abdomen It is usually described as burning or gnawing in character The pain comes and goes and is related to meals

(b) In duodenal ulcer it usually occurs when the stomach is empty and is relieved by meals The pain of gastric ulcer often comes shortly after eating

(c) Other symptoms are loss of weight, heartburn or vomiting In some patients an ulcer causes no symptoms until a complication such as haemorrhage occurs

(d) An ulcer bleeds slowly and there is melena (black stools) and anemia With a larger haemorrhage there is usually haematemesis, the blood which is vomited is changed to a dark brown colour

(e) The spasm of the pyloric canal can give rise to a characteristic feeling of sickness and distension this prevents some patients from taking food which would relieve their symptoms

(f) Acid output is usually above the normal range in patients with duodenal ulcer, and low or absent in patients with carcinoma of the stomach.

2 **Medical treatment:**

Principles of treatment

(i) Rest, both physical and psychological

(ii) A bland diet, given in small amounts at frequent intervals

(iii) Drugs—antacids and secretory inhibitors

(iv) Giving up smoking

Drugs:

(a) Cimetidine and allied drugs by blocking the H_2 receptors in the gastric mucosa reduce acid secretion This relieves symptoms and may promote healing The drug is given throughout the day for one to three months These drugs have no adverse effects Some physicians keep patients on the drugs for longer in the hope of preventing recurrence

(b) Insoluble antacid powders (aluminimium hydroxide, magnesium oxide or trisilicate) usually bring immediate relief of pain

Giving up smoking: The early deaths are not due to surgery or disease of the stomach but mainly to diseases known to be associated with smoking (carcinoma of the lung, chronic bronchitis and coronary heart disease)

Diet:

(a) Patients should avoid large meals by taking small amounts at a time for a number of times This will reduce the risk of exposing the gastric and duodenal mucosa to excessive amounts of acid

(b) When a patient has severe symptoms with pylorospasm or has had haemorrhage he should be given milk, eggs and fruit juice as the main ingredients.

(c) It is important to take Vitamin C since some patients have low reserves as a result of previous self imposed dietary restrictions

Rest:

(a) When a patient curtails his business and social activities, symptoms are often relieved

(b) Both physical and mental rest promote healing of an ulcer

(c) Because of anxiety and emotional difficulties, they often need simple

psychological support and hence they are admitted to hospital. Otherwise not required to be admitted to hospital.

Complications of medical treatment

(a) Scurvy may result from the intake of milk diets for a long period by adults. An excess of soluble alkalis can lead to alkalosis with tetany. A condition known as the milk alkali syndrome may occur in patients who have taken large amounts of milk (more than 1 litre daily) and soluble alkali over long periods. Weakness, anorexia and lethargy are the characteristic features and there may be psychological distrubances

(b) Hypercalcaemia may give rise to calcification in the kidneys and elsewhere.

Gastritis

(a) Ingestion of alcohol, drugs or other chemical irritants may be responsible for gastritis.

(b) The commonest drug causing gastritis is aspirin, often taken for headaches and menstrual pain.

(c) Atrophic gastritis may be due to an autoimmune reaction and this is responsible for the failure to secrete intrinsic factor and HCl in pernicious anaemia. It is also present in patients with severe iron deficiency anaemia.

(d) It may also be present in metabolic disorders, e.g., Uremia, Carcinoma of the stomach.

(e) Clinical features are mild anorexia, vague discomfort, nausea and heartburn to severe and repeated vomiting accompanied by diarrhoea if there is associated enteritis. Sometimes the clinical picture may stimulate acute peptic ulcer and massive gastric haemorrhage may occur. Nausea, abdominal fullness, heartburn and pain occurring before breakfast and improving as the day goes on.

Acute gastritis

(a) The symptoms are nausea, pain and vomiting and commonly follow an excess of alcohol, aspirin or other drugs.

(b) Treatment consists of stopping alcohol or the drug, sometimes washing out the stomach and giving alkalis.

(c) Water and electrolyte losses can be replaced by an oral rehydration fluid. With improvement of the condition the patient is given small feeds of milk and gradually returns to a normal diet within 1 or 2 days.

Disorders of the intestine

The small intestine is the main site of absorption of nutrients. Normally absorption of all nutrients begins in the jejunum and is completed in the ileum except that of water and electrolytes which is completed in the colon.

Diarrhoea leads to depletion of water and electrolytes and may be due to disorders of the small or large intestine. The *malabsorption syndrome* arises when there is failure of digestion and absorption due to disorders of the small intestine. Failure to absorb fat is the main feature leading to *steatorrhoea*. Disorders of the colon leads to *constipation*.

Acute diarrhoeal diseases

These diseases are caused by infections of the small and large intestine by pathogenic viruses, bacteria or protozoa. The term covers illnesses such as acute gastroenteritis, bacterial food poisoning, traveller's diarrhoea, infantile diarrhoea and weanling diarrhoea, as well as the specific infections, bacillary dysentery and cholera.

The losses of water and electrolytes in the diarrhoea lead to dehydration of the body If the diarrhoea is severe, death from dehydration may occur within one or two days, especially in young children, in the very old and in the undernourished

The serious dehydration can be prevented by giving an oral dehydration solution (ORS) early in the disease The solutions used consist mainly of common salt, sodium bicarbonate and glucose or other source of carbohydrate They are cheap and can be administered safely by any mother in a primitive home

1 Clinical features

(a) The diarrhoea is usually accompanied by abdominal discomfort and nausea and often by vomiting

(b) Fever, if present, is seldom high

(c) When there is severe dehydration, there is circulatory collapse with a marked fall in blood pressure This may be fatal or lead to death later from acute renal failure

(d) Infections not only interfere with intestinal absorption but the cholera toxin causes active secretion of chloride by the intestinal mucosa Then with the chloride, sodium and water flow out from the tissues into the lumen of the gut In severe cholera as much as 1 litre of fluid may be lost in an hour By this way death from cholera may arise within a few hours of the onset of the disease

(e) Vomiting also increases the fluid loss and the accompanying loss of acid leads to an alkalosis which is a cause of drowsiness

(f) Sweating may lead to a loss of a litre or more during the course of a hot day in the tropics by an adult, even when at rest Fever also leads to sweating

(g) An attack of diarrhoea reduces food intake Repeated attacks in a young child cause protein energy malnutrition In this way infections are responsible for retarded growth and development of young children in poor communities and also for a large part of the deaths from malnutrition

2 Treatment

(a) WHO has recommended an oral rehydration solution which comprises of the followings

Sodium chloride (table salt)	3 5 g	Dissolve in 1 litre of potable water
Sodium bicarbonate (baking soda)	2 5 g	
or trisodium citrate, dihydrate	2 9 g	
Potassium chloride	1 5 g	
Glucose	20g	

The solution was first developed in Bangladesh for the treatment of cholera and weanling diarrhoea It is there firmly established

(b) Absorption of oral fluids is fully dependent on a good blood supply to the alimentary canal Circulatory collapse, detected by a fall in blood pressure, indicates a need to replace fluid intravenously This is also necessary when vomiting is severe

(c) In young children a lower concentration of NaCl may be desirable to avoid any risk of hypernatremia

(d) Intestinal loss of potassium should be replaced and sodium bicarbonate ($NaHCO_3$) may be needed to correct acidosis caused by starvation, rarely NH_4Cl may be required to correct alkalosis arising from vomiting

(e) Most physicians do not prescribe any antibiotic drug for an attack of acute diarrhoea They select the appropriate drug after microbiological diagnosis

Malabsorption Syndrome

Lack of digestive secretions and injury to the epithelial surface of the small intestine impair absorption of nutrients The clinical features of undernutrition and malnutrition for a long time known as the malabsorption syndrome

1 Clinical features

(a) Loss of weight and oedema in cases of long standing

(b) Chronic diarrhoea with abdominal discomfort and distension

(c) *Steatorrhoea* increased fat in the feces, is frequently present, its severity depends on the amount of fat in the diet

(d) There is usually diarrhoea with a bulky stool that has an offensive smell and floats on water

(e) Anemia is usually present due to impaired absorption of iron and folic acid

(f) Nutritional glossitis, angular stomatitis and peripheral neuropathy arise from the deficiency of the B group vitamins

(g) Prolonged failure of calcium absorption may lead to evidence of osteomalacia and to tetany

(h) Haemorrhages may occur due to Vitamin K deficiency

Tropical Sprue

Sprue is the name given to a tropical disease in which the presenting features are sore mouth, fatty diarrhoea and associated secondary manifestations of undernutrition and malnutrition

Although there is defective absorption of fat, the absorption of water, electrolytes, glucose, vitamins and minerals is also impaired There defects are associated with atrophy of the jejunal villi

Sprue is a serious disease and may prove fatal without proper medical and dietary care Full recovery is possible with proper treatment and if they leave the tropics

Intestinal obstruction

(a) The cause of intestinal obstruction is mechanical or due to paralysis of the intestinal muscle (paralytic ileus)

(b) The common causes of mechanical obstruction are external hernias, volvulus, tumours of the colon, adhesions due to previous inflammatory disease or operation

(c) Paralytic ileus is usually a consequence of peritonitis, resulting from any cause, e g a gastric or intestinal perforation or an abdominal operation.

1 Features

(a) The chief features are vomiting, complete constipation and colicky pain which may be absent or slight in paralytic ileus

(b) A serious loss of water and electrolytes results from the vomiting and from the stagnation of intestinal secretions in the dilated paralysed loops.

(c) The loss of fluid from the circulation from the latter source may be several litres in 24 hours and this may lead to prerenal uremia

(d) The loss of potassium causes apathy, mental confusion and muscular weakness.

2 Treatment

(a) Since intestinal obstruction is always serious it should be treated only in a hospital where surgical and biochemical help are available

(b) Immediate operation is required for the relief of mechanical obstruction, while it is strongly contraindicated in paralytic ileus

(c) In paralytic ileus the distension of the paralysed gut must be treated by continuous suction through a tube passed into the stomach or jejunum, and continued until the bowel recovers from its paralysed state

(d) In both types of obstruction the loss of fluid and electrolytes must be made good by appropriate infusions and intravenous feeding is often needed

CONSTIPATION

Constipation is delay in passage of the feces The presence of food in the stomach is a stimulus to a gastrocolic reflex which causes movements of the colon and these may lead to feces entering the rectum The reflex usually occurs after the first meal of the day In some people the presence of liquid in the stomach initiates the reflex and a drink on rising may be sufficient to stimulate defecation Some healthy people do not defecate every day and a few do so once or twice a week They should not be considered constipated, and constipation should only be diagnosed when delay in defecation causes discomfort and indigestion

The two common causes of constipation are a small fecal bulk and persistent neglect of the call to defecate Many diseases are associated with constipation

The bulk of the feces is mainly water and the amount of water depends on the amount of dietary fibre present and the capacity of the fibre to bind water Low fibre diets cause constipation

If the call to defecate is persistently neglected, the reflex mechanism becomes less sensitive and constipation results Going to the toilet should become a habit early in life

Gastrointestinal diseases give rise to constipation and the irritable bowel syndrome Carcinoma of the colon and rectum sometimes present as constipation Constipation is common in psychiatric disorders which cause depression Any neurological disease causing lesions in the lumbar cord may affect the reflex centres responsible for defecation and lead to constipation

Pregnant women and old people are often constipated The pressure of the gravid uterus on the colon may delay movements of the contents In old people the sensitivity of the neuromuscular reflexes in the colon may be impaired or they may become less aware of the presence of feces in the rectum

When constipation occurs due to low intake of dietary fibres, there is often pain in the left side of the abdomen along the line of descending colon and the feces may be passed as hard pellets Passage of feces relieves the pain When the call to defecate is repeatedly ignored, a mass of inspissated fecal matter may accumulate in the descending colon The fluid contents of the colon they may run down the side of the mass and cause a watery diarrhoea

Treatment

(a) The intake of dietary fibre should be increased by the consumption of fruit and vegetables The fibre has got water holding capacity The fruits and vegetables are oranges, apples, carrots and cabbages

(b) A young child should be advised to go to the toilet at a regular time each day An adult should not neglect to go to the toilet due to laziness, hurry or a lack of suitable accommodation Once the reflex has been lost, it cannot be regained to move the bowels at the same time each day

(c) Many laxatives are available Their continued use may lead to excessive losses of potassium, sodium and water in the feces and is not recommended Two mild laxatives that are recommended for short periods are lactulose and senna Lactulose is a sugar which is not absorbed in the small intestine but passes to the colon where it is partially broken down by bacteria It reduces the absorp-

tion of water from the colon and increases the bulk of the feces Senna is a glycoside which is broken down in the small intestine to emodin, this is absorbed into the blood stream and stimulates the muscles of the colon for 6 to 12 hours after administration of the senna

DISEASES OF THE LIVER, BILIARY TRACT AND PANCREAS

Many disorders and diseases damage liver function Moderate damage causes jaundice Serious damage is caused by many infections and toxic chemicals Persistent infection causes liver failure Dietary therapy is an important part of the treatment of liver failure and of its serious complications

Liver damage

1 Hepatitis

(a) The liver becomes tender and enlarged following continued excessive intake of alcohol The patient may become jaundiced

(b) If alcohol intake is continued, acute liver failure may arise with deep jaundice and several cerebral symptoms

(c) The patients may complain of general malaise, anorexia and a variety of digestive disturbances and become progressively undernourished

(d) During the course of chronic hepatitis there is slow progressive fibrosis leading to major disturbances of liver structure and function

2 Cirrhosis of the liver

(a) It is now referred to any diffuse chronic disease of the liver

(b) Venous congestion leads to enlargement of the spleen which is detected by palpation It leads to varicosities which may bleed

(c) Oesophageal bleeding is a common and serious consequence of cirrhosis of the liver

(d) Damage to liver cells leads to jaundice that may be severe The clinical features are disordered sleep rhythm, restlessness or drowsiness, impaired intellectual function with confusion and, in severe causes, stupor and coma The onset may be sudden Sometimes these features are the first manifestations of liver failure to appear and then they may be mistaken for a psychiatric disorder The condition is due to unidentified toxic substances reaching the brain in the systemic circulation These may arise from disturbances of intermediary metabolism or to substances absorbed from the gut that the damaged hepatocytes are unable to detoxify

Jaundice

(a) This is caused by the increase in the bilirubin in the blood. The skin and sclera of the eyes appear yellow and the urine is usually dark yellow or brown It is not detectable until the plasma bilirubin rises above 8 mg/l

(b) There are three types of jaundice *Hepatocellular* jaundice is due to damage to hepatocytes by toxic or infectious agents interfering with the uptake and conjugation of bilirubin by the cells or to blocking of the bile canaliculi (cholestatic jaundice)

Prehepatic (hemolytic) jaundice is due to increased bilirubin from excessive destruction of R.B C It may arise from congenital defects causing the erythrocytes to be unduly fragile (spherocytosis, sickle cell anemia, thalassemia or from some drugs, incompatible blood transfusions)

Posthepatic jaundice is due to an obstruction of bile flow between the liver and duodenum Common causes are impacted gall stones and cancer of the head of the pancreas The excess of bilirubin in the plasma is then in the conjugated form, whereas in prehepatic jaundice it is unconjugated

(c) Jaundice is sometimes accompanied by itching (pruritus) and this may be severe The cause is retention of bile salts in the blood and it may be relieved by cholestyramine This is an anion exchange resin which binds bile salts is the gut and so interrupts the enterohepatic circulation and increases fecal excretion of bile salts

Agent damaging the bile salts

1 **Dietary deficiency**

Low protein intake causes the fatty changes in the liver in kwashiorkor and there is evidence of reduced capacity to secrete β lipoproteins but not of choline deficiency in man Choline deficiency is not at all responsible for liver damage in man

Fatty changes in the liver are common whenever there is a high proportion of fat in the metabolic mixture, e g in uncontrolled diabetes, in starvation, in some cases of obesity

2 **Infective agents**

Hepatitis A virus is excreted in the stools and spread by the fecal oral route Children or adolescents are affected by this infection where hygienic condition is low The initial symptoms are loss of appetite, nausea and malaise and usually mild fever Jaundice appears after 4 to 7 days and the liver is enlarged and tender The symptoms usually last from 2 to 6 weeks but complete recovery may take many months

Very rarely there is fulminant hepatitis with symptoms of acute liver failure and the death usually occurs within 10 days

Some healthy people, who have no clinical history of the attack of the disease, excrete the virus in their feces Such carriers may cause epidemic Some of the cases of cirrhosis in which no cause can be found may be due to long continuing exposure of the liver to the virus

Hepatitis B virus produces a disease known as homologous serum jaundice because it arises after transfusion of blood It can also be spread by the use of one needle to give injections to a series of patients without effective sterilisation The virus is never spread by the fecal oral route and the infection arises from close personal contact, as between sexual partners They cause similar type of illness as that in Hepatitis A Virus A carrier can be detected by the presence of the antigen of the virus (HBeAg) in the serum

Yellow fever virus has a specific affinity for hepatocytes It is much more virulent More than 20 per cent of patients died of acute liver failure Yellow fever is now very rare, as the mosquito responsible for its transmission, is a domestic species and easily controlled

3 **Other infective agents**

In three diseases the liver is especially involved In Weil's disease leptospira invade all organs and tissues but markedly the liver and jaundice is the common feature

Amoebiasis is primarily a disease of the large intestine but protozoa may migrate up the portal vein and cause amoebic hepatitis and liver abscesses

In schistosomiasis with infection of the large intestine by *Schistosoma mansoni*, ova frequently more up the portal vein and cause multiple granulomata in the liver, these become fibrosed and are a common cause of cirrhosis of the liver

4 **Toxic agents**

(a) Alcohol, other undetected toxic chemicals and infective agents may act synergistically to produce progressive liver damage

(b) Foods may be contaminated by moulds that produce toxin. Aflatoxins are known to cause liver disease

(c) Many drugs can damage the liver in susceptible individuals and produce a clinical and biochemical picture similar to acute viral hepatitis. Examples are paracetamol, a much used analgesic, sometimes used in the treatment of depression. Cholestatic jaundice may follow the use of methyltestosterone and very rarely oral contraceptives

(d) Workers in the chemical industry may be exposed to many chemicals that damage the liver and produce jaundice. Examples are hydrocarbons, carbon tetrachloride, arsenic, trinitrotoluene etc

(e) Excess stores of iron, copper, galactose and glycogen may accumulate in the liver and in time lead to cirrhosis. These conditions are usually due to hereditary defects. Hereditary defects in the transport and conjugation of bilirubin may lead to jaundice. People suffering from Gilbert's disease have no symptoms but jaundice, anorexia, nausea and abdominal pain may arise during a period of stress, such as an infection, fasting or strenuous exercise.

Dietetic management and treatment

1 Acute hepatitis

(a) There is no antiviral agent for use against the hepatitis virus but metronidazole is very effective in amoebic hepatitis. Patients with viral hepatitis do well with rest

(b) No specific dietary treatment is needed but as patients lack appetite and suffer from nausea, meals should be well-cooked and attractively served. Several small meals may be better tolerated than three large ones

(c) Fat intake should be restricted. Carbohydrate and protein diet should be taken. Vitamins and minerals should be given through fruits and vegetables

2 Hepatic failure

(a) Hemorrhages as a result of lack of clotting factors synthesized by the liver lead to shock and renal failure. Profound hypoglycemia and potassium depletion occur

(b) Intravenous infusion of hypertonic glucose and electrolytes is required with repeated monitoring of blood level

(c) Sedatives may be needed for delirium but are potentially dangerous due to the state of the liver

(d) Despite all treatment majority of the patients die

3 Chronic hepatitis

This is common in alcoholics and occasionally follows viral hepatitis. When it is due to alcoholics, most patients do well when alcohol intake is stopped.

Some chronic alcoholics are malnourished because they eat irregularly and seldom take proper meal. They need to change dietary habits and require professional advice

4 Cirrhosis

(a) Many patients with cirrhosis are seriously malnourished, especially those who are alcoholic and require nutritional rehabilitation with a high energy, high protein diet. This may be difficult to achieve on account of poor appetite and admission to hospital is necessary

(b) The fat-soluble vitamins are poorly absorbed because of the poor absorption of fat owing to the absence of bile salts. Therefore, supplements may be needed. A monthly intramuscular injection of Vitamin K (10 mg), of Vitamin A

(30 mg) and of Vitamin D (25 mg) provides for maintenance As bleeding is common and often severe, Vitamin K should be given at once and continued

(c) Osteomalacia is a well known complication of chronic liver disease and also reserves of Vitamin A in the liver is low Vitamin A and D should be given in proper doses and should not be given more which may cause toxic effect

5 Ascites

(a) Fluid accumulates in the peritoneal cavity because of increased capillary pressure in the portal vessels and decreased oncotic pressure owing to a failure of the liver to synthesize plasma albumin Sodium is retained in the fluid

(b) The ascitic fluid often amounts to 10 litres and contains protein in concentrations of 10 to 20 g/l

(c) It depletes plasma proteins and carries a risk of introducing infection

(d) Treatment consists of a diet rich in protein and low in sodium, and also diuretics to increase urinary output of sodium and fluid With all of them there is a danger of electrolyte imbalance Overdosage may lead to sodium deficiency and also to potassium depletion

(e) The patient should avoid salt rich foods

6 Portal hypertension

Bleeding from oesophageal and gastric varices is common and often serious and a cause of death The anemia is treated with iron and repeated blood transfusion are often necessary Pressure in the veins can be relieved by a surgical operation but such operations are dangerous and increase the risk of death from liver failure

7 Biliary cirrhosis

Stagnation of bile in the canaliculi of the liver may lead to hepatitis and subsequently to cirrhosis This may be a consequence of unrelieved obstruction of the bile duct and then secondary bacterial infection is often an additional factor This disease occurs most commonly in middle aged woman

Prompt surgical treatment prevents any damage to the liver from progressing jaundice fluctuates in intensity as does pruritis which is often serious and then may be relieved by cholestyramine

Diseases of the gall bladder and bile ducts

The function of the gall bladder and bile ducts is to concentrate, store and deliver bile into the duodenum at suitable times to assist digestion, hormonal and nervous factors play a part in this process The stimulus for this activity is the entry of food into the small intestine, this causes the mucosa of the duodenum and jejunum to secrete a hormone, cholecystokinin, which is carried in the blood to the gall bladder and causes it to contract Fats and foods rich in fats are especially effective for this purpose Vagal stimulation causes contraction of the gall-bladder and relaxation of the sphincter, while stimulation of the sympathetic nerves produces the reverse effects

Gall-stones (Cholelithiasis)

The bile is concentrated in the gall bladder and when it is supersaturated gall-stones are likely to form Supersaturation arises when there is an insufficiency of solubilising agents such as bile acids and lecithin to keep cholesterol and bile pigments in solution The most common gall stones are composed of cholesterol, bile pigment and various calcium salts including calcium palmitate In the centre is a protein nidus Thus suggests an infective origin. The stones are usually multiple and their surfaces are faceted Pigment gall stones are more prevalent

in the eastern world and may be due to increased hemolysis arising from the sickle cell trait and thalassemia

Gall stones are more common in women than in men Advancing age, repeated pregnancies and sedentary life and the use of oral contraceptives are contributing factors

The formation of cholesterol gall stones depends on the concentration in the bile of cholesterol relative to that of the conjugated bile acids and lecithin High cholesterol concentration may arise from either excess of cholesterol or lack of bile acids Excess cholesterol may be due to increased activity of HMG CoA reductase Deficiency of bile acid may be due to deficiency of the enzyme that controls their formation (cholesterol 7α hydroxylase)

The symptoms due to gall stones are removed surgically often a stone causes no symptoms and then there is no strong case for its removal

Some gall stones can be dissolved in the gall bladder by administration of the bile acid, chenodeoxycholic acid In a large trial complete dissolution occurred in 13 5 per cent of cases after two years with some adverse effects-diarrhoea and a slight rise of plasma cholesterol So it is only for treatment of elderly, frail or who are losing weight before operation.

Acute cholecystitis

It is rarely due to infection of the gall bladder but almost always occurs in association with obstruction to the cystic duct or neck of the gall bladder, upon which infection is usually superimposed In most cases gall stones are the cause of obstruction.

1 Treatment

(a) Cholelithiasis with accompanying cholecystitis is the choice of the surgeon to postpone operation until the acute infection has subsided.

(b) The patient should be in bed and given suitable analgesics and antibiotics

(c) Heat should be applied to the gall bladder region and ample fluids given intravenously if the patient is vomiting

(d) So long as the gall bladder is acutely inflamed, it is advisable to keep the organ at rest as far as possible

(e) For acute cases an entirely fluid diet of at least 2 to 3 litres daily, given in small feeds at hourly or two-hourly intervals is advisable for a few days

(f) When the condition is improved within two or three days, clear soups, milk, fruit jellies, and cereals may be added and the diet is rapidly built up to normal

(g) After recovery if there are gall stones or non functioning gall bladder, cholecystectomy should be advised

Chronic cholecystitis

If it is decided not to remove the gall bladder or if for any reason operation is suspended dietetic treatment should be given

(a) Foods which aggravate the symptoms should be avoided

(b) The cooked meats rich in fat and fried foods be given

(c) Milk, butter and cream cheese is permitted and may promote drainage of the gall bladder

(d) Eggs may be permitted in moderation if they do not cause symptoms

(e) The diet should be bland and contain adequate protein.

(f) Care should be taken to avoid large meals and indigestible articles of food and ample quantities of fluids should be taken in the morning and between meals

Diseases of the pancreas

Acute pancreatitis

This is a serious disorder which may lead to hemorrhagic necrosis of the pancreas, peritonitis and death It usually occurs in middle-aged and elderly persons The main symptom is the sudden onset of agonising pain in the epigastrium which may radiate to the back It may follow a heavy meal or an excess of alcohol Nausea and vomiting are frequently present Moderate fever occurs and jaundice may develop In addition there are signs of peritonitis and profound shock

1 **Treatment**

(a) Medical management consists of the relief of pain and the control of shock

(b) Continuous gastrointestinal suction is essential to reduce vomiting and distension

(c) It also removes acid gastric juice, a stimulus to pancreatic secretion

(d) Production of gastric juice is inhibited by cimetidine and ranitidine

(e) Antibiotics should be administered to prevent secondary infection

THE ANEMIAS

There are three main causes of anemia

1 Loss of blood from the circulation, i e external or internal hemorrhage.
2 Hemolysis, i e increased destruction of R B C
3 Reduced production of erythrocytes and hemoglobin dyshaemopoiesis

For the production of R B C many nutrients are needed The most important are iron, folic acid, and Vitamin B_{12}, but others are protein, pyridoxine, Vit C, copper and Vit E The anemia occurs in a healthy person as a result of poor diet The diet often contains insufficient of one or more of the essential nutrients to meet increased needs caused by chronic hemorrhage, infection and genetic defects affecting the R B C Disorders of the alimentary tract often leads to impaired absorption of the essential nutrients and so to anemia So the extra needs are to be met by increased nutrients

1 **Clinical features of anemia**

(a) Symptoms of anemia arise when the transport of oxygen by the blood is insufficient to meet the needs of the body The need for oxygen is related to physical activity A person leading a sedentary life may have a moderate degree of anemia but entirely free of symptoms

(b) The severity of the clinical features does not depend on the degree of anemia but depends on the rapidity of its development

(c) Common symptoms are general fatigue and lassitude, breathlessness on exertion, giddiness, dimness of vision, headache, insomnia, pallor of the skin, palpitation, anorexia and dyspepsia, tingling and 'pins and needles' in the fingers and toes

(d) Angina pectoris (due to myocardial hypoxia) is sometimes present

(e) Physical signs include palor of mucous membranes and fingernails, tachycardia, functional systolic murmurs, evidence of cardiac dilatation and in severe cases, oedema of the ankles and crepitations at the bases of the lungs

(f) There are signs of nutritional deficiency, particularly angular stomatitis, koilonychia and glossitis

(g) Atrophy of the papillae and mucous membranes gives the tongue a smooth glazed appearance The atrophy begins at the edges and later affects the whole tongue As a result the tongue appears moist and exceptionally clean

2 **Diet**

(a) The most valuable dietary sources are meats and liver, they should perferably be eaten once a day

(b) Eggs also have a high iron content but this is poorly absorbed because of phospholipid inhibitors in the yolk.

(c) Less expensive sources of iron are beans, especially soyabeans and nuts

(d) Fresh fruits and vegetables are of greater value because of their ascorbic acid content which facilitates iron absorption

(e) Milk is a poor source of dietary iron

3 **Genetics defects of R.B C**

There are hereditary defects of R B C that make them more susceptible to hemolysis and persons who carry these genes are liable to become anemic Heterozygotes are at increased risk of becoming severely anemic

(i) **Thalassemia** (Greek Thalassameans sea)

(a It is defin d by a defect in the synthesis of part of the polypeptide chain of hemoglobin A which is partially compensated by persisting synthesis of fetal hemoglobin (Hb F)

(b) There are also abnormalities of the red cell membrane

(c) Homozygotes have thalassemia majer, a severe hemolytic anemia and rarely survive into adult life

(d) Heterozygotes may or may not have a mild anemia, thalassemia minor

(ii) **Sickle cell trait**

(a) This is due to an abnormal hemoglobin, hemoglobin S, differing only in having a single molecule of valine instead of one of glutamic acid in one of the polypeptide chains

(b) The configuration of the molecules of Hb-S distort the R.B C into a characte ristic sickle shape Such corpuscles are abnormally sensitive to hypoxia

(c) Persons with the sickle cell trait are at risk of hemolytic crisis when flying and cabin pressure is reduced

(d) Heterozygotes have the sickle cell trait and are only occasionally anemic.

(e) Homozygotes have a severe anemia, sickle cell disease

(iii) **Spherocytosis**

(a) This is the commonest congenital defect of R B C

(b) The abnormality lies in the cell membranes which are more than normally permeable to sodium ions

(c) The cells assume the shape of spheres which are more easily trapped in the microcirculation of the spleen where hemolysis takes place

(d) In most cases the increased loss of cells by hemolysis is slight and can be made good by increased production in the bone marrow

(e) When anemia develops removal of the spleen reduces hemolysis and cures the anemia but does not change the defect

(iv) **Glucose-6-phosphate dehydrogenase deficiency**

Persons with the defect are liable to develop anemia when treated with oxidant drugs, these include antimalarials sulphonamides, antipyretics and analgesics

Anaemia in tropical countries

The demands for iron may be greatly increased by the loss of hemoglobin in the feces and urine resulting from hemorrhage due to parasitic diseases such as hookworm In addition significant amounts of iron may be lost in the sweat during muscular work in hot climates The protozoal, helminthic infections, and bacterial infections which are common in the tropics may be responsible for anemia The nutritional megaloblastic anemias of tropical climates are always associated with diets poor in animal protein and fresh vegetables, and hence low in Vitamin B_{12} and especially folate

1 Malaria

(a) An attack of malarial fever due to plasmodium falciparum is always accompanied by hemolysis and in a severe or prolonged attack severe anemia may ensure

(b) After the parasites have been removed from the blood, hemolysis may continue, due to a complement mediated immune response, and there may also be a mild depression of erythropoiesis

(c) Malarial infection, if associated with pregnancy and malnutrition the anemia may present as megaloblastic anemia due to folate deficiency, but is more usually hypochromic and microcytic because of iron deficiency

(d) Since the blood destruction is intravascular, most of the iron liberated from the destroyed red cells is retained in the body and can be used again for synthesis of hemoglobin

(e) A vicious circle develops in communities suffering from chronic malaria sickness, weakness and anemia, economic inefficiency, poverty, malnutrition, bad housing and social conditions, reinfection

2 Hookworm infection

(a) Infection with hookworm is a common cause of anemia where there is 'wet' cultivation of the land

(b) Hemorrhages occur at the site of the attachment of the worms to the intestinal mucous membrane These are certainly in part responsible for the anemia

(c) A patient with a heavy infection, namely about 1000 worms can sustain a heavy loss of blood and anemia may quickly develop

(d) Heavy hookworm infection usually occurs in populations whose dietary intake of iron is unsatisfactory

(e) The hypochromic anemia is in part due to a poor diet and in part to the worms This combination causes much ill health, it may reduce greatly the working capacity of all and in these directly responsible for the poverty of many families in the tropics

(f) Heavy infections may cause severe anemia with hemoglobin levels below 4 g/100 ml In such seriously ill patients, before administering a vermifuge, blood transfusion should be given and the general condition of the patient improved by bed rest, diet and medical iron

Other causes of anemia

(a) A mild or moderate anemia may develop due to chronic infection, particularly if fever is present

(b) Many drugs can cause anemia by impairing red cell formation, by causing hemolysis or by loading to bleeding Small repeated hemorrhages in the stomach due to aspirin is a common example

(c) In chronic renal disease anemia is common but the bone marrow remains cellular until renal damage is marked Deficiency of erythropoietin is a possible cause

(d) If megaloblastic anemia occurs this is probably due to primary malnutrition and is found in chronic alcoholics with cirrhosis

(e) Malignant disease causes anemia They include impaired appetite malabsorption or blood loss from the alimentary tract, multiple deposit in the bone marrow and increased hemolysis

(f) Abnormal utilization of iron by the marrow may cause anemia Some cases respond to pyridoxine therapy

DISEASES OF THE NERVOUS, RESPIRATORY SYSTEMS AND OF THE SKIN

Epilepsy

Epilepsy is a common disorder liable to periodic attacks associated with a disturbance of consciousness and commonly manifest as fits In a small minority of cases a local lesion in the brain or a generalised metabolic disturbance is responsible In the great majority of patients the cause is unknown and they are said to have idiopathic epilepsy

Antiepileptic drugs are very effective in preventing the seizures A period of starvation was effective to reduce their frequency Such benefits may be due to a depression of the brain by the accompanying ketoacidosis Ketogenic diets are given when the fits are not controlled by drugs

Childhood myoclonic epilepsy are resistant to drugs and associated with brain damage and patients have many attacks throughout the day Growing child should not be ketoacidotic A diet low in carbohydrate and high in fat may help to control the fits

Antiepileptic drugs cause changes in liver metabolism which may increase requirements of Vitamin D and folic acid Patients taking doses over the years should be checked regularly for signs of anemia and rickets or osteomalacia

Patients with epilepsy should take normal well balanced meals at regular intervals Epileptic children should not be allowed to take very large meals as these may predispose them to fits

Psychiatric disorders

Deficiencies of thiamin niacin and Vitamin B_{12} may each be associated with psychological disorders The psychological symptoms are occasionally so marked and other symptoms and signs of the deficiency so slight that the correct diagnosis is missed and the patient is treated as if he is a neurotic or has a psychotic disorder

Some of the patients have requirements for a vitamin in excess of the recommended intake Psychiatric disorders may lead to abnormal feeding behaviour and so to anorexia nervosa and obesity

CHRONIC RHEUMATIC DISEASES

The main members of this groups are rheumatoid arthritis, osteoarthrosis, and non articular rheumatism The rheumatic group of diseases is second only to bronchitis in men and first in women

1 Rheumatoid arthritis

(a) It occurs in people of all ages The peak incidence is at the age of 40 It occurs in women at least three times as in men.

(b) It is mainly a disease of temperate climates which are associated with cold and damp

(c) The principal tissue affected is the synovial membrane of joints which becomes inflamed and thickened

(d) Muscular stiffness develops first and is followed later by pain and swelling of many joints, starting frequently with the small joints of the hands and feet.

(e) During the active stage of the disease the patient suffers from general malaise and fatigue; fever is sometimes persent and the appetite is poor.

(f) There is loss of weight and some degree of anemia.

Treatment

(a) Anemia and loss of weight by diet should be corrected.

(b) Foods rich in protein, iron and ascorbic acid should be tried first; additional vitamins and minerals should be prescribed.

2. Osteoarthrosis

(a) Osteoarthrosis is characterised by degeneration of the articular cartilage and the formation of bony outgrowths at the edges of the joints. A generalised form of the disease occurs normally in middle-aged women in whom the small joints of the fingers, the carpometacarpal joint of the thumb and the interfacetal joints of the spine are particularly affected.

(b) When one joint is particularly affected, there is frequently a history of an injury to that joint some years before.

(c) Following fractures of the long bones gives rise to osteoarthrosis in adjacent joints.

(d) Symptoms are prove to develop in the weight-bearing joints or those joints subjected to excessive strain at work.

(e) Obesity imposes to osteoarthrosis of the weight-bearing joints in the lower half of the body.

(f) The joints involved are those of the spine, the hips, knees, elbows, and the terminal joints of the fingers.

(g) The symptoms are gradual in onset. Pain is at first intermittent and of the aching character, appearing after the joint has been used, and relieved by rest.

(h) As the disease progresses, movement in the affected joints become increasingly limited, at first by muscular spasm and later by the loss of joint cartilage and the formation of osteophytes. General health is usually excellent.

Treatment

(a) Rest, graduated physical exercises and physiotherapy.

(b) Analgesic should be advised according to the patient's needs.

(c) Prevention and correction of obesity is needed.

3. Non-articular Rheumatism

(a) This is characterised by pain and stiffness, often of sudden onset, affecting mainly the neck, shoulders, back and gluteal regions.

(b) Exposure to cold and damp, excessive or unaccustomed muscular activity, injury to muscles and tendons and poor posture may each be held responsible.

(c) Muscular pain and stiffness arise from strain or injury to ligamentous or articular structures.

(d) Most cases of brachial neuralgia; lumbago and sciatica may result from degenerative changes in the intervertebral discs.

(e) In the acute stage the patient may be severely incapacitated by pain and stiffness.

(f) In the more chronic stage pain is felt most often after rest and improve with moderate activity

(g) Muscular spasm may be marked and movement limited

Treatment

(a) Rest in bed is essential, together with heat to the affected part and analgesics in ample amounts

(b) At a later stage physiotherapy (heat, massage and graduated exercise) is essential

(c) In cases with lesions of the intervertebral disc causing pressure, operation should be considered

(d) Diet has no role in curative treatment but if the patient is obese, reduction in weight is the relief of symptoms

RESPIRATORY SYSTEM

The main causes of disease in the respiratory system are (i) infection, (ii) inhaled irritants' (iii) allergy, (iv) vascular accidents, (v) malignant disease

(i) **Infection :** Infection may be caused by a virus as in the case of influenza, and virus pneumonia, or by bacteria, as in bronchitis, bronchopneumonia, lobar pneumonia and tuberculosis

(ii) **Irritants :** Exposure to tobacco smoke, industrial pollution or other inhaled irritants over many years leads to chronic bronchitis When severe and prolonged and complicated by heart failure, this may lead to severe undernutrition and a state of cachexia This is probably due to anorexia caused by chronic hypoxemia

(iii) **Allergy :** Allergy plays an important role in bronchial asthma Besides inhaled dust or pollen, ingested foods such as eggs, milk, wheat, etc. can be responsible for asthma The detection of a food allergen and its exclusion from the diet may be an important factor in the treatment of asthma

(iv) **Vascular accidents :** The vascular accident in the lung is embolism from a thrombosis formed usually in the veins of the lower limb or pelvis. If this dislodges it travels through the right side of the heart and obstructs one of the, pulmonary arteries It occurs especially in elderly people confined to bed, e g after an operation

(v) **Malignant disease :** Malignant disease of the bronchial tree and lungs has greatly increased in recent years Tobacco smoke and pollution of the atmosphere with smoke and fumes are of great importance. There is little evidence that nutritional factors play any part in the causation of bronchial carcinoma

1 Describe the important principles of balanced diet and the role of each component contained it it (Mith 62S)

2 Define balanced diet Describe briefly the basic principles followed for the preparation balanced diet for a woman in late pregnancy (Mith 71S)

3 What factors are to be considered in planning a balanced diet ? Give the composition of such a diet for an average Indian man of sedentary habits (M U 75A)

4 Describe the principle to be followed to prepare the balanced diet (i) for an active adult of 25 years age (ii) in late pregnancy (Bh U 75S)

5 What are the essential nutritive principles of human diet ? Describe briefly the role and effects of inadequate intake of protein in the diet (R U 64A)

6 Discuss the role of protective substances in nutrition (R U 68A)

7 Discuss the role of protein in nutrition (Bh U 76A , R U 73A)

8 Discuss the role of fat in human nutrition (R U 69A)

9. Describe a diet for a normal adult in terms of total calorie protein, fat and carbobydrate requirements Give reasons which determine the proportion of these food substances. (Pun 65A)

CHAPTER 33

TOXICANTS IN FOODS

Toxic compounds are naturally occurring in some plants and animal foods or foods may be contaminated by toxicants during storage These toxicants cause severe illness in man and in some cases lead to death even. The toxicants are mainly divided into three classes· (A) Naturally occurring toxicants. (B) Toxicants from pathogenic microorganisms (C) Contamination of food with toxic chemicals and pesticides

A. Naturally occurring toxicants.

Lathyrism:

1 This is a crippling disease accompanied by paralysis of the leg muscles occurring mostly in adults who consume large quantities of the seeds of L-sativus or other lathyrus species for a long period.

2 The disease is found to occur in Bihar, Uttarpradesh and Madhyapradesh as well as spain, Algeria, France, and Italy.

Symptoms:

1 First of all weakness in the lower limbs with spasticity of leg muscles. As a result, the movement of the ankle and knee joints are restricted and painful

2 Flexion of the knee is prominent in the second stage and there is inversion of foot with a tendency to walk on toes

3 In the third stage, the above symptoms become more prominent and the individual can walk only with the help of sticks.

4 In the fourth stage, the knee becomes completely flexed and walking becomes quite impossible The thigh and leg muscles become atrophy.

Ackee fruit poisoning:

1 The ackee fruit is cultivated in Nigeria This fruit is consumed after boiling for 15 to 20 minutes

2 The poisonous properties of the fruit are due to unusual amino acid, hypoglycin A, hypoglycin B Both hypoglycin A and B have strong hypoglycemic action resulting in coma and death.

3 The signs and symptoms of this fruit poisoning in young children are found that the children sometimes vomit and they show drowsiness, convulsions or coma These symptoms are due to severe hypoglycemia. They are treated by intravenous glucose for recovery.

Goitrogens:

1 Many food stuffs contain organic compounds which have goitrogenic properties

2 The active goitrogenic principle present in brassicae family in 1, 5-vinyl-2-thio-oxazolidone which is present in cobbage and turnip

3 Certain oilseeds namely rapeseed, mustard etc contain thioglycosides which act as goitrogens

4 The red skin of groundnut contains phenolic glycosides which possesses goitrogenic properties

Pressure Amines:

1 A number of amines namely histamine, tyramine, serotonin and norepinephrine found in some foods have profound physiological activity. Most of them are inactivated by mono-amino oxidase in the intestinal tract. The poisoning effect of pressure amines due to consumption of aged cheese has been reported in patients receiving mono-amino oxidase inhibiting drugs

2 In Africa, the serotonin intake from plantains as a staple food may reach 100 to 200 mg per day The endomyocardial fibrosis may occur as a result of large amount of serotonin

3 The pressure amine foods are mainly plantains (green and ripe), juices of Pineapple and tomato, Bananas, lemons etc

Argemone seed oil poisoning:

1 During the harvest of rapeseed, argemone seeds are mixed up with rapeseed which grow as weeds The rapeseed oil obtained from a mixture of rapeseed and argemone seed causes epidemic dropsy in man

2 The toxic substance in argemone seed is sanguinarine

B **Toxicants from microorganisms.**

1 The harmful microorganisms contaminate the raw foods such as meat, fish, milk etc purchased from the market

2 These microorganisms are destroyed during cooking or processing but some of the microorganisms survive due to insufficient heat

3 Pathogenic fungus infect foodgrains and oilseeds when stored in humid atmosphere causing serious illness

The pathogenic microorganisms responsible for contaminating foods are

Salmonella: This group frequently causes food poisoning developing the symptoms like vomiting, diarrhea, rise in body temperature Infection is transmitted mainly to the processed animal foods

Staphylococci. The contaminated food is poisoned owing to the presence of exotoxin produced by this organism Precooked animal foods cause poisoning developing the symptoms such as vomiting, diarrhea, and abdominal pain

Streptococci: This group is the agent for sore throat producing the symptoms like vomiting, diarrhea, and abdominal pain

Shigella: Bacillary dysentery is caused by this group Loose motions with mucus and blood and abdominal pain are the main symptoms Flies are the carriers for the bacteria from infected material to food when cooked foods are kept exposed The consumption of raw milk to which unclean water is added also causes the disease

Fungal contamination:

Fungi e g Aspergillus flavus, Penicillium islandicum, Fusariums, Clavoceps purpurea (ergot) produce a good number of toxic compounds (mycotoxins)

Aspergillus flavus: This fungus has been found to be grown in cotton seed, cereals, moist groundnut, and soyabean It produces a toxic substance named as aflatoxins which can develop cancer and cirrohosis of the liver in experimental animals The aflatoxin poisoning has recently been occurred in Rajasthan and Gujrat owing to consumption of maize highly contaminated with *Aspergillus flavus*

Penicillium islandicum: The yellow discolouration in rice has been reported in Japan by the contamination of rice by Penicillium islandicum during storage and develops toxic symptoms in man

Clavoceps purpurea (Ergot): This parasitic fungus infects foodgrains such as rye and pear millet during cultivation The disease '*Ergotism*' occurs as a result of the consumption of the contaminated grain The symptoms of this disease are nausea, vomiting, diarrhea, giddiness, severe burning sensation in the extremities, painful cramps in limbs, gangrene in the fingers and toes, depression, weakness, and convulsions The fungus produces the toxic alkaloids causing the disease

Fusarium and cladosporium: The millet is infected by this fungus when *left unharvested in the field during winter* The toxic compound formed by this fungus causes local inflammatory response, acute gastro-enteritis, nause, and vomiting within 1 to 3 days after the consumption of the infected grain The progressive pathological changes in the bone marrow due to the toxin leads to leukopenia, agranulocytosis, and anemia There is also petechial hemorrhages followed by the development of necrotic ulcers of the skin In less advanced cases, the recovery of the disease takes place, in case, the patient is put on a good diet with proper treatment

Parasitic infection:

The contaminated foods transmit some parasitic diseases e g amoebiasis, ascariasis, and hookworm when raw vegetables grown on sewage are consumed.

Contamination of foods with toxic chemicals, pesticides and insecticides.

Lead, mercury, arsenic, antimony, D D T , and B H C etc can contaminate foods due to the following reasons

1 The toxic chemicals such as barium carbonate, arsenic oxides, lead arsenate etc used as rat poison are accidentally mixed with food

2 Accidental contamination of food with pesticides and insecticides

3 Some toxic chemicals or minerals are also present in certain marine foods

4 The presence of large amounts of certain food additives

Toxic metals: The toxic element lead causes toxic symptoms after contamination with food The pathological changes in the kidneys, liver, and arteries are brought about by lead The common signs of lead poisoning are nausea, abdominal pain, anemia, muscular paralysis, and brain damage The toxic effects of methyl mercury are neurological When the brain is affected, the subject becomes blind, deaf, and paralysis of the various muscles makes him cripple Cadmium, arsenic, antimony, cobalt etc are toxic in small doses

Pesticides: The organic pesticides are *D D T , B H C ,* and *malathion* etc. and these are the toxic compounds The presence of large amounts of pesticides causes toxic effects

— **Additives:** In U S A and other western countries, the additives such as *diethyl stilbesterol and antibiotics are added to animal and poultry feeds* These are present in the meat of animals fed on feeds containing these chemicals Stilbesterol can cause leukemia and cancer even in small doses Antibiotics can cause drug resistance and hardening of arteries

Table 33 1. Toxic effects of some metals and chemicals

Metals and Chemicals	Foods	Toxic effect
Arsenic	Fruits sprayed by lead arsenic	Chills, Cramps, Paralysis
Barium	Foods contaminated by rat poison	Muscular twitching and convulsions
Cadmium	Fruit juices, soft drinks	Excessive salivation, liver and kidney damage, prostate cancer

Cobalt	Water, beer	Cardiac failure
Lead	Some processed foods	Paralysis, brain damage
Mercury	Mercury fungicide treated seed gains	Paralysis, brain damage
Tin	Canned foods	Colic, vomiting
Zinc	Foods stored in galvanised iron ware	Dizziness, vomiting
Copper	Acid foods in contact with tarnished copper ware	Vomiting, diarrhea, abdominal pain
Pesticides	All types of food	Damage to liver, kidney, brain, and nerves leading to death
Diethyl stilbesterol	Present in meat of stilbesterol fed animals and birds	Carcinogenesis
Antibiotics	Meat from animals fed antibiotics	Drug resistance, hardening of arteries, heart disease

Exercise

1 Describe how foods are *toxicated*?

CHAPTER 34

BLOOD, LYMPH AND CEREBROSPINAL FLUID

Blood is a tissue consisting of different types of cells—the red blood cells (RBC) the white blood cells (WBC) and the platelets suspended in a liquid medium called plasma It circulates in a closed system of blood vessels The red colour of blood is due to hemoglobin present in the RBC

Functions of blood

1 Blood transports oxygen from the lungs to the tissues and CO_2 from the tissues to the lungs

2 It transports absorbed food materials to the tissues

3 It transports metabolic waste products to the kidneys, lungs, skin and intestines for removal

4 In association with the kidneys and lungs it maintains the acid—base equilibrium of the body by its efficient buffering action

5 It maintains the steady osmotic pressure in the tissues and fluids of the body being assisted by the kidneys and the skin

6 The plasma proteins assist in the exchange of water in the body from the tissue to blood and vice versa

7 It maintains the body temperature at a constant level during its circulation

8 It transports hormones from the site of production to different tissues

9 By clotting it protects body from hemorrhage

10 The WBC form a defence against micro-organisms

11 It transports metabolites from one tissue to another e g. lactic acid formed in muscle is transported to liver and so on

12 Other substances in blood combat toxic agents , they are antitoxins, agglutinins, precipitins

NORMAL BLOOD

Blood volume The blood volume in the adult is about 7% of the body weight. An adult weighing 70 kg has about 5 litres of blood.

Plasma volume The plasma volume is about 4% of the body weight and is about 2 83 litres in an adult weighing 70 kg

Red blood cells (RBC) It is circular, non nucleated bi-concave disc The count of RBC in adult male is about 5 5 millions and in the adult female is 4 8 millions per cu mm. The life span is about 120 days The formation and destruction of RBC are going on continuously

Packed cell volume and hematocrit ratio This ratio is 45/55

Hemoglobin In normal males and females, the hemoglobin contents of blood are 15 8 g and 13 7 g per 100 ml of blood respectively One gram of hemoglobin can carry 1 34 ml oxygen under optimal conditions

Table 1 : Components of blood

I	Cellular fraction (Volume 45 per cent)	
	1 Red blood corpuscles (RBC)	5 000,000 per cu mm
	2 White blood corpuscles (WBC)	6,000 per cu mm
	3 Platelets	2,50,000 per cu mm
II	Plasma fraction (Volnme · 55 per cent)	
	A Non diffusible constituents	
	1 Albumin 2 Globulin 3 Fibrinogen 4 Enzymes, lipids etc	
	B Diffusible constituents	
	1 Hormones, vitamins etc	
	2 Anabolic constituents Glucose aminoacids, creatine etc	
	3 Electrolytes Na^+, K^+, Ca^{++}, Mg^{++}, Cl^- HCO_3^- HPO_4^- etc	
	4 Catabolic products Urea, creatinine, uric acid etc	

Chemistry

Table 2 Constituents present in Normal Blood

Constituents	*Values per* 100 *ml or dl*	*SI units*
Hemoglobin		
Men	14—18 g	1 86—2 48 mmol/L
Women	12—16 g	2 17—2 79 mmol/L.
Plasma Proteins		
Total protein	5—8 g	60—80 g /L
Albumin	3 5—5 5 g	0 54—0 847 mmol/L
Globulin	1 5—3 0 g	15—30 g /L
Fibrinogen	0 2—0 6 g	5 8—6 8 μmol/L
Nutrients		
Glucose (true) Fasting	60—100 mg	3 3—5 5 mmol/L.
Glucose (Folin)		
Fasting	80—120 mg	4 4—6 6 mmol/L
Free amino acid	3—5 5 mg	2 1—3 9 mmol/L
Lipids ·		
Total lip ds	570—820 mg	
Cholesterol (total)	150—280 mg	3 9—7 3 mmol/L
Cholesterol esters	50—66% of total cholesterol	
Phospholipid	145—200 mg	1 87—2 58 mmol/L
Free fatty acids	150—500 mg	

Constituents	*Values per 100 ml or dl*	*SI units*
Minerals in serum :		
Calcium	9—11 mg	2.25—2 65 mmol/L.
	4 5—>4 mEq/L	
Chloride	350—375 mg	100—106 mmol/L.
	100—106 mEq/L.	
Bicarbonate (HCO_3)	55—70 mg.	
Iroo	65—175 μg.	11 6—31 3 μmol/L.
Iodine, total	3—6 5 μg	0.24—0.51 μmol/L.
Iodioe protein bouod	4—8 μg	0.32—0 63 μmol/L.
Magnesium	1—3 mg.	0 75—1 25 mmol/L.
	1 5—2.5 mEq/L.	
Phosphorus, inorganic	3—4 5 mg (adult)	1—1.5 mmol/L.
	4—7 mg (children)	
Potassium	14—20 mg.	2.5—5 0 mmol/L.
	2.5—5 0 mEq/L.	
Sodium	310—340 mg	136—145 mmol/L.
	136—145 mEq/L.	
Copper	100—200 Mg	16—31 μmol/L.
Vitamins :		
Vitamio A	24—60 μg	0 84—2.1 μmol/L.
	24—60 I U	
Ascorbic acid (fasting)	0 4—1 5 mg	23—85 μmol/L.
Folic acid, free	0 6—2.0 μg.	
Vitamin B_{12}	100—800 μμg.	
Waste products :		
Urea	20—40 mg	
Uric acid	3—7 5 mg.	0 18—0.29 mmol/L.
Bilirubin	0 2—0 7 mg. (Indirect)	3 42—11 97 μmol L.
	0 1—0 4 mg (Direct)	1 71—5 84 μmol/L.
Creatinine	0 7—1.5 mg	60—130 μmol/L.
Enzymes .		
Serum amylase	80—180 somogyl units	2.48—5.58 μkat/L.
	0 8—3.2 I U /L.	
Lactic dehydrogenase	90—200 I U./L.	1.50—3.34 μkat/L.
Acid phosphatase	1—5 Units (K.A.)	4 48—17.94 μkat/L.
Alkaline phosphatase	5—13 Units (K.A.)	59—153 4 μkat/L.
Lipase .	0 2—1 5 units	0 93—6 96 μkat/L.

Constituents	*Values per 100 ml or dl*	*SI units*
Transaminases		
SGOT	5—40 units	40 1—320 8 nkat/L
	6—25 I U /L	
SGPT	5—35 units	40 1—280 7 nkat/L.
	3—26 I U /L	
I actic acid	0 44—1 8 mmol/L	0 44—1 28 μmol/L
	4—16 mg	
Nonprotein nitrogen	15—35 mg	10 7—25 mmol/L
Pyruvic acid	0 7—2 mg	79 8—228 μmol/L

PROPERTIES OF BLOOD

Specific gravity The specific gravity of normal blood usually lies between 1 05 and 1 06 The specific gravity of plasma lies between 1 024 and 1 038 and roughly proportional to the protein content

Viscosity The viscosity of blood is important in the sense that it determines the blood pressure Human blood is 5 times as thick as water The high viscos ty is due to the cells plasma has a very much lower viscosity The viscosity of blood is affected by the change in the numbers or size of red cells or white cells Abnormal values are found in leukemias severe hemorrhage and pernicious anemia

P^H Blood is slightly alkaline and its P^H lies between 7 3 and 7 5 In the resting individuals the arterial blood is very slightly more alkaline (about 0 02) than venous blood This difference is increased by muscular exercise due to the more formation of lactic acid Under normal conditions the P^H of blood of an individual is maintained in the region of 7 4 The P^H of an individual is below 7 3 is considered in a condition of *acidosis* and over P^H 7 5 is under the condition of *alkalosis*

Osmotic pressure Normally the osmotic pressure of blood is constant which is equal to 0 945% Nacl The osmotic pressure of blood is slightly reduced on the ingestion of large amounts of water and increased on strenuous exercise

Clotting of blood Blood is clotted or coagulated within 5 or 10 minutes after shedding if it is left undisturbed Different factors are involved in blood clotting which is discussed elaborately in physiology

Composition Sugar and urea are fairly equally distributed between plasma and corpuscles but others are almost entirely confined to one or the other e g Na and Ca in the plasma and K in corpuscles Bromide iodide indican enzymes anti enzymes hormones vitamins and various antibodies are circulating in blood Plasma contains 8% to 9% solids composed largely of proteins The constituents of plasma are as follows

Proteins	7 0%
Lipids	0 7%
Inorganic substances	0 75%
Other organic substances	0 15%

In normal individuals, the plasma proteins vary from 6.0% to 8.5%. The proteins are as follows

Serum albumin	4.5% of plasma.
Serum globulin	2.2% „ „
Fibrinogen	0.3% „ „

Other proteins such as glycoproteins, lipoproteins, enzymes and hormones are present in small amounts

PLASMA PROTEINS

Chemistry :

1. The human plasma proteins are a mixture of simple proteins, glycoproteins, lipoproteins and other conjugated proteins

2. These proteins are separated by salt precipitation, electrophoresis and immunoelectrophoresis

3. The three major plasma proteins (albumin, globulin and fibrinogen) are separated by the use of varying concentrations of sodium or ammonium sulfates These fractions are separated further by electrophoresis. These are as follows

Albumin	52—65% of total plasma protein.
Globulin	29.5—54%
α_1—Globulin	2.5—5 %
α_2—Globulin	7—13%
β—Globulin	8—14%
γ—Globulin	12—22%
Fibrinogen	6.5%

A/G ratio 1·2 : 1

The electrophoretic representation of normal human plasma is shown below :

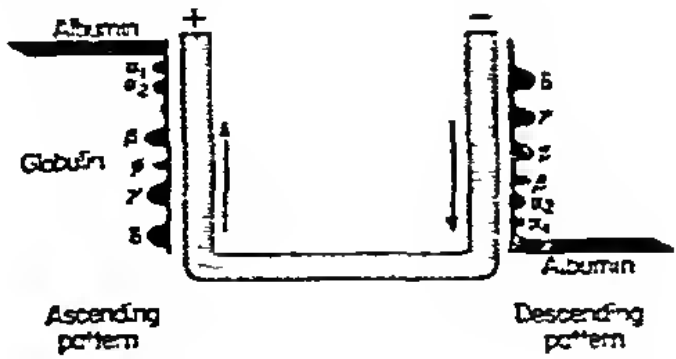


Fig. 34.1 Electrophoresis of normal human plasma.

4. The characteristics of individual plasma proteins are as follows :

I *Albumin :*

(i) About half of the total plasma proteins is albumin.

(ii) It consists of 610 amino acids arranged in a single peptide chain.

(iii) It has a molecular weight of about 69,000 and is synthesized in the liver

(iv) It is precipitated by full-saturation with ammonium sulphate

(v) The normal concentration of serum albumin is 4 5 gm/100 ml by precipitation method

(vi) It exerts 80 per cent of the colloid osmotic pressure of plasma

(vii) It plays an important role in the exchange of water between tissue fluid and blood .

(viii) Its concentration decreases in severe protein deficiency, liver diseases and nephritis leading to the development of edema

II Globulins :

(i) Globulins are separated by half-saturation with ammonium sulphate

(ii) The molecular weight ranges from 90,000 to 13,00,000

(iii) The normal concentration of serum globulin is 2 5 gm/100 ml by precipitation method

(iv) Globulins are separated into α_1^-, α_2^-, β^- and γ^- globulin fractions α^- and β^- globulins are synthesized in liver but γ-globulins are formed in the cells of reticulo-endothelial system

(v) The nature of different globulin fractions are discussed below :

α_1 Globulins : This fraction is of several complex proteins containing carbohydrates and lipids These are orosomucoid, α_1-glycoprotein and α_1 lipoproteins Its normal concentration in serum is 0 42 gm/100 ml

α_2 Globulins This fraction also includes complex proteins such as α_2 glycoprotein plasminogen, prothrombin, haptoglobulin, ceruloplasmin and α_2-macroglobulin The normal concentration of it in serum is 0 67 gm/100 ml

β Globulins : This fraction consists of lipid containing three different β-lipoproteins It also contains siderophilin Its normal concentration in serum is 0 91 gm/100 ml

γ Globulins These are immunoglobulins having antibody activity They are classified as IgG, IgA and IgM on the basis of their electrophoretic mobility

Mucoproteins and glycoproteins : In addition to globulins they contain carbohydrates such as galactose, mannose, fructose and hexosamine They are found in α_1 and α_2 globulin fractions Those which contain more than 4% hexosamine are designated as mucoproteins and those which contain less than 4% hexosamine are glycoproteins Some glycoproteins have specific binding function for thyroxine and cortisol

Lipoproteins : Two lipoproteins (α and β) are found in the α- and β globulin fractions Human serum lipoproteins are separated into different fractions Fraction A contains the β lipoproteins with densities less than 1 063 , fraction B contains the α_2-lipoproteins with densities of 1 063—1 107 , and fraction C contains the α -lipoproteins with densities of 1 107—1 220 The lipoproteins are large molecules having molecular weights of 1,300 000 They are the major carriers of the lipids of the plasma

Metalloproteins : The two important metalloproteins are transferrin (containing iron) and ceruloplasmin (containing copper) Transferrin transports iron and ceruloplasmin transports copper into different tissues Transferrin is found in the β globulin fraction while ceruloplasmin in α_2 globulin fraction Metalloprotein concentration is reduced in pernicious anemia, chronic infections or liver diseases and its concentration is increased in iron deficiency or pregnancy.

III. Fibrinogen :

(i) Its molecular weight is between 350,000 and 450,000

(ii) It is the precursor of fibrin (the substance of the blood clot)

(iii) Like globulin, it is precipitated by half saturation with ammonium sulphate.

(iv) It normally constitutes 4—6% of the total proteins of the plasma.

(v) It is formed in the liver and its concentration in blood falls rapidly in the excessive destruction of liver tissue

(vi) It is a large asymmetrical molecule which is highly elongated having an axial ratio of about 20 1

Functions :

1 Nutritive : Albumin is largely involved in the nutritive functions of the plasma proteins owing to its high concentration It is effective as a source of protein in hypoproteinemic patients

2. Water distribution : The colloid osmotic pressure of plasma proteins plays an important role in the distribution of water between the blood and the tissues Plasma albumin is responsible for this function due to its low molecular weight and quantitative dominance over other proteins In kidney diseases where protein loss from the body is more, large amonot of water moves to the tissues producing edema.

3 Buffering actions : The serum proteins can comb oe with acids or bases to maintain the p^H of blood They act as acids and combine with cations (mainly sodium) at the normal p^H of the blood

4 Transport The plasma proteins transport lipids and the fat-soluble vitamins (e g A, D & E) Bilirubin is associated with albumin and also with fractions of the α globulins. Thus bilirubin is transported along with them β_1-metal combining globulins (Siderophilin) is responsible for the transport of iron in the plasma Thyroxine is transported in association with an α globulin (thyroxine binding protein, TBP) and cortisol by a mucoprotein (transcortin) Many drugs and dyes are transported in the plasma in combination with al bumin Half of the calcium of plasma is bound to protein for transport. Hemoglobin liberated in intravascularly is carried to the reticuloendothelial system by complexing with the hepatoglobins

5 Viscosity : Because of the presence of protein in plasma, it is a viscous fluid the viscocity of blood provides resistance to flow of blood in the blood vessels to maintain blood pressure at normal level

6 Coagulation : Plasma contains prothrombin, fibrinogen and other factors involved in coagulation of blood.

7 Immunity : γ-globulins are present in plasma and these γ globulins protect body against bacterial infections

Enzymes : Plasma contains several enzymes of diagnostic importances in some diseases

Leukocytes .

1 They contain proteins, nucleoproteins, fats, lecithin, cholesterol, purines enzymes and inorganic salts

2 They are highly supplied with proteases

Blood platelets :

1 They are unnucleated and so contain no DNA, but RNA is present

2 They contain glycogen, ADP, ATP, and the enzymes of glycolysis.

3 Large amounts of catecholamines, 5 hydroxytryptamine (serotonin), and histamine are present in them

4 Phospholipids are also present in the form of a lipoproteins which activates prothrombin in blood coagulation

5 They also contain a contractile protein (thrombosthenin) which is involved in the process of clot retraction

BLOOD GROUPS

Human blood is classified into 4 main groups and several sub groups Agglutinogens are neutral nitrogenous mucopoly-saccharides with molecular weights ranging from 200,000 to 300 000 The four main groups are A, B, AB and O The minor groups are M, N, P and Rh

The plasma contains antibodies called agglutinin The distribution of agglutinogens in RBC and agglutinins in plasma in the four groups are noted below

Blood group	Agglutinogen in RBC.	Agglutinin in plasma	Description of the group
A	A	β(anti—B)	Aβ
B	B	α(anti—A)	Bα
AB	A and B	Nil	AB
0	Nil	$\alpha+\beta$ (anti-A+anti B)	0$\alpha\beta$

To determine the blood group of an individual, an isotonic saline suspension of RBC is mixed with a test serum containing agglutinin α or agglutinin β on a

slide When no agglutination occurs the cells separate and evenly distributed When agglutination occurs the cells clump together The results are interpreted as follows :

1 Blood of group A is agglutinated by plasma of group B containing α-agglutinin.

2. Blood of group B is agglutinated by plasma of group A containing β-agglutinin

3 Blood of group AB is agglutinated by the plasma of blood group A and B containing agglutinins β and α respectively

4 Blood of group O is not agglutinated by the plasma of group A, B or AB Therefore, persons of group O are called universal donors Anybody can receive their blood

Rh blood groups :

It is an antigen of the Rhesus monkey and is present in the blood of 85% of white people and may be transmitted from father to child. The commonest Rh antigen is D and its antibody is anti D If the mother is Rh negative, she develops antibodies to it and these antibodies pass through the placenta to the fetus and cause severe destruction of red blood cells in the new born child.

HEMOLYSIS

Red blood cells (RBC) are destroyed by hemolysis. Hemolysis can take place in the following ways :

1 **Osmotically :**

(i) The RBC membrane is permeable to water. When the blood is placed in a hypotonic solution, red cells swell owing to water passing in If the solution is more hypotonic, the red cells swell up more and hemoglobin comes out of the red cell due to the rupture of its membrane

(ii) If the cells are placed in a hypertonic solution, water passes out of the cell and the cells shrink due to reduction in volume The process is called *crenation*

2 By hemotoxins :

(i) Snake venom and certain other bacteria can produce hemolysis in vivo The hemolytic effect is due to the presence of the enzyme phospholipase A which hydrolyzes phospholipids and thus disrupts the cell membrane.

(ii) The W R (Wassermann reaction) for syphilis employs a hemolytic system, produced by the injection of foreign red cells into an animal such as a rabbit

3 *By certain drugs* Quinine, nitrites and chlorates cause hemolysis.

4 *By lipid solvents* Alcohol chloroform, ether, bile salts and saponin cause hemolysis due to their solvent action on the lipid of the plasma membrane

5 *By mechanical means* Grinding, vigorous stirring or shaking causes destruction of erythrocytes

6 *Other hemolytic agents* Heat, pH changes, ultraviolet light etc can cause hemolysis

BLOOD CLOTTING

Stop of bleeding is said to be *hemostasis* There are four phases of hemostasis (i) The first phase is the constriction of the injured vessel to reduce blood flow, (ii) The second phase consists of formation of a loose platelet plug or white thrombus at the site of injury Collagen is exposed at the site of injury and acts as a binding site for platelets (iii) The third phase is the formation of red thrombus (blood clot) (iv) The fourth phase is the partial or complete dissolution of the clot

Clots are of three types The white thrombus is composed of platelets and fibrin and is poor in erythrocytes It is formed at the site of injury, particularly in areas of rapid blood flow (arteries) The second type of thrombus is deposited in small vessels (capillaries) The third type is the red thrombus and consists of red cells and fibrin

Initiation of clot formation to tissue injury is carried out by *extrinsic pathway* and the initiation of the pure red thrombus in an area of restricted blood flow without tissue injury is carried out by *intrinsic pathway* The intrinsic and the extrinsic pathway merge into a final *common pathway*—the activation of Prothrombin to thrombin and thrombin catalyzed fibrinogen to fibrin clot

The blood clotting factors are mentioned below

Factor	Name
I	Fibrinogen
II	Prothrombin
IV	Calcium
V	Labile factor, proaccelerin, accelerator (AC) globulin
VII	Proconvertin, serum prothrombin conversion accelerator (SPCA), cothromboplastin, autoprothrombin I
VIII	Antihemophilic factor, antihemophilic globulin (AHG)
IX	Plasma thromboplastin component (PTC) (christmas factor)
X	Stuart Power factor
XI	Plasma thromboplastin antecedent (PTA)
XII	Hageman factor
XIII	Laki-Lorand factor (LLF)

The Conversion of Fibrinogen to Fibrin by Thrombin.

1 Fibrinogen is a soluble plasma glycoprotein whose length is 46 nm Its molecular weight is 340,000 and it consists of 6 polypeptide chains synthesized in liver The 6 chains are two $A\alpha$ chains, two $B\beta$ chains, and two γ chain making the structure $A\alpha_2\ B\beta_2\ \gamma_2$ All three genes ($A\alpha$, $B\beta$ and γ) are genetically linked

2 The ends of the fiber-shaped fibrinogen molecule are *highly negatively charged* These negatively charged termini of the fibrinogen molecules contribute to its water solubility and also repulse the termini of other fibrinogen molecule, for which aggregation is prevented

3 The molecular weight of *Thrombin* is 34,000 and it consists of two polypeptide chains and hydrolyzes four peptide bonds in fibrinogen Removal of A and B portions of the fibrinogen molecule releases the negatively charged, *fibrinopeptides* and generates the *fibrin monomer* The long insoluble fibrin monomers form the insoluble *fibrin polymer clot*—which traps red cells, platelets, and other components to form the red thrombus or the white thrombus (platelet plug) The initial fibrin clot is weak and held together only by fibrin monomers

4 Thrombin also converts factor XIII to active factor XIII (XIII$_a$) which is a *transglutaminase*. This transglutaminase covalently cross-links fibrin monomers. Persons with an inherited deficiency of factor XIII have a bleeding tendency due to unstable fibrin clot.

5 Prothrombin is a single chain glycoprotein with a molecular weight of 72 000. It is activated on the platelet and its activation requires platelet anionic phospholipid, Ca^{++}, factor Va, and factor Xa. The phospholipids bind Ca^{++} and prothrombin. The platelets also contain factor V and is activated as Va which binds to specific receptors in the platelet membrane. Factor Va also acts as a receptor for factor Xa which in turn binds prothrombin. Factor Xa is a serine protease and cleaves the catalytically inactive prothrombin and the amino portion of prothrombin is released.

6 Factor Va, generated by thrombin, is subsequently *inactivated by thrombin* providing a means of limiting the activation of prothrombin to thrombin.

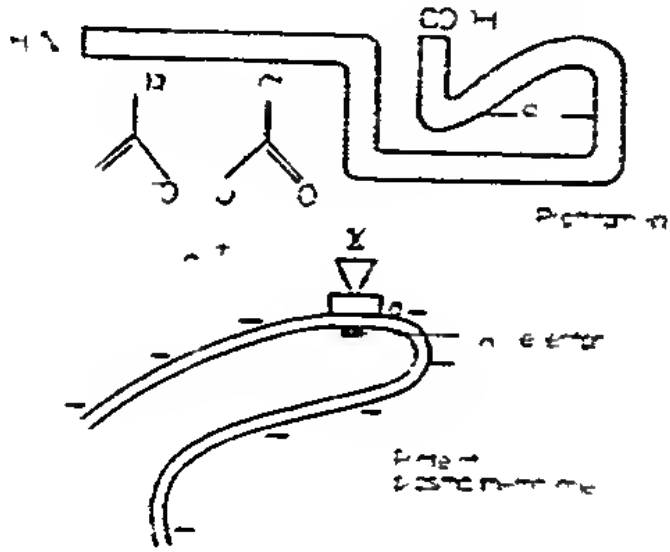


Fig 34.2 Diagrammatic representation of the binding of factors Va, Xa, Ca^{++}, and prothrombin to the platelet plasma membrane.
[Gla=γ-carboxyglutamic acid.]

Activation of factor Xa.

1 Activation of factor Xa takes place at the conceptual site where the intrinsic and extrinsic pathways join to form the *final common pathway*.

2 Factor X is a zymogen having molecular weight of 55,000 of a serine protease and contains Gla residues.

3 The Gla residues of factor X are responsible for the calcium mediated binding of factor X to the acidic phospholipids of platelet membranes.

The extrinsic pathway for generating Factor Xa.

1 The extrinsic pathway is very rapid in response to tissue injury.

2 Factor VII is the precursor of factor VIIa and it is the Gla-containing glycoprotein synthesized in the liver. It can also be cleaved by thrombin or factor Xa. It is a zymogen and has high endogenous activity.

3. The tissue factor necessary to accelerate the attack of factor VII or VIIa on factor X is abundant in placenta, lung, and brain.

4. There is only 0·01 mg of factor X per ml of plasma. This requires the amplification provided by the clotting system, conversion of factor X to Xa is an autocatalytic process and therefore an *amplification system.*

The Intrinsic Pathway for Generating Factor Xa.

1 The intrinsic pathway for the generation of Xa starts with the exposure of *prekallikrein*, high molecular-weight *kininogen*, *factor XII*, and *factor XI* to an activating surface

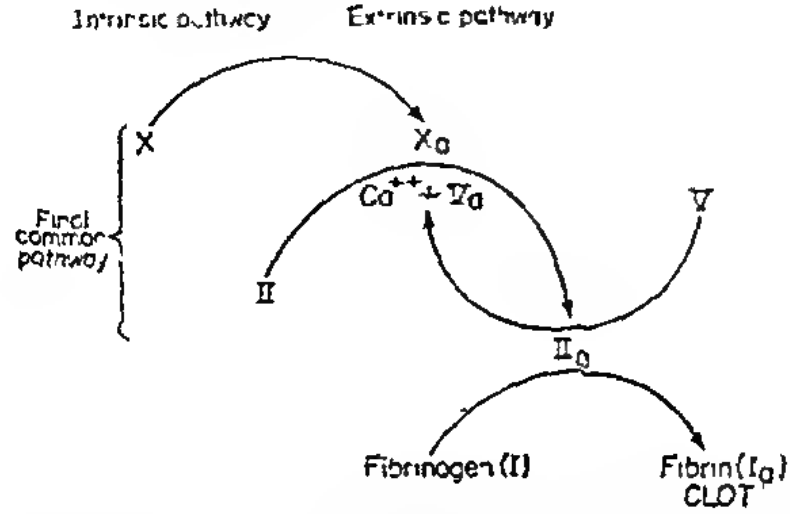


Fig 34 3. Relationship between the intrinsic/extrinsic and final common pathways of blood clotting.

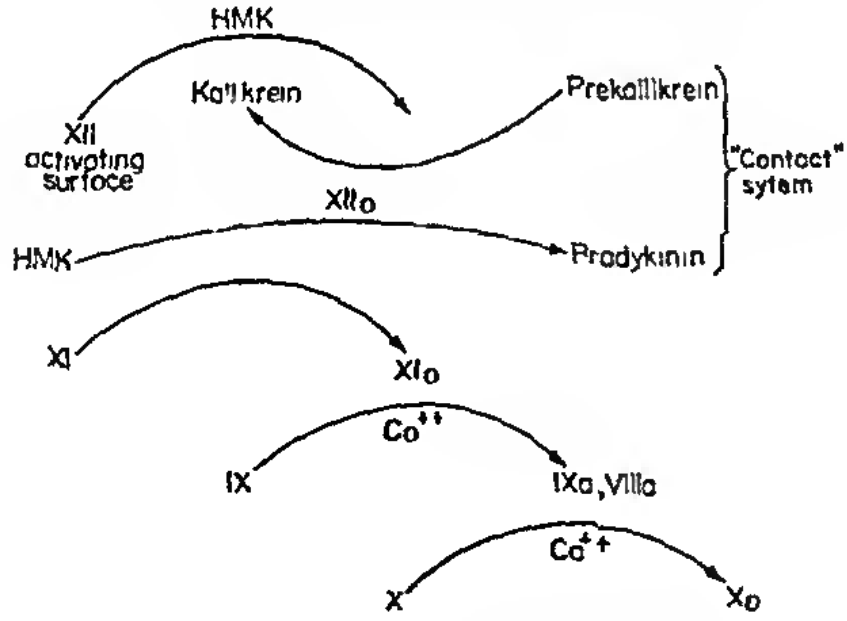


Fig. 34 4. The intrinsic pathway for activating factor X to Xa.
HMK is high molecular weight Kininogen.

2 Factor XIIa is generated by kallikrein and attacks prekallikrein to generate more kallikrein, setting up a reciprocal activation It releases bradykinin from high molecular weight kininogen and activates factor XI to XIa

3 Factor IX, a Gla—containing zymogen, is activated in a two step reaction by factor XIa

4 Factor IXa slowly activates factor X by clearing the same Arg IIe bond that factor VIIa of the extrinsic system hydrolyzes in the presence of calcium and acid phospholipids The factor IXa catalyzed activation of factor X is accelerated about 500 fold by the presence of factor VIII or VIIIa

5 Factor VIII requires activation by minute quantities of thrombin to form factor VIIIa Factor VIII is not a protease but serves as a receptor for factor IXa.

6 The intrinsic pathway is slow because it involves many factors operating in a *cascade mechanism* to generate factor Xa

Hemorrhagic disorders and their abnormalities

Hemophilia A The common deficiency of factor VIII produces a disease known as hemophilia A The X chromosome linked deficiency of factor VIII has played a major role in the history of the royal families of Europe

Von Willebrand's disease This disease is caused by the defect in *platelet adherence* and a deficiency of factor VIII clotting activity (an antigenic material) The platelet adherence factor (Von Willebrand factor) is a large glycoprotein synthesized in vascular endothelial cells and megakaryocytes (platelet precursor cells) It exists in plasma and platelets tightly associated with the factor VIII molecule Platelet surfaces seem to possess a glycoprotein receptor for the factor VIII/Von Willebrand factor complex The Von Willebrand factor also stabilizes factor VIII procoagulant activity This disease may be an inherited defect in a specific oligosaccharide moiety on the Von Willebrand glycoprotein factor The abnormal oligosaccharide may prevent normal platelet adherence and destabilize factor VIII Hemophilia A is a factor VIII protein defect that interrupts its clotting function but does not adversely affect the platelet adherence function of the Von Willebrand factor

Anticoagulants

1 Normal plasma contains three antithrombin activities α_1-antitrypsin has a minor antithrombin activity α_2 globulin is responsible for about 25 per cent of the antithrombin activity The α_2 globulin forms an irreversible complex with thrombin and other proteases and thereby prevents their binding to their neutral substrates Therefore, it is referred to as α_2 plasmin inhibitor

2 **Antithrombin III** has a major antithrombin activity It has some endogenous activity but is greatly activated by the presence of *heparin* which is strongly anionic proteoglycan Heparin binds to a specific cationic site of antithrombin III inducing a conformational change that accelerates the binding of antithrombin III to all serine proteases including trypsin, chymotrypsin, and plasmin Antithrombin III inhibits the activity of thrombin, IXa, Xa, XIa, and XIIa Antithrombin III deficiencies develop frequent and severe widespread clots

3 ***Heparin*** is frequently used in clinical medicine to inhibit clotting The high-dose heparin therapy may cause the prothrombin time prolonged but usually is not prolonged by continuous intravenous administration of heparin The low-doses of heparin administration may coat the endothelial lining of vessels and thereby reduces the activation of the intrinsic pathway Heparin can be antagonized by the use of strongly cationic polypeptides such as protamine to compete with the antithrombin III cationic region for the binding of the polyanionic heparin

4 The **coumarin** drugs inhibit the vitamin K-dependent carboxylation of Glu to Gla residues at the amino-terminal regions of factors II, VII, IX, and X These factors, synthesized in the liver, are dependent upon the Gla residues for maturation and do normal function in the intrinsic, extrinsic, and final common pathways The coumarin drugs inhibit reduction of the quinonx derivatives of vitamin K to the active hydroquinone forms Therefore, the administrat on of vitamin K bypasses the coumarin indueed block and allows maturation of the Gla-dependent clotting factors in the liver to occur

Fibrinolysis·

1 **Plasmin** is a serine protease capable of digesting both fibrinogen and fibrin as well as factor V, VIII, complement, and various polypeptide hormones

2 **Plasmin** normally exists in plasma in the inactive form *plasminogen* Upon formation of fibrin, plasminogen activator cleaves plasminogen to gene rate plasmin When plasmin digests fibrin, the plasminogen activator no longer remains active and proteolysis stops providing a well regulated fibrinolytic process

3 **Urokinase,** the proteolytic enzyme of urine, is also a serine protease anr' can cleave plasminogen at two sites activatiog the protease activity of plasmin

4 **Plasminogen** normally coprecipitates with fibrin and is incorporated into fibrin deposits When it is activated, it digests the fibrin in clots to soluble fragments dissolving the clot Cross linked fibrin clots are less sensitive to dissolu tion by plasmin

5 The plasminogen activators increase in cancers and shock In cirrhosis of the liver, the antiplasmin activities are also impaired Some bacterial products can activate plasminogen without cleavage and are responsible for the diffuse hemorrhage sometimes observed in patients with disseminated bacterial infec tions

Clotting Tests:

1 **Prothrombin time:** This is a test of the extrinsic pathway and the final common patyway of clotting It is performed by adding a tissue factor to the unknown plasma and determining the time necessary for the formation of visible clot The tissue factor along with factor VII activates factor X to Xa in the presen e of factor Va, Ca^{++}, and platelet phospholipids The complex converts protrombin to thrombin which in turn catalyzes the formation of fibrin from fibrinogen The deficiency of factor II, V, VII, or X or a severe deficiency of fibrinogen prolongs the prothrombin time

2 **The clotting time** This is determined by introducing freshly drawn whole blood into small glass tubes Care must be taken that no tissue factor is introduced from the site of venipuncture The blood is gently agitated at regular intervals to determine the time required for coagulation The intrinsic pathway is activated by exposure of whole blood to the glass surface of the tube The clotting time is dependent upon all clotting factors except factors VII and XIII This test is not sensitive to mild deficiencies of factors VIII, IX or XI

3 **The activated partial thromboplastin time:** This involves the addi tion of acid phospholipids and kaolin to plasma to make certain that platelets are not rate-limiting and that factors XI and XII are fully activated. The normal respond time is 35-45 seconds This is more useful than the clotting time deter mination.

4. **The thromboplastin generation tests:** This test activates the intrinsic system to form a prothrombinase or "plasma-thromboplastin" The prothrombinase does not itself clot the fibrinogen but it is capable of activating prothrombin to thrombin which in turn forms fibrin clot from fibrinogen Factor VII is not involved in this test This test involves mixing the patient's plasma after adsorption (containing factors I, V, VIII, XI, XII) with the patient's serum (containing factors VII, IX, X, XI, XIII) and normal platelets and Ca^{++} If no clot forms and the addition of normal serum or normal plasma corrects the defect, there is sure of the missing factor or factors If plasma alone and serum alone both correct the defect, factor XI or XII must be missing from both the patient s serum and plasma If only normal serum corrects the defect factor IX or X must be defective in the patient's serum If only normal plasma corrects the defect, factor V or VIII must be defective in the patient's plasma Antisera specific to the various factors are also available for determining which specific component of the clotting system is missing or defective

LYMPH

1 The term 'lymph" denotes a fluid not only present in the lymphatic vessels but also the fluid which bathes the cells, the tissue or interstitial fluid

2 It resembles plasma in its content of substances which can permeate the capillary wall but there are some differences in electrolyte concentrations

3 About 2 litres of lymph are drained into the blood stream per day Lymph flow is very slow

4 The protein content of lymph varies widely The fluid from the leg contains 2 3 % protein , whereas that from the intestines contain 4-6% and that from the liver 6-8%

5 The lacteals absorb the majority of fat from the intestine

6 The ratio between albumin and globulins in lymph is the same as in plasma

7 The protein present in entire plasma in an adult human is about 210 grams About one-third of this leaks out into the lymph through the interstitial fluid and is returned to the blood at the thoracic duct This process goes on continuously If the thoracic lymph is drained off, the concentration of plasma-proteins falls and the blood volume decreases

CEREBROSPINAL FLUID

Chemistry

1 The cerebrospinal fluid is formed by the ultrafiltration of the plasma by the choroid plexuses of the brain

2 The normal fluid is water clear with a specific gravity of 1 003—1 008 and p^H 7 4

3 Normally, the protein content is low about 20—45 mg 100 ml With an albumin-globulin ratio of 3 I

4 It contains only a small number (less than 5) of lymphocytes per cu mm

5 The total volume of C S F in an adult human is about 130 ml and this is renewed about 6 to 8 times a day

6 The sugar in C S F is somewhat less than in blood 50—85 mg/100 ml in the fasting adult

7 The chloride concentration is normally 700—750mg/100ml of the fluid (expressed as NaCl)

8 The concentration of calcium in normal human C S F is 2 43±0 05 mEq/L that of magnesium 2 40±0 14 mEq/L The ratio of calcium to magnesium is 1 01±0 06

Functions

1 The C S F acts as a buffer

2 It acts as a reservoir to regulate the contents of the cranium If the quantity of blood supply to brain increases the C S F drains away

3 It serves to a limited extent as a medium for nutrient exchange in the nervous system even though the brain derives its nutrients mainly from blood

Clinical significance :

1 In the inflammatory lesions of the central nervous system the protein content of the fluid is increased as high as 125 mg to over 1g /100 ml of the fluid

2 In uremia pneumonia and typhoid fever the protein content is raised in the fluid In neurosyphilis, encephalitis abscess and tumor of the brain, the concentration of protein in C S F is elevated above normal of 20 300mg/ 100 ml

3 In the various forms of suppurative meningitis the total protein content of C S F is high (125 to 300 mg per 100 ml) consisting of globulin, albumin, small amounts of fibrinogen and many other proteins High values are also found in tuberculous meningitis (200 to 2000 mg) and in acute luetic meningitis (180—540 mg) In some cases of early meningitis the fluid protein may be normal

4 In leukemic and carcinomatous infiltration of the meningitis, the sugar content can be very low and the protein level is high

5 The protein content of C S F is increased in myxedema Values as high as 100—200 mg 100 ml are observed

6 The sugar content of C S F is raised in encephalitis, central nervous system syphilis, abscesses and tumors It is decreased in purulent meningitis

7 In most forms of meningitis the chloride is decreased, the reduction is most marked in tuberculous meningitis The chloride content is unchanged in syphilis, encephalitis, poliomyelitis and other diseases of the central nervous system

8 Increased level of calcium is observed in all cases of meningitis and epidemic encephalitis

9 Aminotransferases lactate dehydrogenase and glucose phosphate isomerase also occur in C S F Isomerase activity is increased in malignant brain tumors Elevations also occur in meningitis and cerebral thrombosis Lactate dehydrogenase and aspartate aminotransferase are also increased in cerebral vascular accidents In convulsive disorders increased levels of creatine kinase, aspartate aminotransferase and lactate dehydrogenase are found in C S F

Exercise

1 Discuss the chemistry composition and functions of blood (Pun. 61S)

2 Describe the chemistry and functions of plasma proteins (Math 64A , P U 62S)

3 Write short notes on

(a) Blood groups (R. U 66A)
(b) Hemolysis (M U 73S)
(c) Lymph (Bhag. U 74A)
(d) C S F (P U 68A)

4 Discuss the mechanism of blood clotting

CHAPTER 35
EXCRETION

The kidneys

A large number of waste products are produced in the body as a result of metabolic activities The main waste products are carbon dioxide, water, and nitrogenous compounds The retention of these products produces a harmful effect on the normal health Therefore, the removal of these products from the body is a must Carbon dioxide is removed mainly through lungs and water as well as nitrogenous compounds are removed through urogenital system. The kidneys are the most important component of this system

The kidneys are two in number, usually bean shaped, and exist behind the peritoneum on either side of the vertebral column extending from the 12th thoracic to the 3rd lumbar vertebra Each kidney weighs about 120-170 grams and is about 11-13 cms long, the left being larger than the right one

Each kidney is found to consist of two main parts by section The outer part is called cortex and the inner one is medulla. The cortex consists of a large number of glomeruli and convoluted tubules The medulla is composed of renal tubules

projecting into a cavity towards the inner region of the kidney called the pelvis, the region where the renal artery and vein enters and leaves the kidney respectively.

Nephron: It is a functional basic unit of kidney. Each kidney is provided with about one million nephrons. Each nephron contains the glomerulus and the tubule. The glomerulus is a network of affarent and efferent capillaries. Each glomerulus is surrounded by a double-walled epithelial sac known as *'Bowman's Capsule* which leads to the tubule which is divided into three parts—proximal convoluted tubule, loop of Henle, and the distal convoluted tubule.

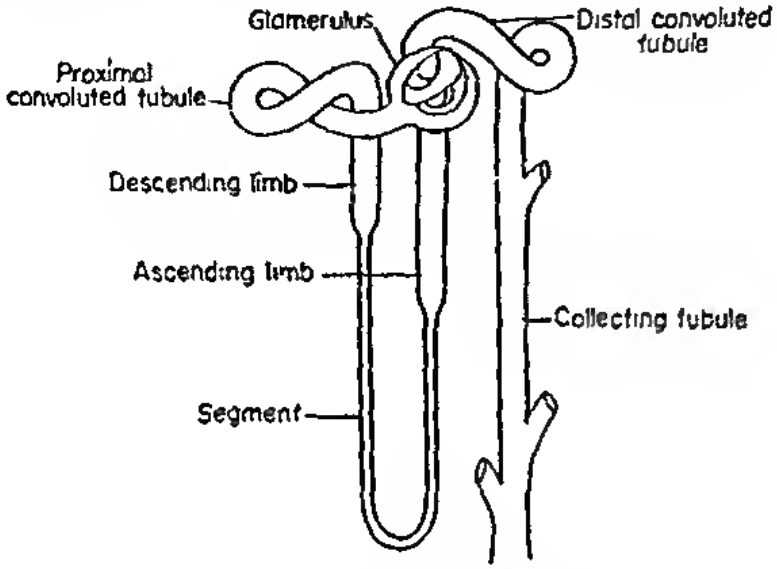


Fig 35 1 Diagram of nephron

The proximal convoluted tubule (PCT) is about.45 mm. long and 50 mm. in diameter This lies in the cortex along with glomerulus. Its luman is continuous with that of the Bowman's Capsule. It consists of cells with scalloped outline and brush border. The brush border is formed by numerous microvilli which increases the surface enormously for absorption.

The loop of Henle consists of three parts—the descending limb, a thin segment, and an ascending limb. The proximal convoluted tubule opens into the descending limb which is continued into the thin segment from where the ascending limb arises. The whole loop of Henle is lined by a single layer of flattened epithelial cells.

The ascending limb of the loop of Henle continues into the distal convoluted tubule (DCT) which finally opens into a collecting tubule or duct which carries the urine to the renal pelvis from where it is carried to the bladder by the ureter. The distal convoluted tubule commences near the pole of the glomerulus and establishes a close proximity to the afferent arteriole of its parent glomerulus. The DCT contains cuboidal epithelium.

Nephrons are mainly of two types—cortical and juxtamedullary. The loop of Henle of the juxtamedullary is long and dips deep into the substances of the medulla. But the loop of Henle of cortical is short and only a very small part of it dips into the medullary tissue and the greater part remains embedded in the cortical substances. Moreover, the glomeruli of the juxtamedullary lie very close to the medulla while those of cortical lie close to the surface of the kidney. The juxtamedullary nephrons constitute 20 per cent of nephrons, while the cortical

nephrons constitute 80 per cent of the total nephrons. These two types of nephrons have the same common function.

Blood supply of the kidneys: The short renal artery arising from the abdominal aorta supplies the blood to the kidney. The renal artery after entering the kidney divides into a number of arterioles—*the afferent arterioles* which further branch into capillaries and enter into each glomerulus. These capillaries then join to form aoother arteriole—*the efferent arteriole* which opens ioto another set of capillaries called peritubular capillaries surrounding the proximal tubule, the loop of Henle, and the distal tubule. Ultimately, the capillary set opens into a venule which joins with other venules to form the renal vein. The renal vein then opens into the inferior vena cava.

Blood flow through the nephron: The blood flows through both the kidneys of an adult weighing 70 kg. at the rate of about 1200 ml./mt. The portion of the total cardiac output (about 560 ml./mt.) which passes through the kidneys is called the *renal fraction.* This is about 560/1200 ml. per minute, i.e. about 21 per cent.

There are two sets of capillaries—the glomerulus and the peritubular. These two capillaries are separated from each other by the efferent arteriole which contributes sufficient resistance to blood flow. The glomerular capillary bed provides a high pressure about 70 mm. Hg., while the peritubular bed provides a low pressure about 13 mm. Hg. The pressures in the artery and vein are 100 mm. Hg. and 8 mm. Hg. respectively. The high pressure in the glomerulus exerts the filtering of fluids continually into the Bowman's Capsule. The low pressure in the peritubular capillaries system, on the other hand, functions in the same way as the usual venous ends of the tissue capillaries with the fluid being absorbed continually into the capillaries.

Functions of kidney:

1. Kidney eliminates excess of certain nutrients such as sugar and amino acids when their concentratioo increases in the blood.
2. It removes certain non-volatile waste products such as urea, urie acid, creatinine, and sulphates etc. from the body.
3. It eliminates certain foreign or toxie substances such as iodides, pigments, drugs, and bacteria etc. from the blood.
4. It regulates hydrogen ion concentration of the blood by removing excess of non-volatile acids and bases.
5. It maintains the osmotic pressure of the blood by regulating the excretion of water and inorganic salts and thus preserves the constant volume of the circulating blood.
6. It regulates the arterial blood pressure by causing the secretion of the hormone renin.
7. It maintains the erythrocyte production by excreting the secretion of the hormone erythropoietin.

Urine formation:

The regulatory activities of kidneys form urine as a by product. Urine formation involves three main steps—the glomeruler filtration, the tubular reabsorption, and the tubular secretion.

1. **Glomerular filtration (Ultrafiltration):** Glomerulus filters out substances of low molecular weight from the blood with the retention of substances of high molecular weight, especially the proteins. Therefore, proteins are retained

in the glomeruli and are not normally found in urine If protein is detected in the urine, it indicates the kidney damage or other disease which effect the glomerular membrane. In normal adult, two million nephrons filter one litre of blood each minute to give about 1200 ml of glomerular filtrate (primary urine) at Bowman's Capsule Therefore, the glomerular filtration rate (GFR) in adult is about 120 ml per minute

The hydrostatic pressure of the blood in the glomerular capillaries (Pg) is the main force for driving the fluid (water and solute) out of the glomerulus The pressure is opposed by two forces—

(i) the hydrostatic pressure of the Bowman's Capsule fluid (P_{BC}), (ii) the osmotic pressure of the plasma proteins (P_{PP}) Therefore, *the effective filtration pressure* (Pef) is calculated by the following relation

$$Pef = Pg - (P_{PP} + P_{BC})$$

$$Pef = 74 - (30 + 20) \text{ mm Hg}$$

$$Pef = 25 \text{ mm Hg}$$

Thus, by substituting the normal values of the various forces, it has been found that the calculated effective (net) filtration pressure (Pef) is 25 mm Hg

A fall in blood pressure may reduce the Pef which results in less amount of urine When the aortic systolic pressure is reduced to 70 mm Hg, the hydrostatic pressure of the blood in glomerular capillaries is reduced to 50 mm Hg This reduces the Pef to Zero [50—(50)] and thus filtration will be ceased. Under such circumstances, urine will not be formed (anuria) until the blood pressure is maintained

2. Tubular reabsorption. The rate of formation of the primary urine is 120 ml /minute, while the rate of urine passing to the bladder under the same condition is 1 2 ml /minutes Therefore, it indicates that about 99 per cent of the glomerular filtrate is reabsorbed during its passage through the different segments of the renal tubule Although, the glomerular filtrate contains nearly the same concentration of glucose as in plasma, the urine contains nil or very little glucose. Hence, glucose is also practically completely reabsorbed in the tubules when the blood sugar level is normal The capacity of reabsorption depends on the renal threshold of that substance

The reabsorption of different solids takes place at different sites in the renal tubules Amino acids, glucose, and small amounts of protein that pass through the glomerulus are reabsorbed in the first part of the proximal tubule. Sodium, chloride, and bicarbonate are reabsorbed uniformly along the entire length of the proximal tubule and also in the distal tubule Potassium is reabsorbed in the proximal and secreted in the distal tubule

The glomerular filtrate produces about 170 litres in a day, whereas the tubules reabsorb about 168 5 litres of water, 170 gms of glucose, 100 gms of NaCl, 360 gms of $NaHCO_3$, and small amounts of phosphate, sulphate, amino acids, urea, uric acid etc and excrete about 60 gms of NaCl, urea and other waste products in about 1 5 litres of urine Most of these solids are reabsorbed by active transport mechanism, while some (e g urea) are reabsorbed by passive transport mechanism

In diseases, the reabsorption mechanism is altered developing glycosuria, phosphaturia, and amino aciduria

3 **Tubular secretion**· Although, most of the substances are reabsorbed by the tubular cells, some substances are actively transported or actively excreted into the tubular lumen The secreted substance by the tubular epithelium in man are creatinine and potassium The tubular epithelium also removes a number of foreign substances that are introduced into the body for therapeutic or diagnostic purposes These foreign substances are penicillin, P-amino-salicylic acid, phenosulphonphthalein (PSP), P aminohippuric acid, and diodrast The hydrogen ions and ammonia formed in the distal tubular cells are also actively excreted into tubular lumen and thus pass to urine

Hormonal regulation· The function of kidney is regulated by three important hormones These hormones are aldosterone (from adrenal cortex), parathormone (from parathyroid), and vasopressin (from hypophyseal posterior lobe)

Aldosterone restricts the excretion of Na^+ and stimulates the excretion of K^+ *Parathormone* stimulates excretion of phosphate *Vasopressin*, the antidiuretic hormone, is held responsible mainly for the reabsorption of water In the absence of this hormone, a large amount of very dilute urine is excreted

Composition of urine

A Volume ·

1 A normal adult excretes daily from 1000 ml to 1800 ml of urine The average is 1500 ml containing 60 g of solids

2 The quantity depends on the water intake external temperature the diet and the individual s mental and physical condition

3 A high protein diet increases excretion because the urea formed as a result of catabolism of protein has a diuretic action

4 The diuretic action of tea coffee and cocoa is due to caffeine

5 The decreased volume of urine in hot weather is due to an increased loss of water by perspiration

6 Nervousness or excitement causes increased urinary volume

7 Increased urine volume are observed in diabetes insipidus, diabetes mellitus and certain types of kidney diseases and decreased volume are found in acute nephritis fevers diseases of the heart diarrhea and vomiting

B Specific Gravity ·

1 The specific gravity of urine in 24 hours lies between 1 003 and 1 030 and varies according to concentration of solutes in the urine The figures in the second and third decimal places multiplied by 2 66 (Long s coefficient) give roughly the total solids in the urine in g/L 60 gms of solids are the average normal for the day

2 The specific gravity of the urine varies with the food water intake and the activity of the individual

3 In chronic interstitial nephritis, the specific gravity is lowered

4 The specific gravity is increased in the excretion of abnormal substances such as albumin or glucose (e g diabetes mellitus)

C. Colour .

1 Normal urine is pale yellow or amber The colour is roughly proportional to the specific gravity Very dilute urine is colourless

2 *Terochrome*, composed of a polypeptide and urobilin, is the chief pigment of urine. Traces of *coproporphyrin, urobilinogen* and *uroerythrin* are also found in urine

3 Reddish urine is due to the ingestion of naturally coloured foods (e g beetroot, blackberries) In fever, the urine may be dark yellow or brownish because of concentration In liver disease, the urine may be green brown, or deep yellow due to bile pigments Blood or haemoglobin develops smoky to red colour The urine is dark brown due to methemoglobin and homogentisic acid Methylene blue gives the urine a green appearance

4 The urine is transparent A turbidity is developed in alkaline urine by precipitation of calcium phosphate Strongly acid urine is pink due to the precipitation of uric acid salts

D Odour .

1 Fresh urine is normally aromatic

2 The odour is modified by the ingestion of certain foods or drugs This is noticed after eating asparagus , the odour is due to methyl mercaptan

3 The ammoniacal smell of urine is due to the action of bacteria on urea

4 In ketosis, the odour of excreted acetone is detected

E p^H :

1 The mixed sample of normal urine in 24 hours has a p^H 6 Individual samples vary from 4 7 to 8 0

2 The urine is acid in high protein intake because excess phosphate and sulfate are formed in the catabolism of protein Acidity is also increased in acidosis and in fever

3 The urine becomes alkaline on standing due to the conversion of urea to ammonia and loss of CO_2 to air It may be alkaline in alkalosis such as after excessive vomiting and after meals due to H^+ secretion in the stomach (the "alkaline tide ")

4 The acidity of urine is increased after strenuous muscular exercise (elimination of lactic acid) by ingestion of ammonium salts of strong acids An alkaline urine may be produced by ingestion of sufficient $NaHCO_3$ Ammonium carbonate does not produce an alkaline urine because ammonia is rapidly converted into urea

Normal constituents of urine

A Urea : Nitrogenous constituents :

1 Urea is the main end product of catabolism of protein in mammals Its excretion is directly proportional to the protein intake It consists of 80-90% of the total urinary nitrogen

2. In fever, diabetes, or excess adrenocortical activity, urea excretion is increased due to increased protein catabolism.

3. Decreased urea excretion is due to decreased urea production in the last stages of fatal liver disease.

4. In acidosis, there is decreased urea excretion

B. Ammonia :

1. Ammonia is formed by the kidney from glutamine or amino acids in acidosis.

2. There is a high ammonia output in the urine in uncontrolled diabetes mellitus in which renal function is impaired.

C. Creatinine and creatine :

1 Creatine is excreted by children and pregnant women and much smaller amounts in men. The excretion in men is 6% of the total excretion of creatinine.

2. Creatinine is formed from creatine. It is excreted in relatively constant amounts regardless of diet.

3. The creatinine coefficient is the ratio between the amount of creatinine excreted in 24 hours and the body weight in kg. It is usually 20—26 mg/kg /day in normal men and 14-22 mg/kg/day in normal women.

4. Creatinine excretion is decreased in many pathologic conditions..

5. Creatine excretion is also found in pathologic states such as starvation hyperthyroidism, impaired carbohydrate metabolism and infections

6. Creatine excretion is decreased in hypothyroidism

D. Uric acid :

1. It is the end product of the oxidation of purines in the body. It is not only formed from dietary nucleoprotein but also from the breakdown of cellular nucleoprotein in the body.

2. It is slightly soluble in water and precipitates readily from acid urine on standing.

3. Uric acid excretion is increased in leukemia, severe liver disease and various stages of gout

4. The concentrated urine on cooling forms a brick-red deposit which is mainly acid urate

5. Pure uric acid is colourless. Deposits of uric acid and urates are coloured by absorbed urinary pigments, particularly the red uroerythrin.

6 The specificity of the analysis for uric acid is increased by treatment with uricase, the enzyme (from hog kidney) which converts uric acid to allantoin.

E. Amino acids :

1. About 150—200 mg of amino acid nitrogen are excreted in the urine of adults in 24 hours.

2. The infant at birth excretes about 3 mg amino acid nitrogen per pound of body weight and upto the age of 6 months the value reaches to 1 mg/pound which is maintained throughout childhood. Premature infants excrete 10 times amino acid nitrogen than that of full-term infant.

3 The low excretion of amino acid nitrogen is due to its high renal threshold value

4 Increased amounts of amino acids are excreted in liver disease and in certain types of poisoning

5 In cystinuria 4 amino acids—arginine, cystine, lysine and ornithine are excreted in urine

F Allantoin :

1 It is the partial oxidative product of uric acid Small quantities of allantoin are excreted in human urine

2 In other subprimate mammals, allantoin the principal end product of purine metabolism which is excreted

G Sulphates :

The urine sulphur is derived from sulphur containing amino acids such as methionine and cystine and therefore, its output varies with protein intake The urine sulphur exists in 3 forms

I Inorganic (sulfate) sulfur :

1 This is the completely oxidized sulfur precipitated from urine

2 It is proportionate to the ingested protein with a ratio of 5 1 between urine nitrogen and inorganic sulfate

II Ethereal sulfur (conjugated sulfates) :

1 It is about 10% of the total excreted sulfur This includes the organic combination of sulfur excreted in the urine

2 It consists of the sulphuric esters of certain phenols

3 It forms no precipitate on addition of acidified $BaCl_2$ Some of the phenols are derived from putrefaction of protein in the large intestine

4 Clinically, the ethereal sulfate is that of indoxyl indican which is formed from bacterial decomposition of tryptophan in the large intestine.

5 Normally, 5 20 mg of indican are excreted and the amount increases in constipation In cholera, typhus gangrene of lung sufficient indican is excreted Iodoxyl liberated from iodican is oxidized to iodign blue on exposure to air

III Neutral sulfur :

1 These are unoxidized sulfur and contained in cystine, taurine, thiocyanate or sulfides

2 They do not vary with the diet

3 They are mainly the products of endogenous metabolism

Other organic compounds .

H Chlorides . . These are excreted as NaCl and output varies with intake

I Phosphates

1 The urine phosphates consist of sodium and potassium phosphate as well as calcium and magnesium phosphate

2 The greater part of the excreted phosphates is derived from ingested food which contains organic phosphates, e g nucleoprotein, phosphoprotein and

phospholipids Phosphates of food are not completely absorbed Some phosphate is also derived from cellular breakdown

3 Phosphate excretion is increased in certain bone diseases such as osteomalacia, wasting diseases of the nervous system and in renal tubular rickets

4 Marked increase of phosphate excretion is also observed in hyperparathyroidism and decrease in hypoparathyroidism and in infectious diseases

J Oxalates :

1 The amount of oxalate in the urine is low (20 mg/day) and found as calcium oxalate crystals in urinary deposits

2 The excretion of oxalate is increased by ingestion of fruits and vegetables containing high oxalates (Spinach)

3 Large quantities of oxalate are excreted in urine in inherited metabolic disease

4 The oxalates present in urine are composed of partly unchanged ingested acid and partly oxidative products of other compounds

K. Minerals :

1 The 4 cations of the extracellular fluid—sodium, potassium, calcium and magnesium—are present in the urine

2 Sodium content varies with intake Urine potassium increases when the intake is increased or in excessive tissue catabolism The excretion of potassium is affected by alkalosis Sodium and potassium excretion are also controlled by the activity of the adrenal cortex

3 Calcium and magnesium are not completely absorbed and their presence in the urine is low But their presence in the urine varies in certain pathologic states, particularly those involving bone metabolism

L. Enzymes .

1 Traces of many enzymes are excreted in urine including pancreatic amylase, pepsin, trypsin and a lipase

2 The pancreatic amylase excretion is increased in pancreatic disease

M. Hormones and vitamins :

1 Certain hormones (sex hormones) and vitamins (e g B_1 and C) are found in urine

2 The vitamin needs are assessed by studying the urinary output after test doses The pregnancy test is also performed by the urinary sex hormones

Abnormal constituents of the urine :

A Proteins Proteinuria (albuminuria) is the presence of albumin and globulin in the urine in abnormal concentrations The traces of protein (30—200 mg) present in normal urine cannot be detected by the ordinary simple tests Pathologically, several proteins such as serum albumin, serum globulin, hemoglobin mucus, proteose, Bence Jones proteins are found in urine

1 Physiologic proteinuria : In this condition, less than 0 5% protein is present in urine which occurs after severe exercise, after a high protein meal or as a result of some temporary impairment in renal circulation when a person stands erect In 30—35% of pregnancy, there is proteinuria

2 Pathologic proteinuria : Proteinuria is marked in glomerulonephritis In nephrotic syndrome, a marked proteinuria occurs The proteinuria increases

with the increasing severity of the renal lesion Proteinuria also results in poisoning of the renal tubules by heavy metals like mercury, arsenic or bismuth,

3 Hemoglobin is also present as a result of hematuria due to hemorrhage from the kidneys or urinary tract, clotting may occur due to sufficient fibrinogen on passing of much blood

4 Mucus is the term for an unidentified protein precipitated by acetic acid in the cold It is a mucin The mucus is increased in infection of the bladder.

5 Proteose may be found which is of little clinical significance

6 Bence Jones proteins found in the urine are the peculiar proteins which are light chain fragments of globulins Most commonly they occur in multiple myeloma and rarely in leukemia They are precipitated when the urine warmed to 50-60 C and redissolved almost completely at 100°C and precipited again on cooling

B Glucose .

1 Normal individuals excrete not more than 19 mg of sugar per day which is difficult to detect by simple test It is said to be *glycosuria* when more than this quantity is found in urine

2 There are different causes of glycosuria. Transient glycosuria is observed after emotional stress such as exciting athletic contest 15% of cases of glycosuria are not due to diabetes glycosuria suggesting diabetes must be confirmed by blood glucose studies to eliminate the probability of renal glycosuria

3 The presence of glucose must be tested by Benedict's test But in case of pregnant women and lactating mother, the Osazone test must be performed for urine glucose to eliminate the lactose present in urine

C Other sugars :

1 *Fructosuria* Fructosuria is due to the disturbance in fructose metabolism but not other carbohydrates

2 *Galactosuria and lactosuria* These may occur occasionally in infants pregnant women and lactating mother Glactosuria may occur in inherited diseases due to the nonconversion of galactose to glucose

3 Pentosuria This may occur transiently after intake of food containing large quantities of pentoses, such as grapes, cherries and plums It may take place in inherited diseases in which pentoses are not metabolized

To detect these other sugars in urine it is wise to perform osazone test

D Ketone bodies

1 Only 3—15 mg of ketone bodies are excreted in urine normally in 24 hours

2 Increased amount of ketone bodies are excreted in urine in starvation, diabetes mellitus pregnancy ether anesthesia and some types of alkalosis

3 Excess fat metabolism may induce a ketonuria in many animals.

4 Increased amount of ammonia is excreted in acidosis accompanying ketosis

E *Bilirubin and Bile salts* :

1 Bilirubin is found in the urine in cases of obstructive or hepatic jaundice

2 Bilirubinuria is accompanied by the excretion of bile salts

3 Bile salts may be excreted in urine without bile pigment in certain stages in liver disease

4 In excessive hemolysis traces of bilirubin without bile salts are excreted in urine

F Blood

1 In the lesion of the kidney or urinary tract blood is excreted in the urine in addition to its presence in nephritis

2. Free hemoglobin is also found in urine after quick hemolysis, e.g in black water fever (a complication of malaria) or after severe burns

G Urobilinogen

1 In excessive hemolysis, e g hemolytic jaundice or pernicious anemia, part of the bile pigment formed by breakdown of hemoglobin is excreted in urine as urobilinogen

2 Urobilin is formed from colourless urobilinogen when the urine is exposed to air This gives the urine an orange colour

3 In liver disease or temporarily in constipation, large amounts of urobilin are found in urine

H Porphyrins

1 Coproporphyrins excreted in urine normaly are 60—250 μg/day

2 Coproporphyrins are excreted more in certain liver diseases

3 The increased amount of coproporphyrins in the urine is a characteristic of the urine of patients suffering from porphyria

MECHANISM OF ACTION OF DIURETICS

1 Diuretics, the drugs, enhance losses of water and salt via the urine through interference with normal reabsorptive mechanisms

2 Osmotic diuretics are nonreabsorbable substances which increase tubular osmolarity The osmotic substances which limit the amount of water Osmotic diuresis is responsible for the serious dehydration which accompanies diabetic ketoacidosis

3 Diamox is the inhibitor of carbonic anhydrase It blocks both HCO_3^- reabsorption in the proximal tubule and regeneration in the distal tubule.

4 Thiazide diuretics furosemide, ethacrynic acid and mercurials all inhibit chloride reabsorption in the ascending limb

RENAL FUNCTION TESTS

Clearance is measured to assess quantitatively the rate of excretion of a given substance by the kidney This is a volume of blood or plasma which contains the amount of the substance which is excreted in the urine in one minute

A Inulin clearance

1 Inulin is a polysaccharide which is filtered at the glomerulus but not secreted or reabsorbed by the tubule Therefore it is a measure of glomerular filtration rate *Mannitol* can also be used for the same perpose

2 These clearances vary with the body size The clearance is calculated on the basis of ml./1 73 m^2

3 To measure inulin clearance it is wise to maintain a constant plasma level of the test substance during the period of urine collections The clearance is measured according to the following formula :

$$Cin = \frac{U \times V}{P}$$

Where, Cin=Clearance of inulin (ml/min)
U=Urinary inulin (mg/100 ml)
V=Volume of urine (ml/min)
P=Plasma inulin (mg/100 ml)

B Endogenous creatinine clearance :

1 Creatinine is filtered at the glomerulus but not secreted or reabsorbed by the tubule Its clearance is measured to get the GFR

2 This method is convenient for the estimation of the GFR because it does not require the intravenous administration of a test substance

3. Normal values for creatinine clearance are 95—105 ml/min

C. The phenolsulphonephthalein (PSP) Test :

1 The dye is almost completely eliminated within 2 hours

2 If less than 25 per cent of the dye is not excreted in 15 minutes, it is an indication of impairment of renal function

D. Other functional tests:

1 Dilution test (water excretion test)
2 Urine concentration test (specific gravity test)
3 Vasopressin (ADH) test
4 Urine acidification test
5 Blood NPN, urea and creatinine
6 Urea clearance test

1. Dilution test:

(a) After emptying the bladder of the individual after overnight fast, he is advised to drink 1200 ml water in 30 minutes

(b) During four hours after drinking, the urine is collected at hourly intervals

(c) In normal individuals in cold climates, 1200 ml of urine are excreted in four hours

(d) This test is not applicable to warm climates since the greater part of the ingested water is lost in perspiration during summer

(e) In case of impaired renal function, the amount of water eliminated in four hours will be less than 1200 ml depending on the degree of impairment and specific gravity of urine is often 1 010 or higher in conditions of oliguria

2. Urine concentration test (specific gravity test) :

(a) The bladder is emptied on the day of the test at 7 a m and the urine is discarded

(b) The urine is collected at 8 a.m and the specific gravity is measured. If the sp gr is 1 022, the test may be rejected

(c) If the sp gr is below 1 022, another urine specimen should be collected at 9 a m and the sp gr is determined.

(d) In case, the urine does not have a sp gr of 1 022, it is sure that the renal concentrating power is impaired either due to tubular defects or decreased secretion of ADH (diabetes insipidus) If the urine volume is large and the sp gr is below 1 022, the ADH test must be carried out

3. Vasopressin (ADH) test :

(a) The individual is not allowed any food or water after 6 p.m on the night before the test Vasopressin (5 units) is injected intramuscularly at 7 p m. in the night

(b) The urine is collected at 7 a.m and 8 a.m. and the sp gr is determined. If the sp gr is 1 022, it is quite confident that the individual suffers from diabetes insipidus and ADH injection is effecting in controlling it

4. Urine acidification test :

(a) This test should not be done on individuals who have acidosis or poor liver function

(b) No dietary or other restrictions are involved in carrying out this test. The bladder is emptied at 8 a.m Thereafter, hourly specimens of urine are collected until 6 p m At 10 a.m , ammonium chloride in a dose of 0 1 gram/kg body weight is given A portion of each specimen is transferred to stoppered bottles and sent immediately to the laboratory for P^H determination

(c) In normal individuals, all urine specimens collected after 2 hours from the time of administration of ammonium chloride should have a P^H between 4 6 and 5 0 but in patients with renal tubular acidosis, the P^H does not fall below 5 3

5. Blood non-protein nitrogen:

(a) In acute nephritis, the NPN values are increased and range from a slight increase (NPN—45 mg , urea N—25 mg , creatinine—2 mg per 100 ml) to very high values (NPN—200 mg , urea N—160 mg , creatinine—25 mg per 100 ml)

(b) NPN increase and retention are due to impaired renal function and excessive protein catabolism

CONGENITAL TUBULAR FUNCTION DEFECTS

1 Diabetes insipidus

(a) This disease is developed due to the non production of ADH The individual passes large volume of urine (5-20 litres in 24 hours) The individual has to drink large amount of water to make up the loss

(b) The reabsorption of water in the distal tubules does not take place in the absence of ADH

2. Vitamin D resistant rickets

(a) The tubular reabsorption of phosphate does not take place under this condition

(b) Excessive loss of phosphate in urine leads to the development of a type of rickets which does not respond to usual doses of vitamin D

3. Renal glycosuria:

In this condition, the tubular reabsorption of glucose is affected Although the blood sugar is within normal level but glucose is excreted in urine due to defective reabsorption by the tubules

4. Idiopathic hypercalciuria:

Calcium is not reabsorbed by the renal tubules in this condition Hence, large amounts of calcium are excreted in the urine Renal calculi may be developed owing to the presence of large amounts of calcium in urine

5. Salt losing nephritis:

(a) Large amounts of sodium and chloride ions are excreted in urine in this condition due to the defect in the tubular reabsorption of these ions resulting in severe dehydration, hyponatremia and hypochloremia.

(b) Blood urea is increased due to the reduced glomerular filtration rate

(c) This condition does not respond to aldosterone administration but responds to parenteral administration of sodium chloride solution

6 Renal tubular acidosis:

(a) In this condition, the urine becomes alkaline or neutral due to the defect in the sodium and hydrogen ion exchange mechanism in the distal tubules There is loss of sodium in the urine

(b) The acidosis is accompanied by excessive mobilization and urinary excretion of calcium and potassium

(c) These abnormalities lead to clinical manifestation of dehydration, hypokalemia, defective mineralisation of bones and nephrocalcinosis

7 Fanconi Syndrome :

(a) A number of defects in tubular reabsorption exist in this condition The defects are renal amino acid in renal glycosuria, hyperphosphaturia, metabolic aciduria, with increased urinary excretion of Na, Ca and K

(b) In some individuals, cystonosis prevails due to the abnormality of cystine metabolism in which cystine crystals are deposited in macrophages in the liver, kidney, spleen, bone marrow, lymph nodes and cornea

8. Hartnup Syndrome (Hard Syndrome) :

(a) In this condition, a number of amino acids are not reabsorbed owing to the defect in tubular reabsorption mechanism

(b) Disturbances in tryptophan metabolism is suggested by the presence of increased amounts of tryptophan, indican and indole acetic acid in urine

(c) The clinical symptoms are of niacin deficiency—a pellagra like skin lesions and mental deficiency

9. Nephrogenic diabetes insipidus (water-losing nephritis) :

This condition is due to congenital defect in water reabsorpnon in the distal tubules and may therefore resemble true diabetes insipidus

UREMIA

The renal failure develops the clinical condition uremia This condition occurs both in chronic renal failure and acute failure The concentration of urea and other NPN constituents in plasma are increased depending on the severity of this condition

In chronic renal disease, excretion of acid (hydrogen ion) and also of phosphate ion is impaired This results in the steady development of acidosis in uremia

In acute renal failure, the urine output is very low (300 ml or less in 24 hours) This leads to a steady increase in urea and NPN constituents and electrolytes (K^+ and Na^+) in plasma There is rapid development of acidosis too

The important findings of severe chronic uremia or acute uremia are

1 High concentration of urea and other NPN constituents

2 High serum potassium concentration

3 Water retention leading to generalised edema

4 Acidosis

Uremic coma occurs in serious cases

1. Urea and NPN : The concentration of urea and other NPN constituents of blood are very much increased (i e 10 times the normal level) in severe renal failure

2. Potassium : The potassium ion level may be slightly increased in chronic uremia But in acute uremia, the concentration in serum is very much increased Potassium is released from the cells due to the breakdown of cellular proteins This released potassium passes into the blood and interstitial fluid When the concentration of potassium ion increases to 8 m Eq/litre, it exerts a cardiotoxic effect resulting in the dilation of the heart and when potassium ion concentration reaches to 12 to 15 m Eq/litre, the heart is likely to be stopped This happens in severe uremia

3. Water retention and edema: If the uremic patient drinks water and consumes other fluids, the water is retained in the body If salt is not consumed, water retention increases in both the intracellular and extracellular compartments causing edema in both the compartments If salt is consumed along with water and food, water is retained in the extracellular fluid resulting in extracellular edema

4. Acidosis: The metabolic processes in the body produce daily 50 to 100 m mol of more metabolic acid than alkali This extra metabolic acid is excreted mainly through the kidneys Acidosis develops rapidly in acute uremia The patient faces 'Coma' due to severe acidosis

THE ARTIFICIAL KIDNEY

During recent years, the artificial kidney has been developed to such an extent that several thousand patients with permanent renal insufficiency or even total kidney removal are being maintained in health for years

The artificial kidney passes blood through very minute channels bounded by thin membranes There is a dialyzing fluid on the other side of the membrane into which unwanted substances present in the blood pass by diffusion The blood is pumped continually between two thin sheets of cellophane, the dialyzing fluid is on the outside of the sheets The cellophane is porous enough to allow all constituents of the plasma except the plasma proteins to diffuse freely in both directions—from plasma into the dialyzing fluid and from the dialyzing fluid into the plasma

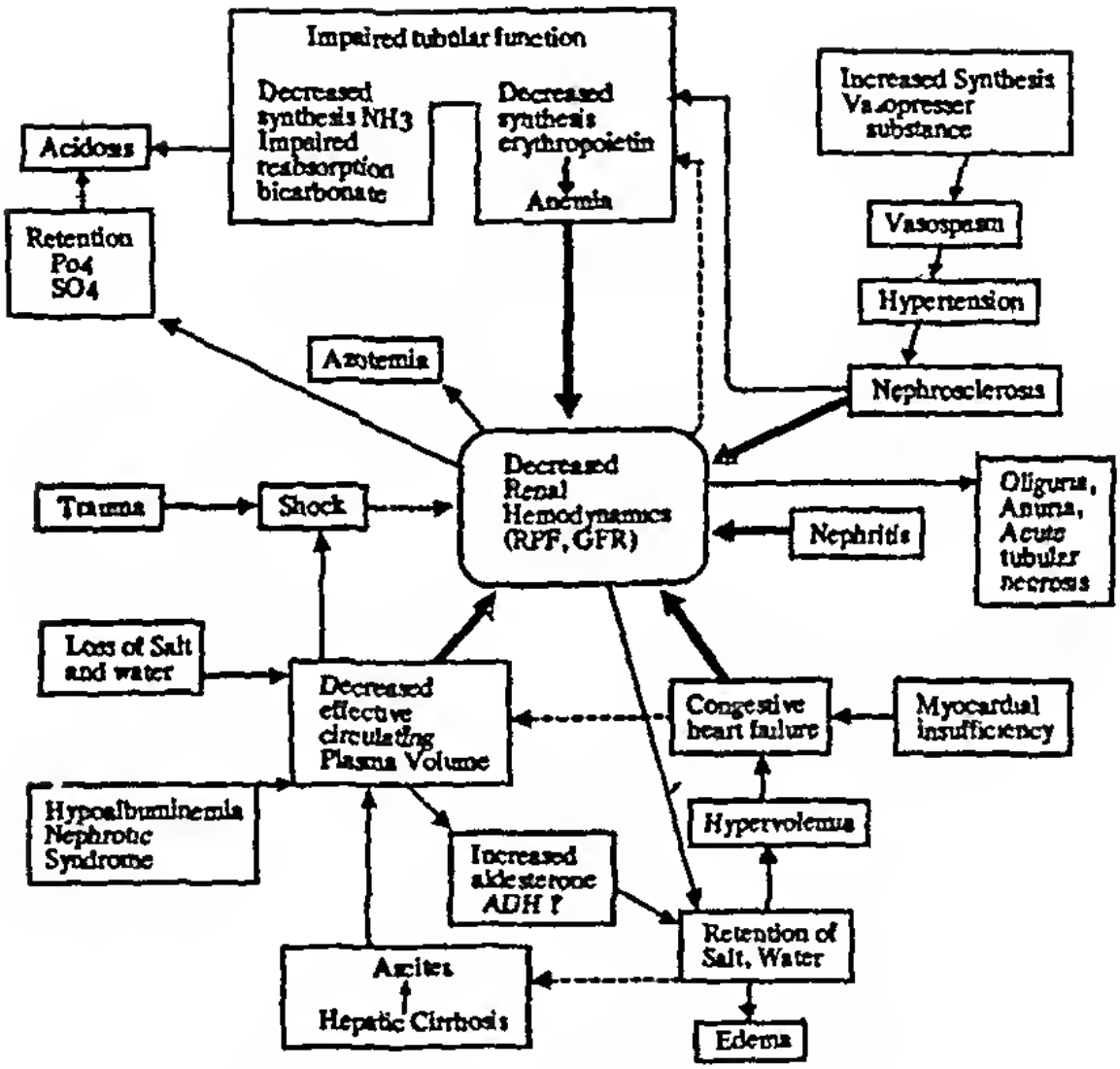


Fig 35.2. Clinical and Physiological disturbances of renal function.
RPF = Renal Plasma Flow. GFR = Glomerular filtration rate.
ADH = Antidiuretic Hormone

The rate of flow of blood through the artificial kidney is several hundred ml. per minute. Heparin is infused into the blood as it enters the kidney to prevent clotting of blood. To prevent bleeding as a result of heparin, an anti-heparin substance, such as protamine, is infused into the blood as it is returned to the patient

The dialyzing fluid: Sodium, potassium and chloride concentrations in the dialyzing fluid and in normal plasma are identical; but in uremic plasma, the potassium and chloride concentrations are considerably greater. These two ions diffuse through the dialyzing membrane so rapidly that their concentrations fall to equal those in the dialyzing fluid within three to four hours, exposure to the dialyzing fluid. On the other hand, there is no phosphate, urea, urate or creatinine in the dialyzing fluid. When the uremic patient is dialyzed, these substances are lost in large quantities into the dialyzing fluid, thereby removing major proportions of them from the plasma Thus, the constituents of the dialyzing fluid are such that those substances in excess in the extracellular fluid in uremia can be removed at rapid rates, while the essential electrolytes remain quite normal.

Utility of artificial kidney: The artificial kidneys can clear 100 to 200 ml. of blood urea per minute which signifies that it can function about twice as rapidly as two normal kidneys together whose urea clearance is only 70 ml. per minute. However, the artificial kidney can be used for not more than 12 hours once in three to four days because of danger from excess heparin and infection to the subject

HORMONES OF THE KIDNEY

1. Not only the kidney performs excretory functions but it acts as an endocrine organ. It liberates many hormones which affect other organs and tissues and some hormones which locally act within the kidney itself. It also destroys several hormones which are liberated from other endocrine organs.

2. The juxtaglomerular cells of the renal cortex produce the proteolytic enzyme *renmin* and secrete it into the blood. Rennin acts on α_2-globulin which is normally present in blood plasma although it is produced in the liver. Rennin splits off a polypeptide fragment called angiotensin I which is a decapeptide containing 10 amino acids. Another enzyme of the lung acts on angiotensin I to split off 2 amino acids and thus form the octapeptide angiotensin II.

Angiotensin increases the force of the heartbeat and constricts the arterioles. It raises blood pressure and causes contraction of smooth muscle. It is destroyed by the enzyme angiotensinases present in normal kidneys, plasma and other tissues. Recent studies suggest that *rennin* angiotensin system is important in the maintenance of normal blood pressure.

3 Prostaglandins are the other hormones of the kidney. They cause relaxation of smooth muscles. They cause vasodilatation and a decrease in blood pressure. They also increase renal blood flow. *Kininogen* which is produced by the kidney has an antihypertensive effect.

4 The two hormones erythropoietin and erythrogenin have an effect on bone marrow to stimulate production of red cells. Kidney plays an important role in the release of erythropoietin and thus in control of red cell production. Hypoxia stimulates producton of erythropoietin.

URINARY DEPOSITS

The commonest deposits are phosphates, oxalates and urates are frequently seen in normal urine.

Phosphates :

1 They are usually found in alkaline urines The commonest is ammonium magnesium phosphate which forms a characteristic crystal

2 A less common form is calcium hydrogen phosphate which forms long prisms

3 Amorphous calcium and magnesium phosphates may be deposited from alkaline urines

The deposition of phosphates is due to a change in p^H after the urine has been passed

Calcium oxalate : This is found in acid urine but may be found in alkaline urine The crystals are of two types—octahedra, dumb bells Calcium oxalate is insoluble in acetic acid

Urates :

1 They are usually found in acid urines

2 Uric acid separates into different forms including prisms, barrels, hexagons and needles which are always pigmented

3 Urates are redissolved on warming the urine

4 The cause of deposition of urates is the cooling of urine after it has been passed.

FECES

Amount :

1 The quantity of feces varies from day to day and with the diet

2 Vegetable food increases the bulk of feces but meat diet which is largely absorbed diminishes the bulk

3 An adult taking a mixed diet passes from 60 to 250 g of moist feces containing 25-45 g of solids per day

4 The young children release high bulk

Composition :

1 Normal adult feces have a water content of 65 80%

2 One third of the dry matter of feces is represented by bacteria The rest are the remainings of intestinal secretions, substances excreted by the large intestine (e g Ca, Fe) and small amounts of food residues The food residues are cellulose, fruit skins and seeds

3. The only substances in feces are fat, nitrogen and mineral elements Dry feces contain the following .

Nitrogen	5—10%
Fat	10—20%
Ash	10—20%

Colour :

1. The normal colour of feces is brown which is due to stercobilin (urobilin)

2 Milk, cereals and chlorophyll-free vegetables give lighter, and meat, coffee, cocoa, black berries etc darker stools.

3 Greenish colour due to excessive consumption of green vegetables

4 The colour of the feces of newly born infants is dark or blackish-green due to biliverdin and porphyrin

5 The stools of young infants on a milk diet is yellow due to bilirubin because of underdevelopment of intestinal bacterial flora.

6 The presence of blood in the stool gives the feces a red colour Excessive hemolysis will give a dark-brown feces

7 Iron or bismuth gives a black stool.

Odour :

1 The normal odour is due to indole and skatole.

2 Mercaptans and H_2S may produce odour

3 A meat diet produces a more intense odour than a vegetable one and a milk diet least of all

pH

1 Feces are normally slightly alkaline pH 7·0—7·5

2. They may be slightly acid with a large proportion of carbohydrate or fat.

Fat :

1 The total fat of feces is divided into two (a) Split fat (fatty acids), (b) Unsplit fat (neutral fats phospholipids, sterols pigments).

2. The amount of fat is independent of the diet. The split fat diminishes in amount on low fat diets

3 In diseases, fecal fat is increased when digestion or absorption of fat is impaired.

Nitrogen :

1 Fecal nitrogen is very little affected by the amount of protein ingested if the protein is well masticated and well assimilated

2 An adult on a mixed diet usually excretes about 1g. of fecal nitrogen per day

3 In diseases, fecal nitrogen is greatly increased by failure of digestion or absorption of protein.

Salts

1 Moist feces contain about 2·3% of salts. Most abundant are calcium and phosphate.

2. Small amounts of magnesium, iron, sodium, potassium, chloride and sulphate are also present in feces. The proportion of calcium is higher on a milk diet and that of magnesium on a meat diet.

SWEAT

1 Sweat is a more dilute fluid and is always hypotonic.

2. The pH is about 4.5 But if the skin is washed and dried, sweat secreted is slightly alkaline (pH 7.0—7.4).

3 It contains most of the diffusible constituents of plasma The most abundant constituent is NaCl

4 The lactic acid content is more than normally found in blood

Exercise

1 Discuss the normal constituents of urine (B U 76A)

2 Discuss the abnormal constituents of urine (R U 71S)

3 Explain fully the composition of urine (P U 68S)

4 Write notes on

(a) Hormones of the kidney (Pun U 67A)

(b) Renal function test (Mith U 72A)

(c) Feces (Bhag U 74S)

CHAPTER 36

LIVER FUNCTION TESTS

Various important metabolic functions are carried out by liver These include the following

1 Destruction of red blood cells and formation and excretion of bile pigments.
2 Secretion of bile.
3 Synthesis of plasma proteins, fibrinogen and prothrombin.
4 Detoxifying functions
5 Carbohydrate metabolism.
6 Fat metabolism
7 Protein metabolism.
8 Storage of glycogen, vitamins and iron
9 Removal or excretion of drugs, hormones and other substances.

The undermentioned tests are required to be done for assessing the functions of liver

1. Tests for bile pigment metabolism in jaundice.
(i) Serum bilirubin estimation and Van den Bergh reaction.
(ii) Biochemical tests for jaundice
(iii) Icteric Index.
(iv) Urine bilirubin.
(v) Urine and fecal stercobilinogen and urobilinogen.
(vi) Congenital hyperbilirubinemia.

2. Tests for carbohydrate metobolism.
(i) Galactose tolerance test.
(ii) Fructose tolerance test

3 Tests for plasma protein concentration.
(i) Plasma albumin, globulin and fibrinogen concentration.
(ii) Flocculation tests
(iii) Amino acids in blood and urine

4. Tests for lipid metabolism,
(i) Serum total, free and ester cholesterol.
(ii) Fecal fat.

5. Tests for—detoxifying functions—excretion of foreign substances.
Hippuric acid synthesis.

6. Tests for excretion of foreign substances.
Bromsulphthalein test.

7. Tests for blood coagulation.
Prothrombin time determination.

8. Tests for serum enzymes derived from liver.
Alkaline phosphatase, transaminases, isocitrate dehydrogenases, pseudocholine esterase and 5-nucleotidase.

9 Tests for conversion of ammonia to urea.
Blood ammonia determination.

10. Determination of glutamine in cerebrospinal fluid.

BILE PIGMENT METABOLISM IN HEALTH AND IN JAUNDICE

When the red blood cells have lived out their life span (averaging 120 days) their cell membranes rupture and the released hemoglobin is phagocytized by the reticuloendothelial cells throughout the body The hemoglobin is first split to heme and globin, and the heme ring is opened to give a straight chain of four pyrrole nuclei from which bile pigments formed The first pigment formed is *biliverdin* which is rapidly reduced to free *bilirubin* This free bilirubin immediately combines very strongly with the plasma albumin and is transported throughout the blood and interstitial fluids Even when bound with the plasma protein, this bilirubin is still called "*free bilirubin*" 80 per cent of the free bilirubin then conjugates in the liver with glucuronic acid to form *bilirubin glucuronide*, 10 per cent conjugates with sulphate to form *bilirubin sulphate* and the rest 10 per cent conjugates with a multiple of other substances This conjugated bilirubin is excreted by an active transport process into the bile canaliculi A small portion of the conjugated bilirubin formed by the hepatic cells returns to he plasma either directly or indirectly by absorption Therefore, a small portion of the conjugated bilirubin is always available in the extracellular fluid

Bilirubin is then converted by bacterial action into *mesobilirubin* which is reduced to *mesobilirubinogen* in the intestine This compound is further reduced to *stercobilinogen* (*Urobilinogen*) which is highly soluble Some of the urobilinogen is reabsorbed through the intestinal mucosa into the blood Most of this is re-excreted by the liver back into the gut, but about 5 per cent of it is excreted by the kidneys into the urine After exposure to air in the urine, the urobilinogen becomes oxidized to *urobilin*. The stercobilinogen in the feces becomes oxidized to *stercobilin* This explanation is represented by the figure 36 1

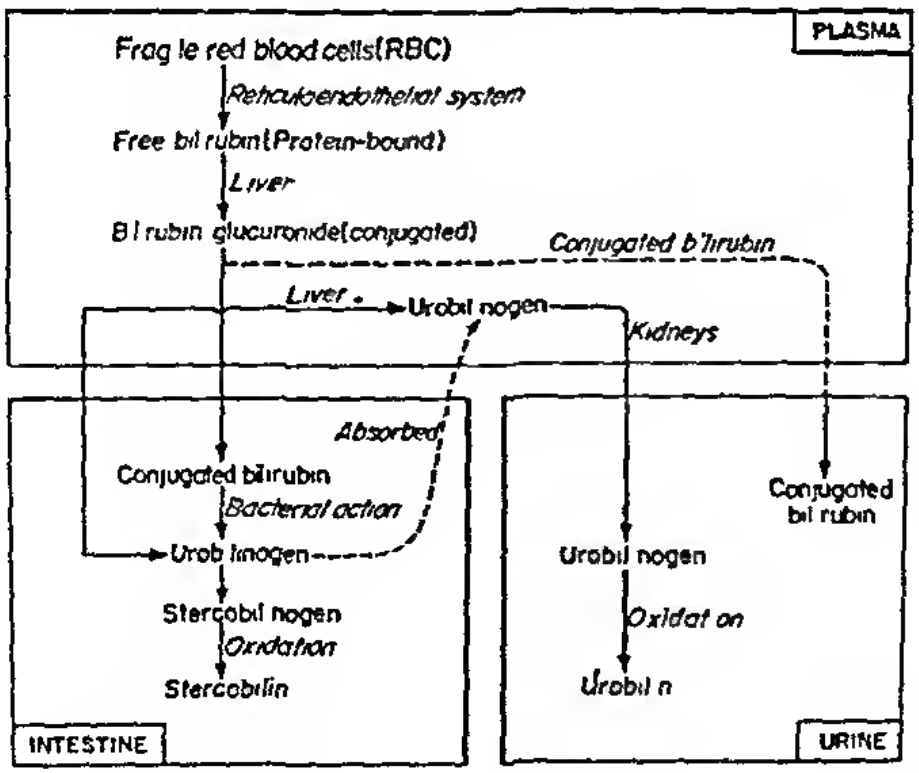


Fig 36 1 Bilirubin formation and excretion

JAUNDICE

"**Jaundice**" means a yellowish tint to the body tissues, including yellowness of the skin and also of the deep tissues The usual cause of jaundice is the increased bilirubin content in the extracellular fluids, either free or conjugated bilirubin The normal plasma concentration of bilirubin (both free and conjugated forms) averages 0 5 mg per 100 ml of plasma But in certain abnormal conditions this can rise to as high as 40 mg per 100 ml The skin becomes yellow when the concentration rises to about three times the normal

Causes·

1 Increased destruction of red blood cells with rapid release of bilirubin into the blood

2 Excessive production of bilirubin beyond the capacity of normal liver to excrete it

3 Dysfunction of liver cells resulting in failure to convert bilirubin into bilirubin glucuronide form in the liver and excrete it in the bile

4 Obstruction of the bile ducts to make usual flow of the bile to the duodenum

Classification·

Rolleston and McNee classified jaundice as follows

1 Hemolytic jaundice 2 Obstructive Jaundice 3 Hepatic Jaundice

Rich classified jaundice as follows

1 Retention jaundice (overproduction of unconjugated bilirubin)

2 Regurgitation jaundice (due to necrosis of liver cells and obstruction of bile ducts)

A Hemolytic jaundice·

1 The excretory function of the liver is not at all impaired

2 The red blood cells are hemolyzed rapidly by hemolytic anemias by the action of some drugs, by malarial or viral infections, and by incompatible blood transfusion The hepatic cells simply cannot excrete the bilirubin as rapidly as it is formed Therefore, the plasma concentration of free bilirubin rises markedly

3 The rate of formation of urobilinogen in the intestine is greatly increased and much of this is absorbed into the blood and later excreted in the urine The colour of the feces becomes dark brown

B Obstructive Jaundice·

1 This happens due to the obstruction in the bile duct preventing the flow of bile into the intestine The obstruction is caused by the blocking of the bile passage by gallstones, by enlarged glands due to tumour of the head of the pancreas, and by stricture or narrowing of the bile duct as a result of surgery.

2 The rate of bilirubin formation is normal, but the bilirubin cannot pass from the blood into the intestines The free bilirubin then enters the liver cells and becomes conjugated in the usual way

3 The conjugated bilirubin is then returned to the blood probably by rupture of the congested bile canaliculi and direct emptying of the bile into the lymph leaving the liver

4 The excess bilirubin is excreted in the urine producing a deep yellow or brownish colour The stools become clay coloured and bulky containing excessive amount of fat The serum alkaline phosphatase concentration is usually high

C Hepatic jaundice (Toxic and infective jaundice):

Hepatic jaundice is caused by infection, toxins and liver poisons Infection with virus is the most common cause The infective organisms cause damage to the liver parenchymal cells

2 The conjugation of bilirubin in the liver is thereby affected Hence, both free and conjugated bilirubin concentration is increased in the serum

3 The urine becomes highly coloured due to the presence of conjugated bilirubin and urobilinogen

(i) Van den Berg reaction to differentiate between hemolytic and obstructive jaundice diagnostically:

If a freshly prepared diazotised sulphanilic acid reagent is added to serum, conjugated bilirubin gives a reddish violet colour within a minute known as the *"direct* Ven den Berg reaction

The free bilirubin (unconjugated bilirubin) of the serum does not develop any colour within one minute but the colour is formed if alcohol is added to the mixture Alcohol precipitates the protein and makes free the free bilirubin from its protein complex so that it can then combine with the Ven den Berg reagent This result is called the *"indirect"* Ven den Berg reaction

Therefore, in hemolytic jaundice an indirect Ven den Berg reaction occurs (increased free bilirubin) and in obstructive jaundice a direct Ven den Berg reaction takes place (increased conjugated bilirubin)

If a faint pink colour is formed after one minute and deepening of the colour results in 2 or 3 minutes in some cases, that indicates clearly that both conjugated and free bilirubin are present in the serum This reaction is said to be *"biphasic"* reaction

In the total obstruction of bile flow, no bilirubin can reach the intestines to be converted into urobilinogen by bacteria Therefore, urobilinogen is not reabsorbed into the blood and is not excreted by the kidneys into the urine So in total obstructive jaundice, tests for urobilinogen in the urine are completely negative The stool becomes clay coloured for *lack of stercobilin*, but not free bilirubin Therefore, in severe obstructive jaundice, large quantities of conjugated bilirubin appear in the urine This can be known by shaking the urine and observing the foam, which becomes intense yellow in colour

(ii) Biochemical tests for jaundice: The biochemical tests are the following

1 Serum bilirubin concentration and nature of Ven den Berg reaction
2 Serum alkaline phosphatase activity and SGPT activity
3 Urine test for urobilinogen and bilirubin
4 Thymol flocculation test and colloidal gold test
5 Feces colour

The biochemical findings in three types of jaundice are given in table 34.2

Serial No	Tests	Hemolytic	Obstructive	Hepatic
1	Serum bilirubin	2 to 3 mg/100 ml (free bilirubin)	Upto 50 mg/100 ml (conjugated bilirubin)	Upto 20 mg /100 ml (both types of bilirubin)
2	Van den berg reaction	Indirect	Direct	Biphasic
3	Test for bile pigments in urine	Urobilinogen (Schlesinger's test positive)	Conjugated bilirubin (Fouchet's test and Gremlin's test positive)	Urobilinogen and conjugated bilirubin
4	Feces colour	Dark brown	Clay colour	Varies
5	SGPT activity	5 to 30 units (normal)	35 to 100 units (Raised slightly)	100 to 300 units (Raised markedly)
6	Serum alkaline phosphatase activity	3 to 12 K.A units and upto 25 in children (normal)	30 to 100 K.A units (Raised markedly)	15 to 30 K.A. units (Raised slightly)
7	Serum 5-nucleotidase activity	Negative	Raised markedly	Raised slightly
8	Thymol flocculation test	Negative	Negative	Positive
9	Colloidal gold test	Negative	Negative	Positive

TABLE 34.2 Biochemical changes in jaundice

(iii) **Icteric Index:** The icteric index shows the degree of jaundice by measuring the intensity of yellow colour of the serum. Serum is diluted with normal saline until it matches the colour of 1 in 10,000 solution of potassium dichromate. The dilution factor is termed *Icteric Index*.

The Icteric Index in normal person is 4 to 6 units. In latent jaundice, it is 3 to 14 units. In clinical jaundice, it is higher than 15 units. Carotene present in serum interferes its determination.

(iv) **Urine bilirubin:** The urobilinogen in urine can be detected by Schlesinger's test. The concentration of urobilinogen is more in hemolytic and hepatic jaundice. Fouchet's and Gmelin's test are both positive in obstructive jaundice. These tests indicate the presence of conjugated bilirubin in urine in obstructive jaundice.

(v) **Bile pigments in feces:** The quantity of stercobilinogen depends on the quantity of bilirubin entering into the intestine. Stercobilinogen content of feces of patients with stone obstruction is nearer the normal level (10 to 150 mg/day in adults) while it is low or absent (0 to 5 mg/day) in those with malignant obstruction.

(vi) **Congenita hyperbilirubinemia:**

(a) **Gilbert's syndrome:** In this condition, there is defective intracellular transport of bilirubin. In some cases, there is defective hepatic conversion of bilirubin to bilirubin diglucuronide. Therefore, the serum free bilirubin concentration is high ranging from 4 to 6 mg/100 ml, sometimes it may rise to 12 mg. The serum shows indirect Ven den Berg reaction.

(b) **Lucey-Driscoll syndrome:** This condition appears in new born infants. The serum bilirubin concentration is very high (upto 60 mg/100 ml). This syndrome occurs due to the presence of a substance (probably steroid) which inhibits the conversion of bilirubin into bilirubin diglucuronide in the liver. This inhibitor disappears one month after the birth of the infants having this syndrome.

(c) **Dubin-Johnson syndrome:** In this condition, there is defective excretion of conjugated bilirubin by liver cells into bile. Conjugated bilirubin is found in urine. Alkaline phosphatase level of serum is normal.

(d) **Grigler-Najjar syndrome:** This syndrome appears in new born infants. The serum bilirubin level rises to 20 mg/100 ml or more within a few days after birth. This condition is a familial incidence. Bilirubin is not converted to bilirubin diglucuronide due to the deficiency of the enzyme glucuronyl transferase in the liver. The bile does not contain conjugated bilirubin. The serum bilirubin level comes down to normal level in those infants who survive.

2. Tests for carbohydrate metabolism:

(i) **Galactose tolerance test:** This test is done in the morning after overnight fast. Fasting blood sample is taken. The individual is then given to ingest a galactose solution containing 40 grams of galactose in 300 ml of water. Blood is drawn at half an hour interval for 2 hours. Galactose content of the blood samples are then determined after removing the glucose by fermenting with yeast.

The normal blood galactose level is 0 to 160 mg/100 ml. In infective and toxic hepatitis, values may go upto 500 mg/100 ml of blood. In Cirrhosis of the liver, values upto 500 mg/100 ml of blood are also found depending on the severity of the disease.

(ii) **Fructose tolerance test:** This test is also performed in the morning after overnight fast. The individual is administered 50 grams of fructose dissolved in 300 ml of water. Fasting blood sample is taken. Blood is also taken at half an hour intervals for 2 hours after fructose ingestion. The total blood sugar (glucose +fructose) is estimated.

In normal subjects, the highest blood sugar level does not exceed the fasting level by more than 30 mg/100 ml. Blood sugar levels upto 150 mg/100 ml are found in patients with infective hepatitis. This test is less sensitive than the galactose tolerance test.

3. Tests for plasma protein concentration:

Albumin, fibrinogen, and some of the α and β globulins are synthesized in the liver. In advanced liver diseases, the albumin content is decreased and globulin content increased. Edema may develop when the plasma albumin level falls below 2.5 per cent. The globulin content may increase upto 5 per cent in some cases. Fibrinogen values in normal persons range from 0.2 to 1.03 per cent and it may fall to 0.1 g in severe liver disorder such as acute hepatic necrosis.

(i) **Electrophoretic separation of plasma proteins:** The percentage of different proteins determined by paper electrophoresis in normal subjects are as follows

Albumin	55·2%	α_2-Globulin	8·7%
Globulin	44·8%	β-Globulin	13·4%
Albumin/Globulin ratio	1·23%	γ-Globulin	11·0%
α_1-Globulin	5·3%	Fibrinogen	6·5%

The following results are obtained in liver diseases:

Chronic infective hepatitis .. γ-Globulin increased.
Cirrhosis of the liver .. Albumin contest decreased.
Necrosis of the liver .. Fibrinogen content decreased.

(ii) **Flocculation tests.**

(a) **Thymol turbidity test:** The degree of turbidity is measured against standards containing 10, 20, 30, 40 ..... 100 mg per 100 ml of protein when serum is mixed with a buffered solution of thymol. A turbidity equal to that of the 10 mg protein standard is taken as 1 unit by McLagan.

In normal subjects, the thymol units range from 0 to 4 units. In infective hepatitis, the values range from 5 to 20 units. In obstructive jaundice, only 8 per cent give positive result. The thymol flocculation test will be positive in all cases in which the turbidity is positive.

(b) **Serum colloidal gold test:** The results obtained in this test in subjects suffering from liver diseases are similar to those obtained with thymol turbidity test.

(c) **Zinc sulphate test:** This test is positive in all cases of infective hepatitis and cirrhosis. In normal persons, serum γ-globulin content is 2 to 8 units but the values rise from 15 to 80 units in infective hepatitis and cirrhosis.

4. Tests for detoxifying functions:

Hippuric acid synthesis test: The liver detoxicates benzoic acid by reacting it with glycine to form hippuric acid which is excreted in urine. The liver is able to synthesise sufficient glycine to conjugate with benzoic acid to form hippuric acid.

The test should begin at least 3 hours after a light breakfast. The patient empties the bladder and drinks sodium benzoate in about 200 ml of water. Urine is collected for a period of 4 hours from the time of ingestion of sodium benzoate. The amount of hippuric acid excreted is determined.

In normal persons, 60 per cent of the benzoic acid taken should be excreted as hippuric acid. The excreted should be 4·5 grams. Smaller quantities are excreted in acute or chronic liver damage.

5. Test for excretion of foreign substances

Bromsulphthalein (BSP) test: When bromsulphthalein dye is injected, it circulates in the blood in combination with albumin. Normal subjects after injection of 5 mg. BSP per kg body weight retain less than 10 per cent of the dye in 30 minutes, and 7 per cent in 45 minutes. At 60 minutes, no dye is retained. This is the most sensitive and dependable liver function test. It is particularly useful for evaluating suspicious or slightly positive results obtained by flocculation tests in the absence of hyperbilirubinemia.

If the liver function is impaired, the dye is excreted slowly and upto 50 per cent of the dye will be retained in the body at the end of 45 minutes after injection. The testis most useful in the diagnosis of liver cell damage without clinical jaundice, in chronic hepatitis and in cirrhosis of the liver.

6. Tests for blood coagulation.

Prothrombin time test: Prothrombin (Factor II) and factors VII, IX and X involved in the coagulation of blood are synthesized in the liver in the presence of vitamin K. Deficiencies of these can occur for two reasons: (i) in the presence

of parenchymal cell damage, synthesis is impaired despite adequate supplies of vitamin K (ii) In the absence of bile as in cholestasis and obstructive jaundice, the vitamin K is not absorbed from the intestines and their synthesis is affected

Shortening of prothrombin time after parental vitamin K therapy suggests cholestasis, while lack of response to vitamin K indicates liver damage

7 **Tests for serum enzymes:** Certain enzymes are released from liver into the blood due to the damage to liver cells The levels of SGOT, SGPT, LDH and isocitrate dehydrogenase are increased The levels of these enzymes are increased in viral hepatitis and reach their maximum soon after the onset of jaundice, and then decrease slowly Very high levels occur in toxic hepatic necrosis Levels in cirrhosis may be moderately raised but only if the process is active Choline esterase levels are decreased in liver cell dysfunction

8 **Test for the conversion of ammonia to urea:** The normal range of blood ammonia is 40 to 75 μg ammonia nitrogen per 100 ml of blood In cirrhosis of the liver, blood ammonia may be increased to over 250 μg/100 ml High values are found in hepatic coma

9 **Glutamine content of cerebrospinal fluid:** The normal range of glutamine in cerebrospinal fluid is from 6 to 14 mg per 100 ml In cirrhosis of the liver, higher values ranging from 16 to 31 mg/100 ml have been reported. In hepatic coma, still higher values ranging from 30 to 54 mg/100 ml have been reported

MISCELLANEOUS METABOLIC FUNCTIONS OF THE LIVER

1 **Storage of vitamins:** The liver has a particular propensity for storing vitamins Vitamin A is stored in the liver to the greatest extent but large quantities of vitamin D and vitamin B_{12} are normally stored as well Sufficient amount of vitamin A can be stored to prevent vitamin A deficiency for one to two years, sufficient vitamin D and vitamin B_{12} can be stored to prevent their deficiency for one to four months

2 **Storage of iron:** Iron is stored in the liver in the form of *ferritin* When the iron in the circulating body fluids reaches a low level, the ferritin releases the iron Therefore, the apoferritin ferritin system of the liver acts as a blood iron buffer and also as an iron storage medium

3 **Removal or excretion of drugs, hormones and other substances:** The liver excretes into the bile many different drugs including sulfonamides, penicillin, ampicillin and erythromycin In the same manner, different hormones are either chemically altered or excreted including thyroxine and essentially all of the steroid hormones such as estrogens, cortisol, aldosterone and so forth Finally, one of the major routes for excreting calcium from the blood is into the bile and then into the feces

Exercise

1 Write short notes on liver function test (M U 682)

CHAPTER 37

MEMBRANES

Membranes are viscous solutions which exist around all living cells They form closed compartments around the protoplasm of the cell and separate one cell from another They act as barriers with selective permeabilities to materials and thus maintain the difference between inside and outside The selective permeabilities are regulated by gates and pumps as well as by specific receptors for enzymes, substrates, and hormones

Membranes are mostly composed of lipids and proteins but also contain carbohydrates Membranes anchore protein molecules where they carry out specific function of the organelle, the cell, and the organism

LIPIDS

The lipid composition of membranes are phospholipids, glycolipids, and sterols—cholesterol in mammalian membranes

Phospholipids.

1 The commonest one is phosphoglycerides which consist of glycerol, two fatty acids, and a phosphorylated alcohol The fatty acids contain even number carbon atoms and they are unbranched and can be saturated or unsaturated.

2 The other phospholipids are the *sphingomyelins* which contain sphingosine rather than glycerol A fatty acid is attached to the amino group of sphingosine. The primary alcoholic group of sphingosine is esterified to phosphorylcholine

Glycolipids.

1 The glycolipids are the cerebrosides and gangliosides which are also derived from sphingosine.

2 A cerebroside contains a single glucose or galactose, but a ganglioside contains a branched chain of seven glucose or galactose

Sterols.

1 **Cholesterol,** the most common sterol in membranes, exists in the plasma membranes of mammalian cells but also found in mitochondria, golgi complex and nuclear membranes in less quantity

2 It is more abundant in the outside of the plasma membrane

3 The major lipids in membranes are amphipathic which contains both hydrophobic and hydrophilic regions If the hydrophobic region is separated from the rest of the molecule, it will be insoluble in water but soluble in oil The reverse happens in case of hydrophilic region Detergents are amphipathic molecules which have much biochemical importance

PROTEINS

1 The phospholipids of the membrane are the solvent for the membrane proteins The functional groups attached to the alpha carbon of twenty amino acids involved in the primary structure of protein are strongly hydrophobic in 6, weakly hydrophobic in a few, and hydrophilic in the remainder

2 The alpha helical structure of protein minimizes the hydrophilic character of the peptide bonds Proteins are, therefore, amphipathic. different proteins perform different functions in membranes

3 The outside location of the carbohydrates attached to membrane proteins provides an inside outside asymmetry

Integral and Peripheral Membrane Proteins

1. The membrane proteins are mostly integral components of the membrane. The integral proteins are usually globular in shape and are amphipathic. They contain two hydrophilic ends separated by a hydrophobic region.

2. The integral proteins are asymmetrically distributed across the membrane bilayer. If they are dissolved in detergent and the detergent is then slowly removed, the phospholipids and the integral proteins will assemble.

3. Peripheral proteins do not interact directly with the phospholipids in the bilayer but are weakly bound to the hydrophilic regions of specific integral proteins.

4. The immunoglobulin molecules on the plasma membranes of lymphocytes are integral membrane proteins and are released by the shedding of small fragments of the membrane.

5. Many *hormone receptor molecules* are integral proteins and the specific polypeptide hormones which bind to the receptor molecules are considered peripheral proteins.

MEMBRANE ASSEMBLY

1. The enzymes responsible for the synthesis of phospholipids reside on the cytoplasmic aspects of the vesicles of endoplasmic reticulum. The lipid vesicles migrate to the golgi apparatus which in turn fuses with the plasma membrane. Both the golgi complex and the endoplasmic reticulum vesicles exhibit transverse asymmetries of both lipid and protein and these asymmetries are maintained during fusion with the plasma membrane. The inside of the vesicle after fusion becomes the outside of the plasma membrane and the cytoplasmic side of the vesicles remains the cytoplasmic side of the membrane.

2. The integration of proteins into membrane can be explained by two hypothesis—The *signal hypothesis* supports that the protein is inserted into the membrane simultaneously with the translation of its mRNA on polyribosomes. As the leader sequence of the protein emerges from the ribosome, it is recognized by a signal recognition particle (SRP) that blocks further translation after about 70 amino acids have been polymerized—40 buried in the large ribosomal complex and 30 exposed. The SRP contains 6 proteins and has associated with it a 7sRNA.

3. Integral membrane proteins do not completely cross the membrane and are prevented by a hydrophilic C-terminal anchor region. Secreted proteins completely traverse the membrane bilayer and are discharged into the lumen of the endoplasmic reticulum. Carbohydrate moieties are already attached before they reach the interior of that vesicle. The secretary proteins are also found in the lumen of the golgi apparatus.

4. The *membrane trigger hypothesis minimizes* the role of catalysis in membrane assembly. This hypothesis does not require specific ribosome membrane interaction but this does not mean that protein synthesis on membranes cannot occur.

5. Both the signal and trigger mechanisms exist even in the same cell. Some membrane proteins and secreted proteins are synthesized on membrane-bound polysomes, while others are formed on free cytoplasmic polysomes.

INTERCELLULAR CONTACT AND COMMUNICATION

Cells have developed specialized regions on their membranes for intercellular communication. *Gap junctions* mediate and regulate the passage of ions and small molecules through a narrow *hydrophilic pore* connecting the cytoplasms of adjacent cells. These pores are composed of subunits called "connexons". Connexons consist of 6 protein subunits that span the membrane and connect with the analo-

gous structures on the adjacent cells In response to specific chemical stimuli, the subunits rearrange themselves relative to one another to provide a tangential central opening about 2 nm in diameter and through this central opening, ions and small molecules can pass from one cytoplasm to another in a regulated manner

Signal transmission.

1 Neurotransmitters, hormones, and immunoglobulins bind to specific receptors (integral proteins) exposed to the outside of cellular membranes and transmit information through these membranes to the cytoplasm

2 The β-adrenergic receptor, which binds catecholamines stereospecifically, is assymmetrically located on the outer aspect of plasma membranes of target cells such as the erythrocytes The binding of the catecholamine on the outside stimulates the catalytic activity of adenylate cyclase which is asymmetrically located on the inside of the membrane Adenylate cyclase generates cAMP from ATP Thus, the information on the outside of the second messenger cAMP

3 The catecholamine when binds to the beta receptor, the latter by conformational change activates *phospholipid methyltransferase I* (an integral enzyme) This enzyme generates phosphotidyl-N monomethylethanolamine from phosphotidylethanolamine The phosphotidyl N monomethylethanolamine flip flops toward the outside of the membrane and is further methylated to phosphatidyl-N-dimethylethanolamine and subsequently to phosphatidylcholine by the integral enzyme *phospholipid methyltransferase* II, located toward the outside of the membrane The increased local concentration of phosphatidylcholine enhances the local *membrane fluidity* The increased fluidity exposes more pre-existing beta receptor molecules to the outside membrane surface

4. Phospholipid methylation serves as an initial common pathway for the transmission of many receptor mediated biologic signals through membranes In some systems, the increased fluidity due to phospholipid methylation allows the rapid influx of calcium which can bind to *calmodulin* and activate specific enzymes including phospholipases The phospholipases from *phosphotidylcholine* can in turn generate free fatty acids including arachidonic acid which is an immediate precursor of *prostaglandins* The prostaglandins are involved in signal transmission in several transmembranes, hormonally responsive systems

Endocytosis

1 Endocytosis is a transport process which allows cells to internalize extracellular material and forms endocytotic vesicles during that process Endocytotic vesicles are formed when segments of the plasma membrane invaginate enclosing a volume of extracellular fluid and pinch off as the fusion of plasma membranes seals the neck of the vesicle on the original site of invagination Subsequent fusion of the endocytotic vesicle with other membrane structures performs the transport of its contents to other cellular compartments or even back to the cell exterior

2 Endocytosis are of two types—Phagocytosis and Pinocytosis *Phagocytosis* occurs only in specialized phagocytic cells present in blood *Pinocytosis* leads to the all cellular uptake of fluid and fluid contents The *adsorptive pinocytosis* is a receptor mediated, selective process primarily responsible for the uptake of macromolecules for which there is a finite number of binding sites on the plasma membrane These high affinity receptors permit pinocytosis to concentrate ligands from the medium and to minimize the uptake of fluid or soluble unbound macromolecules

3 Several hormones, the other macromolecules, are subject to adsorptive pinocytosis and form *receptosomes*, vesicles which avoid lysosomes and deliver their contents to the Golgi system

4 Adsorptive pinocytosis of extracellular glycoproteins requires to glycoproteins that carry specific *carbohydrate recognition signals* These recognition signals are bound by membrane receptor molecules which play a role analogous to that of the LDL receptor Acid hydrolases taken up by adsorptive pinocytosis in fibroblasts are recognized by their mannose-6 phosphate *moieties* The *mannose-6-phosphate* moiety also plays an important role in the *intracellular segregation* of the acid hydrolases to the lysosomes of the cells in which they are synthesized

5 The other type of pinocytosis is a *fluid phase pinocytosis* which is a nonselective fluid phase process in which the uptake of a solute is simply proportionate to its concentration in the surrounding medium This type of pinocytosis forms small vesicles and it is an extraordinarily active process utilizing up to 50 per cent of the plasma membrane per hour in some cells The components of the membrane must be recycled to maintain cellular integrity

DISORDERS OF MEMBRANES

Multiple sclerosis: It is a somewhat confusing neurologic disorder in which nervous tissue is demyelinated by an unkown process The demyelinization removes the neuronal insulation and profoundly decreases the velocity of nerve impulse transmission

Pseudohypoparathyroidism: It is an inherited disorder which signifies defective signaling across the plasma membranes of several types of target cells In that affected cell, the parathormone signal is normally mediated by activation of adenylate cyclase There is a defective coupling protein in the membrane that prevents transmission of the extracellular hormone signal to the adenylate cyclase

I cell disease: It is a recessive disorder due to inability of the patient's fibroblasts to attach the mannose-6-phosphate signal to the acid hydrolases synthesized in various cell types The acid hydrolases do not segregate normally to the primary lysomes but are secreted into the extracellular space leaving the intracellular lysomes deficient in these important hydrolases Thus, the lysosomes accumulate excessive debris and thereby generate intracellular cytoplasmic inclusions (I) These abnormal secreted hydrolases are not subject to adsorptive pinocytosis by the patient's or even by normal fibroblasts, since they lack the necessary mannose-6-phosphate recognition signal

9 Each chain is divided into specific domains or regions that have structural and functional significance The half of the *light chain* (L) toward the carboxy terminus is termed as the *constant region* (CL), while the amino terminal half is the *variable region* (VL) of the light chain About one quarter of the *heavy* (H) *chain* at the amino terminus is termed as variable region (VH), and the other three quarters of the heavy chain are referred to as the constant regions (CH^1, CH^2, CH^3) of that H chain

10 The portion of the immunoglobulin molecule which *binds the specific antigen* is formed by the aminoterminal portions (variable regions) of both the H and L chains i e the *VH* and *VL* domains The domains of the protein chains do not simply exist as linear sequences of amino acids but form globular regions with secondary and tertiary structure

Exercise

1 Discuss in details the membrane
2 Write short notes on
   (a) Membrane assembly
   (b) Disorders of membranes

CHAPTER 38

GLYCOPROTEINS, PROTEOGLYCANS, AND GLYCOSAMINOGLYCANS

These three types of molecules have the common structure, syntheas, degradation, and even function

Glycoproteins differ from other proteins with the oligosaccharide chains covalently attached to their polypeptide backbones

Proteoglycans are also proteins to which oligosaccharide chains are covalently attached to the polypeptide back bone, but the oligosaccharides chemically differ from those of glycoproteins The oligosaccharides consist of repeating units of (i) glucosamine or galactosamine, (ii) a uronic acid (except for keratan sulphate) and (iii) covalently attached sulphate groups (except for hyaluronic acid)

Glycosaminoglycans are oligosaccharide structures that have been removed from the protein backbone of their proteoglycan precursor

All these three classes of molecules are mostly present in the *extracellular space* but are synthesized intracellularly

Glycoprotein	Protein—oligosaccharide
Proteoglycan	Protein—oligosaccharide (uronic acid/or SO_4) —Protein
Glycosaminoglycan	Oligosaccharide (uronic acid/or SO_4)

GLYCOPROTEINS

1 Their molecular weight ranges from 15,000 to over 1 million containing 15 or fewer sugar units per chain

2 Their carbohydrate contents range from 1 to 85 per cent by weights

3 They are present in plants, bacteria, fungi, viruses, and animals The most membrane proteins and secreted proteins are glycoproteins

4 They act as structural molecules in cell walls, collagen, elastin, fibrins, and bone matrix, as lubricants and protective agents in mucins, mucous secretions.

5 They are utilized as transport molecules for vitamins, lipids, minerals and trace elements, as immunologic molecules for immunoglobins, histocompatibility antigens, complement, and interferon, as hormones in chorionic gonadotropin, thyrotropin (TSH), as enzymes in proteases, nucleases, glycosidases, hydrolases, and clotting factors, as recognition sites in cell-cell, virus-cell, bacterium-cell, and hormone receptors

Oligosaccharides of Glycoproteins·

1 The oligosaccharide chains contain *nine* different sugar residues Glucose (Glc) is found only in collagen, but galactose (Gal) and mannose (Man) are more common and widely distributed. The hexoses are N-acetylgalactosamine (Gal NAC) and N acetylglucosamine (Gle NAC) Fucose (Fuc) is a common constituent Two pentoses-arabinose (Ara) and Xylose (xyl) are found and the ninth are the sialic acids (Sial) of which N-acetylneuraminic acid (Nana) is an example The fucose and nana residues are more distal in the chain, frequently at terminal sites,

2 The oligosaccharide chains are attached to the polypeptide backbone at one of five amino acid residues—asparagine (Asn), serine (Ser), threonine (Thr), hydroxylysine (Hyl), or hydroproline (Hyp) Two types of chemical bonds that provide the attachment sites are (a) O-glycosidic links and (b) N-glycosidic links

(a) **O-glycosidic Links:**

(i) The O glycosidic links occur through the free alcoholic groups of Ser or Thr residues of the polypeptide in a tripeptide sequence of Asn-y Ser (Thr), where Y is an amino acid other than aspartic acid

(ii) Gal NAG is the most common sugar residue attached directly to the Ser or Thr residue Six different types of oligosaccharide are attached to this Gal NAC-Ser (Thr) linkage

(iii) The initiation and extension of different types of oligosaccharide chains of glycoproteins occur by the stepwise donation of sugar residues from pyrimidine or purine nucleotide sugars

(iv) Oligosaccharides may be linked to proteins via O glycosidic bonds to Hyl or Hyp which are amino acid residues found in collagens and some fibrous proteins of plants

(b) **N-glycosidic linkage:**

(i) The N-linked oligosaccharide consists of a core region with the structure Man β 1, 4-Glc NAC-β 1, 4-Glc NAG Asn This core region are of two types—The high mannose (simple) type and the complex type A single protein can contain oligosaccharide chains of both high mannose and complex type

(ii) Although all high mannose oligosaccharides are synthesized from nucleotide sugars, there exists an important lipid-linked precursor oligosaccharide that is transferred en bloc from a lipid carrier to the Asn of the protein

(iii) The complex N-linked oligosaccharides also contain the β man-di-N-acetylchitobiose core structure but consist also of a variable number of outer chains containing Sial, Gal, and Fuc residues linked to the core

(iv) Complex N linked oligosaccharide structures are found only in higher animals, whereas the high mannose type are common in primitive organisms

The synthesis of Complex Carbohydrates of Glycoproteins:

(i) The nucleotide of sialic acid, CMP-Sial, is formed from CTP by sialyl-transferases located in the Golgi complex and in the nucleoplasm

(ii) In animal cells, the sugars are linked to the nucleotides by the alpha-linkage with the exception of the beta-linkage of L-fucose to GDP The alpha-bridges are converted to the beta bridges and vice-versa during the transfer of the sugar moiety to the oligosaccharide

(iii) A number of specific glycosyl transferase enzymes catalyze the transfer of the sugar moieties to generate the complex glycoproteins These enzymes require Mn^{++}

(iv) A Golgi localized enzyme, UDP Glc NAG transferase I, can then denote a Glc NAG to a linear or branched alpha-Man moiety to form Glc NAC-β-1, 2-Man linkages A second transferase, UDP Glc NAG transferase II, will denote its Glc NAG moiety only to a branched structure by the transferase I enzyme.

(v) Fucosyltransferases can then act on the products of Glc NAG transferase I or transferase II The galactosyltansferase enzymes are also located on the Golgi complex and attach a galactosyl residue usually to the end of a chain The

galactosyl residues are linked to GlcNAC by beta-1, 4-linkages but occasionally by beta-1, 6-linkages.

(vi) Four different sialyltransferase enzymes are found in the Golgi complex and use CMP-sialic acid as donor for the sialation of protein-linked oligosaccharides.

(vii) The elongation process generating the complex type oligosaccharides of glycoproteins occurs exclusively in the Golgi complex. Each linkage is carried out by a specific glycosyl-transferase; thus, there seems to be a "one linkage, one glycosyltransferase" synthetic arrangement.

BLOOD GROUP ANTIGENS

In 1900, *Landsteiner* described the *ABO blood groups*. Today, there are more than twenty blood group systems expressing more than 100 distinct antigens. These erythrocyte antigens are linked to specific membrane proteins by O-glycosidic bonds in which GalNAC is the most proximal sugar residue. The specific oligosaccharides exist in three forms: (i) as glycosphingolipids and glycoproteins on the surfaces of erythrocytes and other cells, (ii) as oligosaccharides in milk and urine, and (iii) as oligosaccharides attached to mucins secreted in the gastrointestinal, genitourinary, and respiratory tracts.

Four independent gene systems are related to the expressions of these oligosaccharide antigens.

Genetic Locus	*Alleles*
H	H, h.
Secretor	Se, se.
ABO	A, B, O
Lewis	Le, le.

The H Locus: This codes for a fucosyltransferase that attaches a fucose residue in alpha-1, 2-linkage to a Gal residue, itself attached in beta-1, 4-linkage to an oligosaccharide. Fuc-α-1, 2-Gal-β-R is a precursor for the formation of both the A and B oligosaccharide antigens. The h allele of the H locus codes for an inactive fucosyltransferase. Therefore, individuals with the hh genotype cannot generate this necessary precursor of the A and B antigens. Hence hh genotype persons will be type O.

The secretor locus: This controls the appearance of the H-specific Fuc-transferase in some secretory organs, such as the exocrine glands, but not in the erythrocytes. Accordingly, individuals with the Hh or HH genotype and an Se allele will generate the A and B antigen precursor in the exocrine glands that form saliva. The individuals who are SeSe or Sese and possess an H allele will be secretors of the A or B antigens (or both). When the A- or B-specific transferase are present. Individuals who are sese genotype will not secrete A or B antigens; but if they possess an H allele and A or B alleles, their erythrocytes will express the A, B or both antigens.

The ABO locus: This codes for two specific transferases that act to transfer specific Gal moieties to the Fuc-α-1, 2-Gal-β-R precursor oligosaccharide formed by the action of the H allele-coded fucosyltransferase. Persons possessing an A allele will attach a GalNAC moiety to the precursor generated by the H allele transferase and an individual possessing a B allele will transfer a Gal moiety to the same precursor. Individuals possessing both A and B allele will generate both A and B alleles (OO homozygotes) will not attach either GalNAC or Gal to the

ursor When neither Gal NAG nor Gal is at the reducing terminus of this saccharide, it will not be recognized by either anti-A or anti B antisera, and blood group antigen is said to be *type O* Individuals with the hh genotype pable of attaching the Fuc moiety to the appropriate Gal β R oligosaccharide capable of expressing the A or the B antigen determinant and thus is con- red to be of the O type blood group

The Lewis Locus: The lewis dependent fucosyltransferase is not specific ut what is not to the Gal-1, 3 β group When no H allele is present (hh), product of the lewis a-1, 4 focosyl transferase is referred to as the Le^a antigen ch cannot have A or B antigenicity even when the A or B transferases are present When both the H allele and the Le allele fucosyltransferases have d on the Gal-1, 3 R oligosaccharide, the product is referred to as the Le^b igen The Le^b antigen may also exist without A antigenicity or B antigenicity the same molecule The le allele codes for an inactive lewis transferase, and s neither Le^a nor Le^b antigens will be formed in a person with lele genotype

PROTEOGLYCANS

Each polysaccharide of proteoglycans consists of repeating disaccharide ts in which D glucosamine or D-galactosamine is always present Each dis- haride contains a uronic acid, glucuronic acid (GlC UA), L iduronic acid UA) All polysaccharides contain sulphate groups with the exception of aluronic acid

The linkage of the polysaccharides to their polypeptide chain is one of three es

(i) An O-glycosidic bond between Xyl and Ser, a bond that is unique to oteoglycans

(ii) An O-glycosidic bond between GalNAG and Ser (Thr), present in ratan sulphate II

(iii) An N-glycosylamine bond between GlG NAC and the amide nitrogen Asn

The elongation process of chain involves the nucleotidyl sugars acting as nors The reactions are performed by the substrate specifities of the specific ycosyltransferases Thus, "one enzyme, one linkage" relationship holds he specificity of these reactions is dependent upon the nucleotide sugar donor, ie acceptor oligosaccharide

The polysaccharide chain growth termination results from (i) capping effects f isolation by the specific sialyltransferases, (ii) sulfation, particularly at the positions of the sugars, and (iii) the progression of the particular polysaccharide way from the site in the membrane where the catalysis occurs After formation f the polysaccharide chain, numerous chemical modifications take place

Inherited defects in the degradation of the polysaccharide chains lead to he group of diseases known as *mucopolysaccharidoses and mucolipidoses*

Seven types of polysaccharides are covalently attached to the proteins of proteoglycans Six of them contain alternating uronic acid and hexosamine esidues. Except hyaluronic acid all contain sulphated sugars These seven types f polysaccharides are distinguished by their monomer composition, their glycosidic inkage, and the amount and location of their sulphate substituents

Functions of Glycosaminoglycans and Proteoglycans.

I Glycosaminoglycans can interact with extracellular macromolecules, plasma proteins, cell surface components, and intracellular macromolecules

2. Because of their polyanionic nature the binding of this is generally electrostatic.
3. These with IdUA bind proteins with greater affinities than those containing GlcUA as their only uronic acid constituent.
4. The binding between these and other extracellular macromolecules contributes to the structural organization of connective tissue matrix.

A. Interactions with extracellular macromolecules.

(i) All glycosaminoglycans except those that lack sulphate groups or carboxyl groups bind to collagen electrostatically at neutral p^H. Tighter binding is promoted by the presence of IdUA and the proteoglycans interact more strongly than glycosaminoglycans.

(ii) The chondroitin sulphate and keratan sulphate chains of proteoglycans aggregate with hyaluronic acid.

B. Interactions with plasma proteins.

(i) Dermatan sulphate binds plasma lipoproteins and appears to be the major glycosaminoglycan synthesized by arterial smooth muscle cells. This dermatan sulphate may play an important role in the development of atherosclerosis.

(ii) Heparin with its high negative charge density (due to the IdUA and sulphate residues) interacts strongly with several plasma components. It interacts with antithrombin III. Heparin sulphate is also capable of accelerating the action of antithrombin III, but is much less potent than heparin. Heparin can bind to *lipoprotein lipase* present in capillary walls and cause a release of that triglyceride-degrading enzyme into the circulation. Hepatic lipase also binds heparin but with lower affinity.

C. Cell surface molecules.

(i) Heparin associates with blood platelets, arterial endothelial cells, and liver cells. Chondroitin sulphate, dermatan sulphate, and heparan sulphate bind to independent sites on surface of cells such as fibroblasts. At those sites, the glycosaminoglycans and proteoglycans are taken up by fibroblasts and degraded.

(ii) Some proteoglycans serve as receptors and carriers for macromolecules. These proteoglycans are involved in the regulation of cell growth.

D. Intracellular macromolecules.

(i) Proteoglycans and their glycosaminoglycan components have effects on protein synthesis and intranuclear functions. Glycosaminoglycans are found in significant quantities in nuclei from different cell types.

(ii) The acid hydrolases in lysosomes may be naturally complexed with glycosaminoglycans to provide a protected and inactive form. Chondroitin sulphates, dermatan sulphates, and heparin can affect the activities of various lysosomal acid hydrolase in negative or positive ways.

(iii) Many storage or secretory granules such as the chromaffin granules in adrenal medulla, the prolactin secretory granules in the pituitary gland, and the basophilic granules in mast cells contain sulphated glycosaminoglycans. The glycosaminoglycan-peptide complexes that occur in these granules play a role in the release of biogenic amines.

Exercise

1. Write about glycoproteins.
2. What do you know about proteoglycans?
3. Discuss blood group antigens.

CHAPTER 39

BIOCHEMICAL ANALYSIS OF NERVE TISSUE

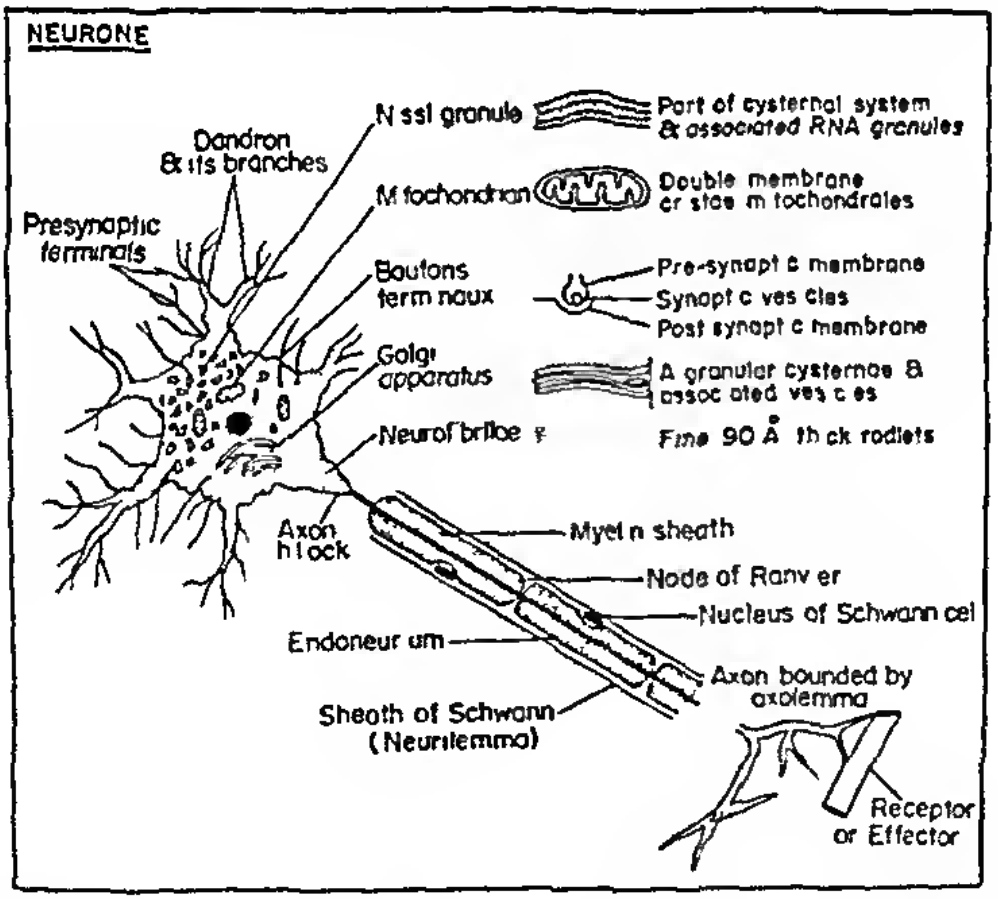
Introduction

Two main systems namely the nervous system and the endocrine system regulate and control the functions of the body The endocrine system mainly regulates the metabolic functions of the body which are comparatively sluggish The nervous system controls the rapid activities of the body namely muscular contractions, respiration, and the rates of secretion of some endocrine glands such as adrenal medula and neurohypophysis

The nervous system includes brain, spinal cord, cranial and peripheral nerves, ganglia and plexuses This system has the capacity to transmit rapidly a number of informations at a time from one part of the body to another Although the detailed informations regarding the functions of nervous system are not yet known, still some of the biochemical informations as regards to the transmission of the nervous system have been discussed below

Structural and functional units:

Nervous system comprises about 2 5 per cent of the total body weight This consists of three sub-systems namely central, peripheral, and autonomic nervous system The central nervous system includes the brain and the spinal cord The peripheral includes various cranial and peripheral nerves The autonomic nervous system is divided into sympathetic and parasympathetic systems The nervous tissue consists of a group of highly specialised cells known as *neurones*



Left Diagrammatic representation of the structure found in the nerve cell under the light microscope *Right* The electron microscopic details of these structures

Fig 39 1

Structure of a neurone:

Each neurone consists of a metabolically active body known as the nerve cell proper, soma —, or perikaryon, and a long filamentous structure arising from it known as the nerve fibre or axon, while the part of the cell body from which axon arises is said to be axon hillock.

1 Perikaryon:

(a) Its diameter varies from 5μ to 125μ

(b) It has a cell membrane, cytoplasm, a large vesicular nucleus containing a single prominent nucleolus, numerous rod like or spherical mitochondria, and a golgi apparatus.

(c) It also contains certain other organelles which are not present in a usual cell of the body These organelles include *dendrites, presynaptic terminals, sex-chromatin, nissl granules,* and *neurofibillae* etc.

Dentrites.

(a) The cell membrane is projected in the form of a number of filamentous structures known as dendrites.

(b) They may vary from almost none to one metre in length (peripheral sensory nerve fibre)

(c) Each dendron contains nissl granules, mitochondria, and neurofibrillae.

(d) Each dendron on leaving the cells goes on branching repeatedly

Presynaptic terminals:

(a) These are small knob like structures which exist on the surfaces of the dendrites and perikaryons.

(b) They are the ends of the nerve fibrils that originate in other neurones next to on which they come to exist.

Sex-chromatin:

(a) It is a large granule often seen adjacent to the nucleolus.

(b) This is found to be present only in females and the sex can be determined by its presence or absence Hence it is termed as sex-chromatin.

Nissl granules:

(a) The cytoplasm of a perikaryon shows the presence of basophilic masses known as nissl granules or bodies on the study of special staining techniques.

(b) These granules under electron microscope appear to be composed of many thin, membrane bounded cavities or cisternae arranged in a parallel fashion.

(c) Their sizes and number vary with the physiological conditions of the cell e g , fatigue, the action of certain poisons. The granular disintegrate into a fine dust which ultimately disappears on the section of the axon.

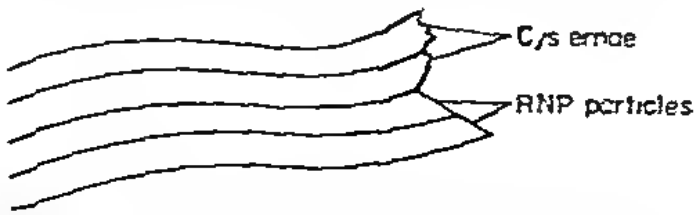


Fig 39.2. Electron microscopic structure of nissl granules.

(d) These granules are similar to the (less numerous) rough surfaced membranes found in the cytoplasm of most cells which collectively make-up the endoplasmic reticulum

(e) The minute particles consist chiefly of ribose nucleo proteins (RNP) which stain with basic dyes

Neurofibrillae:

These are aggregation of thin, thread like structures of variable length and 60—100Å in diameter forming a loose network of fibrils in the cytoplasm

2 **Axon:**

(a) The axon or nerve fibre arises from the axon hillock of the perikaryon which is rich in neurofibrillae but has no nissl granules

(b) It consists of a central core of semifluid axoplasm which flows from the cell body to the axon and not in the reverse direction

(c) Axoplasm also contains mitochondria, nissl granules, neurofibrillae running parallel to the long axis of the axon Axoplasm is bounded by a cell membrane known as axolemma which is continuous with the cell membrane of parent cell body

(d) The axon is further surrounded by a sheath known as myelin sheath or medullary sheath which is not present in all the fibres The fibres having myelin sheath are known as myelinated or medullated nerve fibres and other which have no myelin sheath are known as non myelinated or non medullated nerve fibres. The myelinated nerve fibres are also said to be white nerve fibres on account of the presence of lipids in the myelin sheath. The non myelinated nerve fibres appear grey for which they are known as grey nerve fibres All the post ganglionic fibres of the autonomic nervous system are non medullated, while all the pre-ganglionic fibres are medullated

(e) A thin layer of fine reticular fibres surrounding the myelin sheath forms the endoneurium or sheath of Henle

(f) The axolemma at the terminal end of the axon is also thrown into similar projections—dendrites which come in contact either with the dendrites of the perikaryon of next neuron forming synapses or receptor or effector (e g , myoneurunal junction)

3 **Myelin sheath and Myelinogenesis:**

(a) A specialized set of Schwann cells arranged in a linear manner all around the axolemma forms myelin sheath by a process known as myelinogenesis In this process, the schwann cells grow round the axon and envelop it completely along its entire length so that cell membrane of the Schwann cell can be divided into two parts—one surrounding the axon i e , inner part and other outer part which are connected to one another by the double mesaxon

(b) The Schwann cells start to rotate and go on rotating for several times As a result, many closely packed, helically arranged layers of double membrane are wrapped around the axon as shown in figure 39 3 This set of membranes is known as myelin sheath

(c) Each membrane is composed of two layers of lipid substances sandwiched between layers of protein and they form the myelin sheath of the nerve fibre

(d) The thickness of the myelin sheath is ascertained by the number of membrane layers wrapped round the axon.

(e) The phospholipid sphingomyelin is an excellent insulator that prevents almost all flow of ions outside the nerve fibre

(f) A small uninsulated area at the junction between each two successive Schwann cells along the axon remains where ions can flow with ease between the extracellular fluid and the axon. The area is said to be the *node of Ranvier*

(g) The higher permeability at the nodes of Ranvier causes the impulses to be conducted from node to node by the myelinated nerve fibre rather than continuously along the entire fibre as happens in the unmyelinated nerve fibre. This process is called as *saltatory conduction* and is helpful in the higher velocity of nerve transmission in myelinated fibres than that in unmyelinated fibres

(h) The nerve fibres in the central nervous system are myelinated by a different types of cells known as *oligodendroglia* and by a different process.

(i) The Schwann cells although surround the axon of unmyelinated nerve fibres, they do not spin a myelin sheath around them

(j) Neurones in different parts of the body differ markedly from one another in the size and shape of the cell body, length, size and number of dendrites, the length and size of the axon, the number of presynaptic terminals which may range from hundred to several thousands.

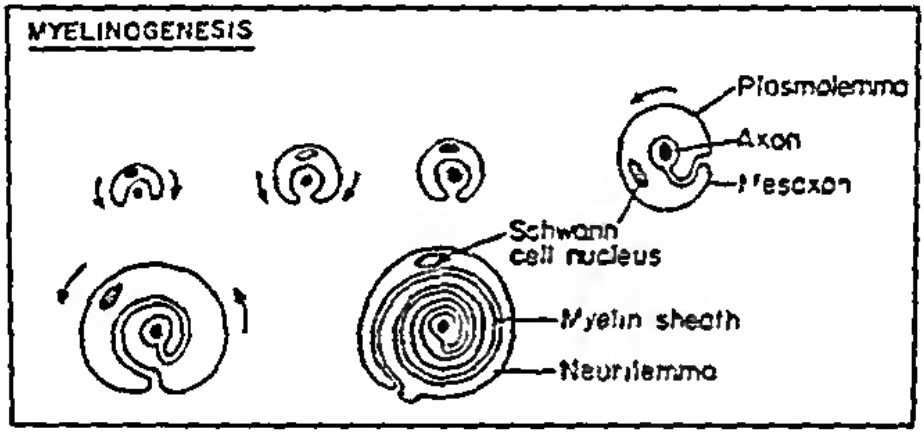


Figure. 39.3 The Myelinogenesis

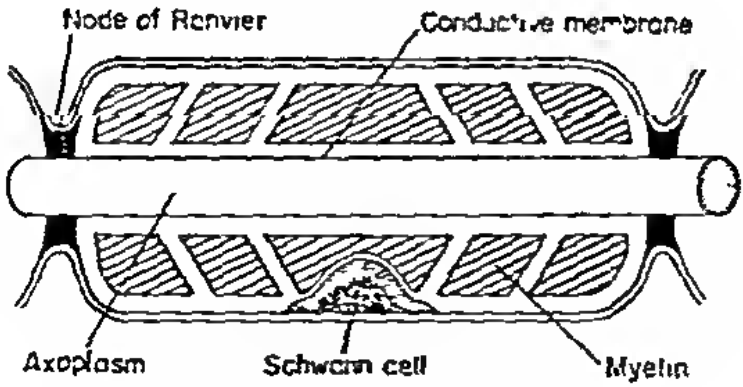


Figure 39.4 Structure of a myelinated nerve fibre.

Structure of a typical nerve:

A nerve consists of groups of nerve fibres Bundles of nerve fibres are enclosed in a connective tissue sheath known as *perineurium*. A number of such bundles are bounded together by another sheath of connective tissue known as the *epineurium* to form a nerve

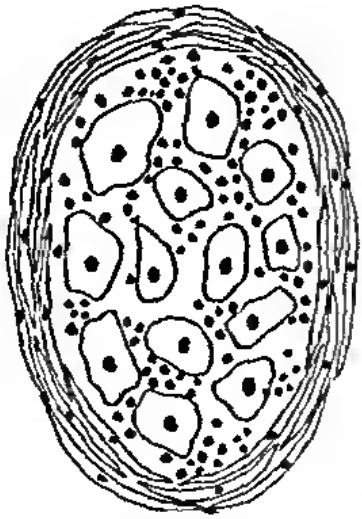


Fig 39 5 Cross section of a small nerve trunk containing myelinated (largely few and comprising most of area) and unmyelinated (Many more, small and lying between large ones) fibres

Chemical composition of the nerve tissue:

Nerve tissue is composed of—

Water	80 per cent
Solids	20 per cent

The solids are mainly composed of proteins, lipids, small amounts of organic extracts and inorganic salts. Proteins are about 38 to 40 per cent of the total solids They include different globulins, nucleoproteins, and a characteristic albuminoid called neurokeratin. The lipid contents are 50 to 54 per cent of the total solids The important lipids are phospholipids, cholesterol, cerebrosides, aminolipids, and suphur containing lipids The principal inorganic salts are potassium phosphate and chloride, with smaller amounts of sodium and other alkaline elements Potassium is highly significant in the nerve impulse

The water content of brain is little more than that of spinal cord In brain too the grey matter which represents a concentration of nerve cell bodies contains more water than the whole matter where the nerve fibres are mainly found A fraction of the brain proteins remains combined with copper forming *ceruloplasmin* An increased deposition of copper is found in the brain tissue in *Wilson's disease* White matter and the peripheral nerves contain a little more cerebrosides, free cholesterol, and sphingomyelin than the brain grey matter The considerable increase in the concentration of sphingomyelin is found in *Niemann Pick disease*

Metabolism of the nerve tissue:

Since the respiratory quotient (R Q) of the metabolic nerve is near about 1 this indicates that the nerve tissues utilize carbohydrates almost exclusively for the energy purposes Lactic acid and pyruvic acid are formed under anaerobic conditions in the nerve tissue as a result of carbohydrate metabolism and these acids disappear very slowly in the presence of oxygen. Hence, the metabolism of carbohydrates in the nerve tissue appear to be similar to that of muscles A minute supply of blood glucose is specially important to the nervous system because of the less glycogen storage in the nerve tissue This may be the vital cause for the prominence of nervous symptoms such as mental confusion, dizziness, delirium etc in hypoglycemia.

As regards to protein metabolism, glutamic acid is the only amino acid metabolized by brain tissue This acid serves as a precursor of γ aminobutyric acid (GABA), one of the chemical transmitters, and as a major acceptor of ammonia produced either by brain itself or delivered to brain when the arterial blood ammonia is increased and thus protects the brain tissue against its toxic effects Different workers have also demonstrated the synthesis and exchange of lipids in nerve tissue

Nerve impulse:

Nerve impulse may be defined as an electro-chemical change which is transmitted by nerve fibres It must not be confused with the stimulus which is the external force (e.g , chemical, physical, biological) which sets up the impulse The chemical changes in the nerve fibres are concerned with the recovery processes which follow the activity The electrical change in the most certain indicator of the development and propagation of the nerve impulse and represents the essential process involved in the transmission of the impulse along the nerve fibre as discussed in the following pages

Electrical or Membrane potential

The difference in the potentials (charges) on the two sides of the membrane is said to be membrane or electrical potentials This can be explained in the following manner Suppose, the extra and intra-cellular fluid as electrolyte solution containing approximately 155 meq /litre of anions and the same concentration of cations then usually an excess number of anions accumulates immediately inside the cell membrane separating the two fluids along its inner surface and an equal number of cations outside the membrane on its outer surface This results in the development of membrane potential or electrical potential

Membrane potential is of two types—resting membrane potential and action potential. Resting membrane potential is the potential which exists during the resting state of membrane Action potential exists during the active state of membrbrane

Electrical potential exists across the membranes of essentially all cells of the body

Resting membrane potential:

The resting membrane potential may be defined as the net different in the charge between inside and outside membrane requiring little or no expenditure of energy In a neurone, this is about—85mv so long as this neurone is in a resting stage and is maintained almost constant It is believed to arise (1) from the unequal distribution of ionic substances on the two sides of the membrane due to sodium and potassium pumps. (2) Due to semi permeability of the membranes

In the resting condition, the concentration of Na^+ is about 142 meq/litre outside the cell and 10 meq/litre inside the cell, while that of K^+ (main diffusible cation) is 5 meq/litre outside the cell and 140 meq/litre inside the cell due to sodium and potassium pump respectively There is also a very high concentration of non-diffusible anions, 150 meq/litre inside the cell in contract to a very low concentration of only 5 meq/litre outside the cell Simultaneously, K^+ are easily diffusible through the membrane, while Na^+ and non-diffusible anions diffuse very poorly due to selective permeability of the membrane Therefore, K^+ diffuses from inside to outside of the membrane removing positive(+) charge from the inner side of the membrane while building up positive (+) charge on outer surface of the membrane This results in overall excess of negativity on the inner side as compared to the positivity on the outerside This comes out to be in between -75 and -95 millivolts with an average of -85 millivolts Thus means that the interior of the axon (or nerve cell body) is about 85 mv negative with respect to the exterior in the resting stage of the nerve.

As this potential is dependent on diffusion of K^+, it is also called as *diffusion electrical potential*. There can be another type of electrical potential due to active transport of ions.

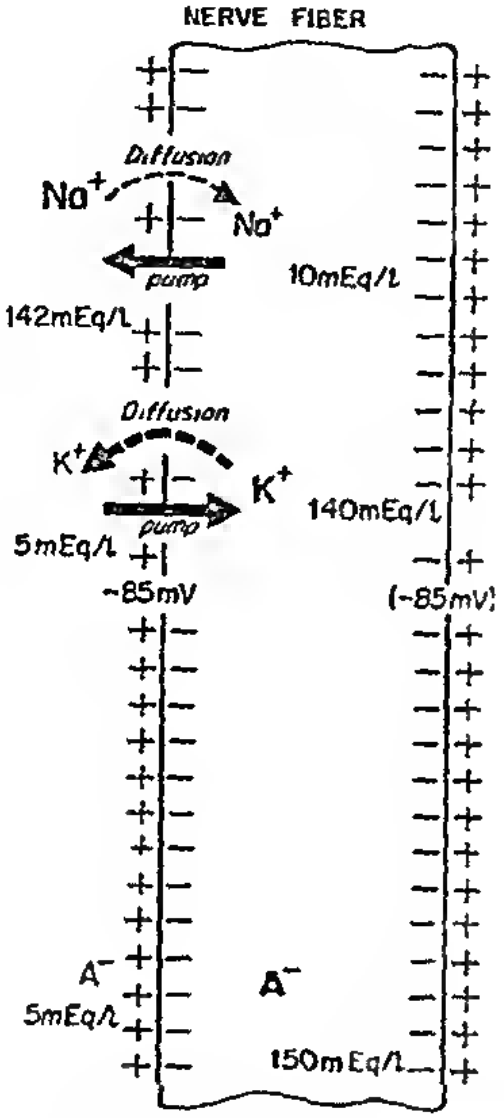


Fig. 39.6. Establishment of a membrane potential of −85 mv in the normal resting nerve fibre and development of concentration difference of Na^+, K^+ and A^- (the non-diffusable protein ions) between the two sides of the membrane. The dashed arrows represents diffusion and the solid arrows represent active transport (Pumps)

Calculation of the membrane potential: The resting membrane potential can be calculated by *Nernst equation*. When a concentration difference of ions across a membrane causes diffusion of ions through the membrane creating a membrane potential, the magnitude of the potential is determined by the difference in tendency of the ions to diffuse in one direction versus in the other direction. This is determined by the following formula:

$$E(mv) = \frac{RT}{\eta F} \times 23 \log_{10} \frac{[C_i]}{[C_o]}$$

where, E(mv) = Membrane potential in millivolts
R = Gas constant = 8 3 joules/°
T = Absolute temperature = 273°
n = Valency of the diffusible ions
F = Faraday = 96,500 Coulombs
C_i = Concentration of diffusible ions inside the cell
C_o = Concentration of diffusible ions outside the cell.

At a temperature of 38°C (body temperature), this equation results in

$$E(mv) = \frac{8\,3 \times (273+38)}{n \times 96{,}500} \times 2\,3 \log_{10} \frac{[C_i]}{[C_o]}$$

$$= \frac{61\,5}{n} \log_{10} \frac{[C_i]}{[C_o]}$$

As K^+ ions are the main diffusible ions and their concentration inside the cell is about 30 times as that outside the cell, so the electrical potential created by this concentration difference of K^+ ions will be

$$E = \frac{61\,5}{1} \log_{10} \frac{[30]}{[1]} = -90 \text{ mv (approximately)}$$

This shows that the calculated value for the K^+ diffusion potential (−90 mv) is normally slightly more than the actual measured value of the resting membrane potential (−85 mv) This is due to non-consideration of Na^+ and non-diffusible protein anions which also have a slight tendency to diffuse to the interior and exterior of the cell respectively Obviously, either of these effects would reduce the potential to slightly below that calculated for potassium diffusion alone

Action potential

So long the membrane of the nerve fibre remains completely undisturbed, the membrane potential remains about −85 mv This is said to be the resting membrane potential But when this membrane is disturbed by a stimulus (e.g., electrical, chemical, mechanical) which can suddenly increase the permeability of this membrane to Na^+ then a sequence of rapid changes in the resting membrane potential takes place. This lasts a minute fraction of a second and is followed immediately by return of the membrane potential to its resting value. This sequence of rapid changes in the electrical potential is said to be the action potential which is discussed below

It has been found that in the resting condition, the external surface of the membrane is positive with respect to its interior When this membrane is suddenly stimulated, the permeability of the membrane to Na^+ increases transiently lowering the relative permeability to K^+ and, therefore, many of the Na^+ rush to the interior of the fibre carrying sufficient positive charge to the interior to cause complete disappearance of the normal resting potential and usually enough charge to develop a positive (+) state inside the fibre instead of the normal negative (−) state. This positive state inside the nerve fibre is called as the reversal (from negative to positive) potential and this process is said to be the *depolarisation of membrane*

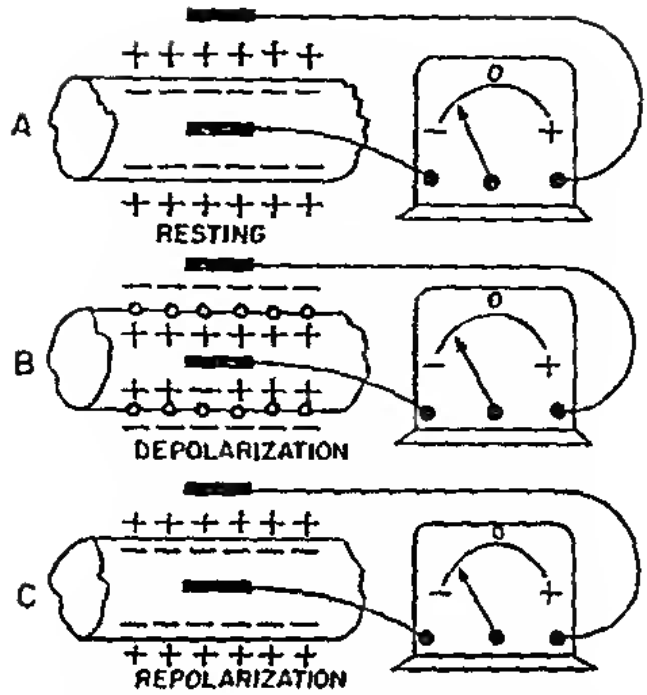


Fig 39 7 Sequential events during the action potential
A=The normal resting potential
B=Development of a reversal potential during deporalisation
C=Re-establishment of the normal resting potential during repolarisation

As the positive potential inside the nerve reaches a peak which is known as the peak potential or spike potential, the process of depolarisation stops and the membrane again becomes almost completely impermeable to Na^+ and relatively more permeable to K^+ so that the K^+ leaves the fibre faster than the Na^+ enters the fibre Hence, the reversal potential inside the fibre disappears and the normal resting membrane potential returns to the site of stimulation and the fibre once more becomes responsive to further stimulation This process of restoration of the resting membrane potential from the reversal potential is said to be the repolarisation of the membrane

Mechanism of action potential:

The exact mechanism responsible for action potential is not yet known However, one of the theories to explain it is as follows

It is postulated that under normal circumstances the *sodium "channels"* in the membrane are lined with Ca^{++} repelling the Na^+ and other cations. Hence, Ca^{++} resists the passage of Na^+ through these sodium channels Further, when the membrane is stimulated, these Ca^{++} are dislodged from their binding sites and some Na^+ begin to move inwards The inwards movement of the in rushing Na^+ theoretically dislodge more and more Ca^{++} from their ending site for which more Na^+ rush inward, and the process is continued till no Ca^{++} remain to resist the passage of Na^+ through Na^+ channels The diffusion of the Na^+ inside the membrane causes *reversal potential* with the development of positivity inside the membrane and negativity outside. This positivity inside the membrane reaching the peak of spike potential opposes further inflow of the positively charged Na^+ allowing the Ca^{++} to begin rebinding with the binding sites along the Na^+ channel and the highly concentrated K^+ begin moving outwards through the so-called *potassium "channels"*—probably separate from the sodium channels. As the Ca^{++} bind, Na^+ conductance decrease

which allows still more Ca^{++} to bind and thus another vicious cycle sets in which operates in the opposite direction and continues until the membrane becomes almost totally impermeable to Na^{+} once again and acquires the similar resting membrane potential.

Propagation of action potential:

The electrical stimulation of a nerve fibre initiates at the point of stimulation action potential which in turn excites the adjacent portion of the membrane resulting in the propagation of nerve impulse.

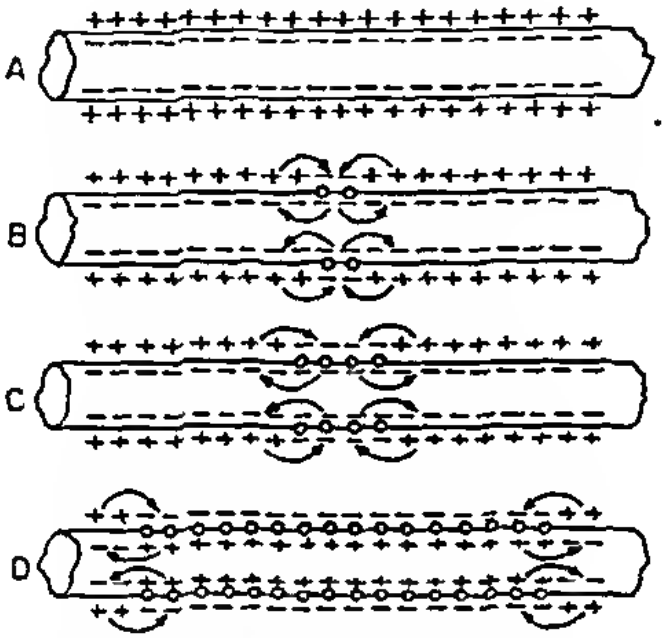


Fig. 39.8 Propagation of action potential in both directions along a conductive fibre.
A=Resting nerve fibre.
B=Same nerve fibre has been excited in its mid portion.
C and D=Depolarisation of successive portion of the same nerve fibre membrane.

The above mentioned figure (Fig. 39.8) shows that when a nerve fibre is stimulated at any point, a sort of current flows inwards through this point and outwards through a part of resting membrane ahead of this thus completing a circuit. In some unknown way, the current flowing through resting membrane now increases the membrane's permeability to sodium which immediately allows Na^{+} to diffuse inward through the membrane and thus the action potential is developed in this area also by the same mechanism as discussed earlier. The newly activated area causes local circuits of current flow still further along the membrane causing progressively more and more depolarisation. Thus, the depolarisation process propagates in both directions from the point of stimulation along the entire length of the nerve fibre. This transmission of the depolarisation process along a nerve or muscle fibre is called as the nerve or muscle impulse.

Within few moments the depolarisation is followed by repolarisation which also propagates in the same direction in that depolarisation had previously propagated as shown figure below:

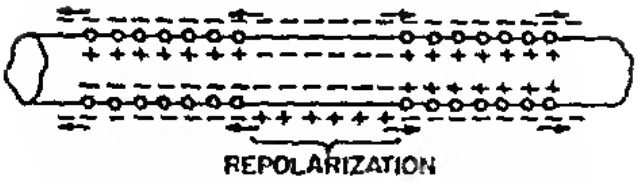


Fig. 59.9 Propagation of repolarisation in both directions along a conductive fibre.

Transmission of nerve impulse from one neurone to other:

The successive neurones are separted from one another by a narrow slit called as *synaptic cleft*; while the junction between one neurone and the next is termed as a *synapse*. Synapse consists of a presynaptic terminal and a part of perikaryon which are separated by a synaptic cleft having an average width of about 200Å.

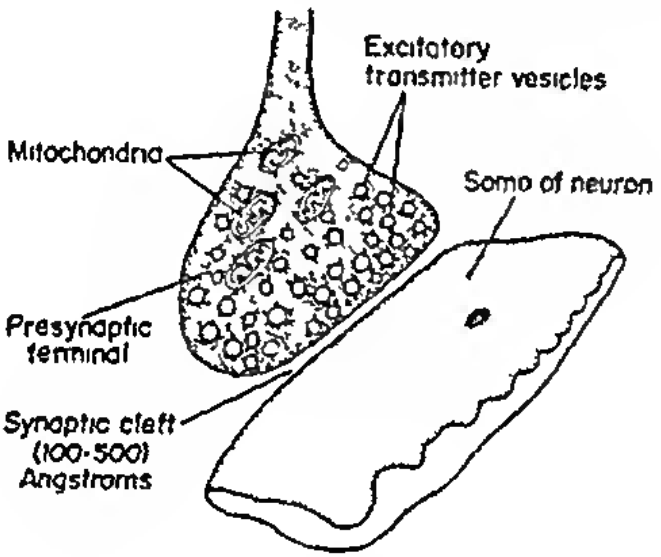


Fig 59.10. Physiologic anatomy of the synapse

The presynaptic terminal contains mitochondria and synaptic vesicles The synaptic vesicles contain chemical transmitter which may be excitatory and then the presynaptic terminal is termed as excitory presynaptic terminal and the parent neurone as excitory neurone or inhibitory to the next neurone and then the presynaptic terminal is called as inhibitory presynaptic terminal and the parent neurone as inhibitory neurone. The action potential reaching the presynaptic terminal causes these vesicles to release transmitter substance in the synaptic cleft. The transmitter immediately increases the permeability of sub-synaptic somal membrame either to the Na^+ when it is excitatory in nature or to K^+ when it is inhibitory in nature. When the permeability of Na^+ increases, Na^+ enters the interior of the perikaryon of the next neurone. Thus, it brings about a sequence of changes resulting in the action potential called as excitatory post synaptic potential (EPSP) and thus this neurone gets excited.

But if the permeability increases to K^+ due to release of inhibitory transmitter, K^+ moves out rapidly through the membrane because of excess of K^+ inside the cell leaving a greater degree of negativity inside the neurone. Thus state is known as *hyperpolarisation* and the potential so developed as Inhibitory Postsynaptic Potential

(IPSP) because this results in the inhibition of the next neurone or the effector cell It is also called as the chemical transmission of nerve impulses due to the involvement of chemical transmitters in the above process

Nature of the chemical transmitters.

Loewi in 1921 first discovered the process of chemical transmission. He applied the fluid from a perfused frog heart to a second heart and showed that when the vagus nerve supplying to the first heart was stimulated, the fluid from this heart also caused the typical vagal effects of depression of the second heart Similarly, the stimulation of sympathetic nerve supply to the first heart also caused the stimulation of second heart Loewi called the substance so liberated by vagus stimulation as 'vagusstoff' and that liberated by sympathetic stimulation as "acceleransstoff" Later on these substances had been shown to be acetylcholine and noradrenaline respectively

A substance to act as a transmitter must satisfy the following needs

1 Nerve stimulation must release the substance (the transmitter) from the nerve terminals

2 Drugs which antagonise the action of that substance at the post-synaptic membrane should also block the effects of the nerve stimulation transmitted by that very substance

3 Substances (drugs) which block the synthesis or storage of this substance should block the effects of nerve stimulation transmitted by this substance.

4 There must be mechanism for the destruction or removal of the proposed transmitter, e g , the enzyme acetyl cholinesterase is needed for the destruction of acetylcholine, so this enzyme must be present in the neurones whose transmitter is acetyl choline

5 It must be synthesized and stored within the neurones.

6 The action of the substance on the innervated organ if added from outside must be the same as the effects on stimulation of the nerves which release that substance.

7 Drugs which affect the destruction or removal of transmitter must also influence the response to nerve stimulation in an appropriate way

The transmitters which satisfy the above needs are acetylcholine, epinephrine, norepinephrine The possible transmitters are γ aminobutyric acid (GABA), serotonin, glutamic acid, glycine and dopamine

Cholinergic and Adrenergic nerve fibres:

Dale expressed that the nerve fibres which form and release acetylcholine as transmitter are termed as cholinergic fibres, while those which form and release noradrenaline are termed as adrenergic fibres Cholinergic nerve fibres are as follows

1 All the pre-ganglionic fibres of both parasympathetic as well as sympathetic systems

2 All the post ganglionic fibres of the para-sympathetic systems

3 Certain post ganglionic fibres of the sympathetic system e.g , nerve fibres to the sweat glands

4 The motor nerve fibres to skeletal muscles.

5 Some of the neurones of the central nervous system

6 Efferent fibres supplying to adrenal medulla

The adrenergic fibres are as follows

1 Most of the post ganglionic fibres of the sympathetic system

2 Some of the neurones of the central nervous system

Transmission of an impulse from the nerve to a skeletal muscle

Figure39.11A shows a junction between a motor nerve fibre and a skeletal muscle fibre which is said to be *neuromuscular junction.* A motor neurone together with the group of muscle fibres which it innervates is called a *motor unit.*

As the myelinated nerve fibre approaches to a skeletal muscle fibre, it loses its medullary sheath and branches at its terminal end into terminal ramifications. At the tips of these terminal ramifications of the nerve fibre are still other structure called as *sole feet* which make an extensive contact with a specialised part of the muscle fibre membrane to form the actual neuromuscular junction. The part of muscle fibre membrane lying in contact with a sole foot is called as *motor end plate* (Fig. 39.11B). It is thrown into several folds which increase the surface area at which the synaptic transmitter can act.

The large aggregates of the enzyme cholinesterase which is capable of destroying the synaptic transmitter (acetylcholine) exist around the rim of the motor end plate. The sole foot is separated from the synaptic gutter (motor end plate) by a gap known as synaptic cleft which remains filled with a gelatinous "ground substance" through which the extracellular fluid diffuses. The sole foot contains a number of small vesicles which store the synaptic transmitter synthesised probably by mitochondria which also supply the energy for synthetis

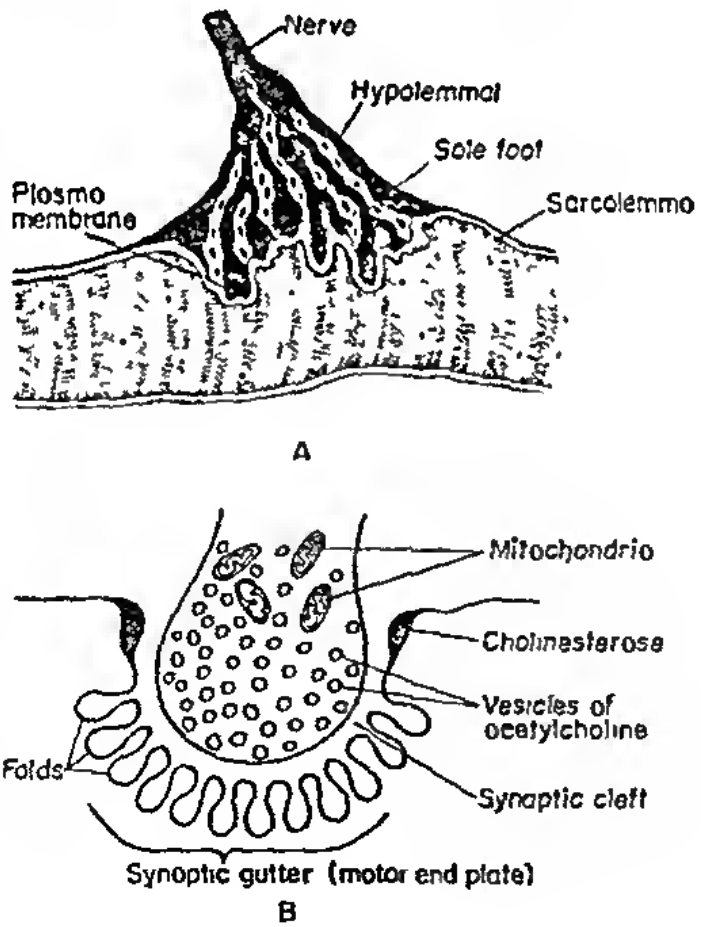


Fig. 39.11.—A. The neuromuscular junction.
B. Invagination of a sole foot into the membrane of the muscle fibre.

As a result of the discussion of the structural details of neuromuscular junction, the sequence of events that occur at the neuromuscular junction during the transmission of a nerve impulse from motor nerve fibre to a skeletal muscle fibre can be discussed as follows

1 Arrival of an impulse at motor nerve terminal

2 Rupture of synaptic vesicles containing acetylcholine by these calcium ion (Ca^{++}) through the membrane of the sole foot and thus release of acetylcholine into the synaptic cleft and its attachment to the end plate receptor (cholinergic receptors) Acetylcholine release is inhibited by magnesium ion (Mg^{++})

3 Movements of Ca^{++} (hypothetical) from the extracellular fluid into membrane of the sole foot

4 Propagation of the so produced end plate potential when it reaches a certain critical magnitude (threshold)

5 Increase in permeability of muscle membrane to Na^{+} allowing rapid influx of Na^{+} into the muscle fibre and thus production of a potential called the *local end-plate potential* which is entirely analogous to the excitatory post synaptic potential

6 Removal of acetylcholine from its receptor site within about two milliseconds partly by diffusion back into sole foot and partly by destruction by cholinesterase The sequence of these events is represented in the figure below

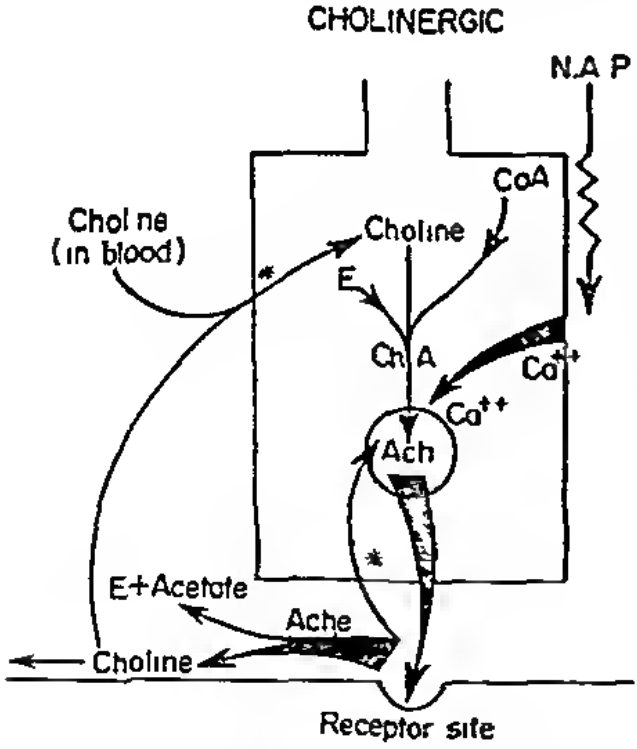


Fig 39 12 Diagrammatic representation of the processes involved in synthesis, release and disposal of acetylcholine at cholinergic nerve terminal and receptor site. Ach=Acetylcholine chA=choline acetylase Ache=Acetylcholinesterase, COA=Coenzyme A E Energy→Sites of active transport NAP=Nerve action potential

When the nerve fibre is stimulated at a rate greater than 150 times per second continuously for many minutes, the quantity of acetylcholine released with each impulse diminishes, so that impulses even fail to pass into the muscle fibre. This is referred to as the *fatigue* of the neuromuscular junction and is analogous to fatigue of a synapse However, under normal conditions, fatigue of the neuromuscular junction does never occur since it is rate that more than 150 impulses per second reach even the most active neuromuscular junction

CHAPTER 40

HISTORY AND ADVANCEMENT OF CLINICAL BIOCHEMISTRY

From the time of Hippocrates (C 460—C 375 B C) the crude examination of body fluids including urine had been done Thomas Willis, in the second half of the 17th century wrote a dissertation on urine and did the sweet taste to differentiate between the two types of diabetes

Boerhaave of Leyden (1688 1738), who had much influence on medicine, taught quite confidently that medical phenomena could be interpreted in terms of chemistry Physicians had long been interested in urinary calculi Richard Bright in 1836 showed the relation of albuminous urine with Kidney disease He did this by heating urine in a spoon and showed that cloudiness developed just before boiling

In nineteenth century, Henry Bence Jones whose name is associated with "protein" and J W L Thudichum became lecturer in chemical pathology at St Thomas' Hospital, London Alfred Garrod, just over 100 years ago, first did quantitative analytical methods applied to blood and at about this time, a number of well known qualitative tests for urine had been done By the end of the nineteenth century much of modern urine testing had already been done

At the commencement of the 20th century "Clinical Chemistry" really began to develop into the discipline which we know today Quantitative tests were carried out in regard to sugar and urea of urine and a series of qualitative tests for acetone, albumin, bile, and sugar were performed

Different techniques now began to appear, mainly in the United States, for quantitative estimations applied to blood In 1912, Folin and Denis described the phosphotungstic acid reagent for uric acid, in 1913, Bang described his micro-method for blood sugar, and Van den Bergh used Ehrlich's diazo-reagent for the determination of bilirubin In 1915, Maclean and Van Slyke published their iodometric method for chloride In the same year, the Liebermann-Burchard reaction was applied to the determination of cholesterol in blood. The newly introduced clinical application of insulin to the treatment of diabetes greatly stimulated urine and blood sugar estimations as well as those of electrolytes

The range of quantitative tests performed in the clinical chemistry laboratory increased remarkably during the years between the two world wars The B M R was also estimated as well as urea concentration tests, the phenol red excretion test, the fractional test meal, and the test for occult blood Pancreatic disease was studied by means of urinary diastase, fecal fat, and muscle fibres in the feces In relation to neurological disease, cerebrospinal fluid was fairly well investigated In 1930, there appeared Harrison's Chemical Methods in Clinical Medicine and Stewart and Dunlop's clinical chemistry in Practical Medicine A year later, the two volumes of Peters and Van Slyke were published. These three publications undoubtedly formed the foundation for the numerous text books on the subject in circulation today

In the late thirties in the United States and in the early forties in the United Kingdom, various types of photoelectric colorimeters had appeared resulting in the speeding up of routine clinical chemical work with accuracy Another great progress, in the middle and late forties, there was the appearance of the

flame photometer which enabled the estimation of sodium and potassium in the body fluids to be done quite quickly and very accurately This had helped the clinicians to treat the diseases such as diabetic coma

In 1938, Callow published the use of the Zimmerman reaction to determine 17—Ketosteroids This was the first of the steroid determinations applied for routine purposes Satisfactory methods had been developed for the estimation of catecholamines and their metabolites, particularly in urine The more recent development of radioimmunological assay and protein binding techniques has led to the estimation of the hormones themselves, both in blood and other biological fluids

Bodansky as well as King and Armstrong, in the early thirties, introduced their techniques for phosphatase estimation Specific inhibition techniques then led to the estimation of prostatic acid phosphatase which played a great part in the diagnosis and control of therapy of prostatic carcinoma The estimation of aminotransferases, lactate dehydrogenase and a number of other intracellular enzymes had been done. Isoenzyme determination had also been performed. Techniques also became available for the estimation of trace elements by absorption spectrophotometer which gave accurate results for routine purposes

Grabar and his associates in 1953 made a firm basis on Immunoelectrophoresis which gave detailed study on Para Proteinemias Individual serum proteins could be investigated also by other immunological techniques, including radioimmunoassay

Tswett in 1906 described absorption chromatography, Adams and Holmes in 1936 ion exchange chromatography, Martin and Synge in 1941 partition chromatography, Consden, Gordon and Martin in 1944 paper chromatography, Mottier, James and Martin in 1952 gas liquid chromatography All these techniques are in current use by clinical chemists and have greatly increased the range of substances which canbe investegated in relation to disease.

In the fifties and sixties, the improvement in the control of respiratory disorders, open heart surgery, and renal dialysis had been made by the use of the Astrup technique Hereditary metabolic disorders had been detected by microbiological methods

Since clinical chemistry had achieved the status of a recognized discipline in its own right national societies devoted to the subject were formed in various countries First formed in the Netherlands in 1947 and was soon followed by those in the United States of America and in the United Kingdom. Societies of clinical chemistry have now grown up all over the world The first journal, *Clinical Chemistry*, was published in 1955 by the United States This was soon followed by *Clinica Chemica Acta*, and now numerous journals have been published in many parts of the world In addition there are a good number of excellent books dealing with details of analytical methodology

Since the national societies had been formed it was realized that problems related to clinical chemistry should be considered at an international level. This has led to the formation of the clinical chemical section of the International Union of Pure and Applied Chemistry (IUPAC) and the International Federation of Clinical Chemistry (IFCC) These organizations have a number of joint committees Much of the account of the history of clinical biochemistry given in this chapter was orginally produced for the joint commission on Education of these two bodies

This discipline is, of course, advancing rapidly High resolution analysis is progressing speedily This includes pressure chromatography and techniques such as mass spectroscopy and electron spin resonance Sophisticated techniques of this kind will enable the clinical chemist to estimate relatively large numbers of metabolites in relatively s all amounts of blood, tissue fluid tissue culture, and biopsy samples Such development must certainly lead to greater understanding of disease processes as well as to more accurate diagnostic procedures and therapeutic control The normal range of these estimations should also be known for detecting diseases It is now becoming almost mandatory to abolish the old-fashioned concept of "normal range" and replace it by "reference values"

"NORMAL RANGE"

The so-called normal ranges were at first determined on specimens obtained from healthy medical students or laboratory staff Factors such as bedrest could effect certain important biochemical values

Whatever method is used to determine ranges, it soon becomes obvious that these values are dependent on a whole variety of factors including method of collection and handling of the sample for analysis, time of collection, seasonal changes, laboratory analytical method employed, laboratory accuracy, patient's age, sex, ethnic group, social class, diet, physiological factors such as pregnancy or environment, and so on There is also the effect of therapeutic agents on the analytical method employed as well as on the actual blood levels of certain constituents. Many of these variables can be standardized, and then it is possible to obtain so-called reference populations These must be defined in terms of the other factors, such as sex, age, ethnic group, social class, etc. It should be pointed out here that in this regard biochemical values resemble many other physical signs obtained at the bedside or by ancillary methods of investigation

It may also be mentioned that there is the effect of disease itself on biochemical values, which in themselves are not diagnostic of that disease For example, in renal disease, in addition to an increase of blood urea there is frequently an increase in blood urate There is a rough clinical correlation The clinician learns to associate certain blood urate levels with appropriate increases in blood urea If the level of the former is too high in relation to the latter, then the possibility of Gout is considered The latter disease can also be associated with an increase in the blood urea as well as of blood urate and a similar reasoning process must be employed

Under the above discussion, it indicates that, in relation to diagnosis, biochemical values are merely additional, and frequently very important, physical signs. Biochemical findings, as with most physical signs, have significance only in relation to the history of the patient's illness and the physician's findings, at the bedside as well as from ancillary methods of investigation

Maximum difficulty arises to obtain normal reference values in the elderly because of the presence of frequent diseases in them Therefore, it is difficult to define the "normal" state The meaningful values are just begun to obtain in pediatrics possibly because of difficulties of sample collection and emotional obstacles

It will be essential to standardize analytical methodology to obtain meaningful "reference intervals" in all groups of patients Necessity will come into force to set up reliable reference methods, by which other methods can be standardized

This is particularly the case in relation to enzyme determination. It is also vital to report laboratory findings in terms of standard units. For this purpose, it has already been decided to adopt *SI* Units.

SI UNITS

The conference Generele des Poids et Mesures, in 1960, confirmed the Systeme International d' Unites (SI), originally adopted in 1954. This system is now based on eight base units related to eigbt basie kinds of quantity.

The katal has been included in the following table 38.1. It should be pointed out that a number of authorities believe that the concept catalytic amount is not really viable and its unit would really be derived rather than hase. One could introduce the concept catalytic activity per liter (derived coherent unit) which would give a figure which would be the same as if the Katal were employed.

The mole and the katal have already been defined. In order to define the Kelvin, it is necessary to state that 273.16 K represents the temperature interval between the absolute zero and the triple point of water. Temperature is more Commonly expressed in terms of celsius temperature, which is the thermodynamic temperature minus 273.16 K, since the celsius temperature at the triple poiht water is 0.01 degree celsius (0.01°C). The former term "centrigrade" should no longer be used. The steam point celsius temperature is 100°C above the celsius triple point of water. Other units can be derived from the base units.

Table 40.1 : Base Units of SI system

Kind of Quantity	*SI Base Unit*	*Symbol for Unit*
Length	meter	m
Mass	kilomegram	kg.
Time	second	S
Electric current	ampere	A
Thermodynamic temperature ..	kelvin	K
Luminous intensity	candela	cd.
Amount of substance	mole	mol.
Katalytic amount	Katal	kat.

Derived Coherent Units:

These are the units constructed exclusively from base units. In this category, unit area (Unit length × unit length) or unit velocity (unit length divided by unit time) can also be included. The coherent unit of volume is the cubic meter. For the purpose of clinical biochemistry, the liter has been retained as the unit and redefined as exactly one-thousandth part of a cubic meter.

Derived Noncoherent Units:

These are the units constructed from base units and numerical factors, for example, milligram per deciliter In other words, the numerical factors are multiples or submultiples, which can be denoted by prefixes as shown in table 38 2

Table 40.2 Prefixes denoting decimal factors.

Factor	*Name*	*Symbol*
10^{12}	tera	T
10^{9}	giga	G
10^{6}	mega	M
10^{3}	kilo	K
10^{2}	hecto	h
10^{1}	deca	da
10^{-1}	deci	d
10^{-2}	centi	c
10^{-3}	milli	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p
10^{-15}	femto	f
10^{-18}	atto	a

Certain derived coherent units are used in clinical biochemistry The unit of force is the newton (N) and 1N=1 Kg m/s^2 The unit of energy is the Joule (J) and 1J=1 Nm 4 2 Kj corresponds to 1 Kcal (Kilocalorie), and the latter unit, which is the medical calorie, will be placed out The unit of power is the Watt (W) and 1W=1 J/S The electrical units are the volt (V), the farad (F) and the ohm (Ω) 1V=1 W/A, 1F=1 A/S/V, 1Ω=1V/A

Concentration can be expressed in two types of unit Mass concentration is the mass component divided by the volume of the mixture (kg /l, g/l, mg/l etc) Substance concentration is the amount of substance of the component divided by the volume of the mixture (mol/l, m mol/l etc.) The latter form of expression is preferable if the molecular weight of the substance is known It can also be used for formula units

Substance concentration has not been expressed in SI units in a number of chapter of this book since expressions of concentration in common clinical usage have been employed (mg/100 ml, mEq/liter etc) To convert these values into substance concentration in SI units (m mol/l, μ mol/l etc.), it is necessary to multiply by certain factors, some of which are shown in table 38 3 below

The multiplication factor is easily derived from the former ways of expressing mass concentration, since it equals $\frac{n}{VXMW}$, where n is the numerical value in a stated volume V (measured in liters) and MW is the molecular weight of the substance concerned When concentration has been expressed as mEq/l, it is merely necessary to divide by the valency of the component being determined

Certain standard abbreviations are also recommended as shown below :

Whole blood	..	B
Arterial blood	..	aB.
Venous blood	..	vB.
Day	..	d (Preferably 24 hr.)
Fasting measurement	..	fpt.
Feces	..	F
Night	..	n
Plasma	..	P
Serum	..	S
Spinal fluid	..	Sp.
Urine	..	U

Table 40.3 Multiplication factors for conversion to substance concentration in Plasma.

Substance	*Current concentration expression*	*Substance concentration*	*Multiplication factor for conversion to substance concentration*
Aminoacid N ..	mg/100 ml.	m mol/l	0.714
Ammonium	µg/100 ml.	µ mol/l	0.587
Ascorbic acid	mg/100 ml.	µ mol/l	56.8
Bilirubin	mg/100 ml.	µ mol/l	17.1
Calcium ion	mg/100 ml.	m mol/l	0.250
	mEq./l	m mol/l	0.5
Cholesterol	mg/100 mol.	m mol/l	0.0259
Copper	µg/100 ml.	µ mol/l	0.157
Creatinine	mg/100 ml.	µ mol/l	88.4
Glucose	mg/100 ml.	m mol/l	0.0555
Iron	µg/100 ml.	µ mol/l	0.179
Lead	µg/100 ml.	µ mol/l	0.0483
Magnesium	mg/100 ml.	m mol/l	0.411
	m Eq/l	m mol/l	0.5
Phosphate (Inorganic P) ..	mg/100 ml.	m mol/l	0.323
Protein-bound iodine ..	µg/100 ml.	m mol/l	78.8
Sodium ion	m Eq/l	m mol/l	1.0
Urate	mg/100 ml.	m mol/l	0.0595
Urea	mg/100 ml.	m mol/l	0.166

System	*Component*	*Kind of Quantity*	*Numberical value*	*Unit*
Serum	Calcium (Total)	Substance Concentration	2 5	*m mol/I*

A laboratory report of a measurement should ideally always indicate

(1) the system
(2) the component
(3) the kind of quantity
(4) the numerical value
(5) the unit

It is also doubtful whether actually the words "substance concentration" will be included in the average laboratory report

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CHAPTER 41

CANCER, ONCOGENES AND GROWTH FACTORS

INTRODUCTION

1 Cancer cells have the following characteristic properties

a They have diminished control of growth
b They invade local tissues
c They spread to other parts of the body

2 The main object is to explain in biochemical terms the uncontrolled growth of cancer cells and their capacity to invade and metastasize

3 The genes controlling growth and interactions with other normal cells are apparently abnormal in structure or regulation in cancer cells

4 Information regarding the control of cell growth (both normal and Pathologic) is limited and knowledge of specific genes involved in growth regulation is even very scanty Little is also known as yet about the biochemical basis of metastasis

5 Some types of cancer (e g , certain leukemias) can be regarded as examples of abnormal differentiation But little is known about the molecular basis of differentiation Many workers in this area think that further research on oncogenes and growth factors will provide information on the nature of the distrubed control of growth, of differentiation, and of cell—cell interaction exhibited by cancer cells

BIOMEDICAL IMPORTANCE

1 The second most common cause of death is cancer after cardiovascular disease

2 Humans of all ages are victims to cancer and a wide variety of organs are affected by this disease

3 Cancer increases with age, so that as people live longer, more will develop the disease

Biochemical Laboratory tests

Many cancers are associated with the abnormal production of enzymes, proteins, and hormones which can be measured in plasma or serum. These molecules are known as *tumor markers* Measurement of some tumor markers is helpful in managing some types of cancer The tumor markers are applied in diagnosis and management of cancer Three major conclusions are derived from the study of tumor markers

1 No single marker is useful for all types of cancer or for all patients with a given type of cancer

2 Markers are most often detected in advanced stages of cancer rather than early stages

3 The most successful use of markers is to monitor the responses to therapy and the detection of early recurrence

Table 41 1. Clinically useful tumor markers.

Marker	Associated Cancer
Carcinoembryonic antigen (CEA)	Colon, Lung, Breast, Pancreas
Alpha fetoprotein (AFP)	Liver, Germ Cell
Human chorionic gonadotropin (HCG)	Tropoblast, Germ Cell
Calcitonin (CT)	Thyroid (medullary carcinoma)
Prostatic acid phosphatase (PAP)	Prostate

CAUSES OF CANCER

The followings are the groups causing cancer.

1. Radiant energy.
2. Chemical compounds.
3. Viruses.

1. Radiant energy

(a) Ultraviolet rays, X-rays, and γ-rays damage DNA in several ways.

(b) Ultraviolet radiation may cause pyrimidine dimers to form.

(c) Apurinic or apyrimidinic sites may form by elimination of corresponding bases. Single—and double—strand breaks or cross-linking of strands may occur.

(d) The basic mechanism of carcinogenicity with radiant energy is to cause damage to DNA.

(e) *Free radicals* are formed in tissues by X-rays and γ-rays. The resultant OH, superoxide, and other radicals can interact with DNA and other macromolecules, leading to molecular damage and thereby probably contributing to carcinogenic effects of radiant energy.

2. Chemical Compounds

(a) It has been estimated that environmental factors, principally chemicals, can cause upto 80 per cent of human cancers.

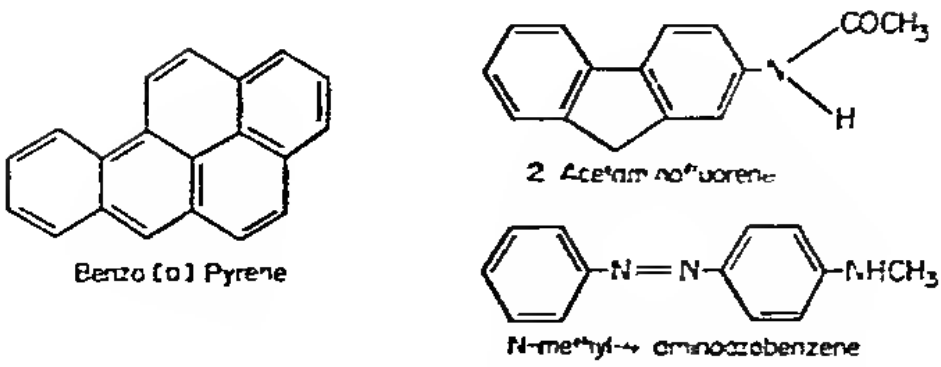
(b) Exposure to such compounds can occur because of a person's *occupation* (e.g., benzene, asbestos); *diet* (e.g., aflatoxin B_1 which is produced by the mold Aspergillus flavus and sometimes found as a contaminant of peanuts and other foodstuffs); *Life style* (e.g., cigarette smoking); or in other ways (e.g., certain therapeutic drugs can be carcinogenic).

(c) The carcinogenic substances may be both organic and inorganic molecules. The following table shows some chemical compounds which may cause cancer.

Table 41.2. Some Chemical Carcinogens

Class	Compound
Polycyclic aromatic hydrocarbons.	Benzo [a] Pyrene.
Aromatic amines.	2—Acetylaminofluorene, N-methyl-4-aminoazobenzene (MAB).
Nitrosamines.	Dimethylnitrosamine, diethylnitrosamine.
Various drugs.	Alkylating agents (e.g., Cyclophosphamide), diethylstilbesterol
Naturally occurring compounds.	Dactinomycin, aflatoxin B_1.
Inorganic compounds.	Arsenic, asbestos, beryllium, cadmium, chromium.

The followings are the structures of three important carcinogenic compounds.



Benzo [a] Pyrene

2 Acetyl aminofluorene

N-methyl-4-aminoazobenzene

Fig 41 1 Structures of three important experimentally used chemical carcinogens

Action

(a) The organic carcinogens such as nitrogen mustard and β-propiolactone interact directly with target molecules (direct carcinogens), but others require prior metabolism to become carcinogenic (*Procarcinogens*) The process by which one or more enzyme-catalyzed reactions convert procarcinogens to active carcinogens is called *metabolic activation* Any intermediate compound formed is *proximate carcinogens*, and the final compound that reacts with cellular components (e.g , DNA) is the *ultimate carcinogen*

Procarcinogen ⟶ Proximate carcinogen A ⟶ Proximate carcinogen B ⟶ Ultimate carcinogen

(b) The ultimate carcinogen is highly reactive and is usually *electrophile* (molecule deficient in electron) This readily attacks nucleophilic (electron-rich) group in DNA, RNA, and protein

Mono-oxygenases and Transferases

(a) Mono oxygenases and transferases cause the metabolism of Procarcinogens and other xenobiotics

(b) The heme—containing mono-oxygenases of the cytochrome P-450 type located in the endoplasmic reticulum are mainly responsible for the metabolic activation of procarcinogens

(c) The mono-oxygenases catalyze the hydroxylation of various procarcinogens and other xenobiotics using molecular oxygen as the source of oxygen and NADPH as a reducing source.

$$R-H+O_2+NADPH+H^+ \longrightarrow R-OH+H_2O+NADP^+$$

(d) At least 6 or many more such mono-oxygenases are present in the endoplasmic reticulum of human liver

(e) The specific mono oxygenase responsible for the metabolism of polycyclic aromatic hydrocarbons is named cytochrome P-448 or aromatic hydrocarbon hydroxylase The reactions catalized by these mono-oxygenases are called *phase* I *reactions of xenobiotic metabolism*

(f) In *phase* 2 *reactions of xenobiotic metabolism*, the hydroxylated xenobiotics are conjugated with various moieties (e g , Glucuronate, sulfate, acetate, glutathione) These reactions usually detoxify the reactivity of the compounds involved and make them ready for excretion, mainly in the urine

(g) In some cases, conjugation actually increases the biologic activity or chemical reactivity of a molecule. The enzymes catalyzing the above conjugation reactions are usually cytosolic in location, although some are also present

in the endoplasmic reticulum The various glutathione transferases use glutathione transferases use glutathione itself as the donor

Factors affecting enzymes metabolizing xenobiotics

The following factors affect the activities of the enzymes metabolizing xenobiotics

(i) The activities of these enzymes may differ substantially among species

(ii) Significant differences are found in enzyme activities among individuals, many of which are due to genetic factrors

(iii) The activities of the some of the enzymes vary according to age and sex

(iv) Intake of phenobarbital, PCBS, or certain hydrocarbons can also increase the activities of many enzymes by a process known as *enzyme induction* Hydrocarbon inhalation from cigarette smoking *during* pregnancy induces the activity of cytochrome P-448 in the placenta altering the amounts of certain metabolites of hydrocarbons to which the fetus is exposed

(v) The metabolites of certain drugs can inhibit the activities of xenobiotic-metabolizing enzymes

Mutagens

(a) Most of the chemical carcinogens are mutagens

(b) The use of bacteria is mutagenicity tests creates a problem that they do not contain the spectrum of mono-oxygenases found in higher animals

Initiation and promotion

(a) The stage of carcinogenesis on the skin of experimental mice caused by the application of benzo [a] pyrene is called initiation and this stage is rapid and irreversible It is supposed to involve an irreversible modification of *DNA* resulting in one or more mutations Benzo[a] pyrene is th is called an initiating agent

(b) The second stage of carcinogenesis, *resulting* from the application of croton oil, is called promotion and croton oil is therefore a *promoter* Promoters can cause initiation

(c) Most carcinogens can act as both initiating, and promoting agents

(d) A good number of compounds including phenobarbital and saccharin can act as promoters in different organs

(e) The active agent of croton oil is a mixture of phorbol esters The most active phorbol ester is 12—0—tetradecanoylphorbol—13—acetate (TPA) which has numerous effects

(f) Protein Kinase C can act as a receptor for TPA The enzyme being stimulated by interaction with TPA may result in the phosphorylation of a number of *membrane proteins resulting* in the effects on transport and other functions

Role of DNA

DNA is the premier target molecule in carcinogenesis which is being established by the following facts

(i) Cancer cells beget cancer cells, i e , the required changes responsible for cancer are transmitted from mother to daughter cells This is consistent with the behaviour of DNA

(ii) DNA is damaged by both irradiation and chemical carcinogens which are capable of causing mutations in DNA

(iii) Many tumor cells exhibit abnormal chromosomes

(iv) Transfection experiments show that purified DNA (oncogenes) from cancer cells can transform normal cells into cancer cells Epigenetic factors may also play a role in carcinogenesis

3. ONCOGENIC VIRUSES

(i) Oncogenic viruses contain either DNA or RNA as their genome

(ii) *Polyomavirus* and *SV 40 viruses* have played an important role in the development of current ideas about viral oncogenesis Both of them are small and their circular genomes code for only about 5—6 proteins Under certain circumstances, appropriate cells being infected with these viruses can result in malignant transformation Specific viral proteins are involved too

(iii) In case of SV 40, these proteins (often called antigens) are known as T and t, and in case of polyomavirus, they are known as T, mid-T, and t (T refers the first of these proteins detected in a tumor)

(iv) The T antigens are to bind tightly to DNA and cause alterations in gene expression These proteins show cooperative effects, suggesting that alteration of more than one reaction or process is required for transformation

(v) Transformation of certain animal cells are caused by some types of adenovirus

(vi) Epstein Barr virus is associated with Burkitt's Lymphoma and nasopharyngeal carcinoma in humans

(vii) Herpes Simplex virus is associated with cancer of the cervix, and hepatitis B virus is also associated with some cases of liver cancer in humans

TRANSFORMATION

The cultured cells undergo malignant transformation when they are infected with certain oncogenic viruses These changes affect cell shape, motility, growth, and a number of biochemical processes They reflect the conversion from the normal to the malignant state Acquisition by cells of the changes collectively known as transformation does not mean that such cells will display the same biologic properties as tumor cells in vivo, cells must yield tumors when injected into a suitable host animal

ONCOGENES

Oncogenes are genes capable of causing cancer These were first recognised as unique genes of tumor—causing viruses that are responsible for the process of transformation (viral oncogenes)

Oncogenes of Rous Sarcoma Virus

(i) The genome of this retrovirus contains four genes named gag, pol, env, and src

(ii) The gag gene codes for group specific antigens of the virus, pol for the reverse transcriptase that characterizes retroviruses, and env for certain glycoproteins of the viral envelope A *protein tyrosine kinase* was shown to be the product of src (i e , the sarcoma-causing gene) that is responsible for transformation

(iii) Certain *glycolytic enzymes* become target proteins for the src protein-tyrosine kinase This shows that transformed cells often show increases rates of glycolysis The product of src may also catalyze phosphorylation of phosphatidylinositol to phosphatidylinositol mono- and biphosphate

(vi) When phosphatidylinositol 4, 5—biphosphate is hydrolyzed by the action of phespholipase C, 2 second messengers are released inositol triphos

phate and diacylglycerol The first compound mediates release of Ca++ from intracellular sites of storage (e g , the endoplasmic reticulum)

(v) Diacylglycerol stimulates the activity of the plasma membrane bound protein kinase C which in turn phosphorylase a number of proteins, some of which may be components of iron pumps

(vi) Mild alkalinization of the cell brought about by activation of an Na^+/H^+ antiport system can play a role in stimulating mitosis

(vii) The product of src may, therefore, affect a large number of cellular processes by its ability to phosphorylate various target proteins and enzymes and by stimulating the pathway of synthesis of the polyphosphoinositides

Oncogenes of other Retroviruses

(i) About 20 oncogenes of other retroviruses have been identified Almost half of the products are protein kinases, mostly of the tyrosine type

(ii) Some of these encode protein kinases, the remainder encode various other proteins with interesting biologic activities

(iii) The product of the ras oncogene of murine sarcoma viruses binds GTP, has GTPase activity, and is related to the proteins that regulate the activity of the important plasma membrane enzyme, adenylate cyclase

Mechanism by which proto-oncogenes become Oncogenes

A Promoter insertion

(i) When the particular viruses infect cells, a DNA copy (cDNA) of their RNA genome is synthesized by reverse transcriptase, and the cDNA is integrated into the host genome The integrated double-stranded cDNA is called a *Provirus*

(ii) Following infection of chicken B lymphocytes by certain avian leukemia viruses, their proviruses become integrated near the myc gene The myc gene is activated by an upstream, adjacent viral long terminal repeat acting as a promoter, resulting in transcription of both the corresponding myc mRNA and translation of its product in such cells

B. Enhancer Insertions

(i) In certain cases, the provirus is inserted downstream from the myc gene or upstream from it but oriented in the reverse direction, the myc gene never becomes activitated Such activation cannot be due to promoter insertion

(ii) Enhancer sequences present in the long terminal repeat sequences of the retroviruses

(iii) The above two mechanisms – promoter and enhancer insertion – commonly operate in viral oncogenesis

C. Chromosomal Translocations

(i) Many tumor cells show chromosomal abnormalities Translocation is a type of chromosomal change seen in cancer cells

(ii) A piece of one chromosome being split off join to another chromosome and if the second chromosome donates material to the first, the translocation is said to be *reciprocal*

(iii) A number of tumor cells show characteristic translocations One important translocation is the philadelphia chromosome occurring in chronic granulocytic leukemia

(iv) *Burkitt's Lymphoma* is a fast growing cancer of human B Lymphocytes

(v) Synthesis of greatly increased amounts of the DNA–binding protein

coded for by the myc gene acts to "drive" or "force" the cell towards becoming malignant by an effect on the regulation of mitosis

D. Gene Amplification

(i) One method is shown in respect of gene amplification in tumors by administration of the anticancer drug methotrexate, an inhibitor of the enzyme dihydrofolate reductase. Tumor cells can become resistant to the action of this drug

(ii) Certain cellular oncogenes can also be amplified and are thus activated.

(iii) Increased amounts of the products of certain oncogenes produced by gene amplification may play a role in the progression of tumor cells to a more malignant state

E. Single-point mutation

(i) The product of murine retroviruses, a protein of MW 21000 is related to the G proteins that modulate the activity of adenylate cyclase and thus play a key role in cellular responses to many hormones and drugs

(ii) The lower activity of GTPase can result in chronic stimulation of the activity of adenylate cyclase which normally is diminished when GDP is formed from GTP. The resulting stimulation of the activity of adenylate cyclase can result in a number of effects on cellular metabolism exerted by the increased amount of cAMP affecting the activities of various cAMP-dependent protein kinases

General comments on Activation of Oncogenes

(i) Increased amounts of the product of an oncogene may be sufficient to push a cell towards becoming malignant.

(ii) The presence of a structurally abnormal key regulatory protein in a cell may also be sufficient to tip the scales toward cancer

(iii) Oncogenes have been isolated from only about 15 per cent of human tumors

(iv) Recent work has shown that activation of C-ras in rat mammary cancers induced by nitrosomethylurea was apparently due to a specific G→A transition type of mutation, demonstrating that oncogenes are probably involved in chemical carcinogenesis

(v) Further research is essential to examine the possible involvement of oncogenes in the phenomena of initiation, promotion, tumor progression, and metastasis

Mechanisms of Action of Oncogenes

(i) They may act on key intracellular pathways involved in growth control uncoupling them from the need for an exogenous stimulus

(ii) The products of oncogenes may also imitate the action of a polypeptide growth factor

(iii) The products may also imitate an occupied receptor for a growth factor

POLYPEPTIDE GROWTH FACTORS

(i) The growth factors affect many different types of cells, e.g., cells from the blood, nervous system, mesenchymal tissues, and epithelial tissues

(ii) They exert a mitogenic response on their target cells

(iii) Platelet-derived growth factor (PDGF) released from the alpha gra-

nules of platelets plays a role in normal wound healing Various growth factors play key roles in regulating differentiation of stem cells to form various types of mature hematopoietic cells Growth inhibitory factors also exist Thus, chronic exposure to increased amounts of a growth inhibitory factor can alter the balance of cellular growth

ENDOCRINE, PARACRINE, AND AUTOCRINE ACTIONS OF GROWTH FACTORS

Growth factors may act in the following ways

(i) Their effects may be endocrine, like hormones, they may be synthesized elsewhere in the body and pass in the circulation to their target cells

(ii) They may be synthesized in certain cells and secreted from them to affect neighbouring cells The cells that synthesize the growth factor are not themselves affected because they lack suitable receptors This mode of action is called *paracrine*

(iii) Some growth factors can affect the cells that synthesize them This third mode of action is called *autocrine*

BIOCHEMICAL MECHANISMS OF ACTION OF GROWTH FACTORS

(i) The growth factors like hormones must transmit a message across the plasma membrane to the interior of the cells The message ultimately affects one or more processes involved in mitosis

(ii) Most growth factors have high affinity protein receptors on the plasma membrane of target cells The genes for the receptors for epidermal growth factor (EGF) and insulin have been cloned

(iii) A number of receptors have been found to exhibit protein tyrosine kinase activities The kinase activity located in the cytoplasmic domains causes autophosphorylation of the receptor protein and also phosphorylates other target proteins The receptor-lig-and complexes are subjected to endocytosis in coated vesicles

(iv) Phospholipase C is stimulated following exposure of cells to PDGF resulting in hydrolysis of phosphatidylinositol 4, 5-biphosphate to form inositol triphosphate and diacylglycerol These two second messengers can effect intra cellular release of Ca^{++} and stimulation of the activity of protein kinase C respectively, thus affecting a larger number of cellular reactions

(v) The hydrolysis of diacylglycerol of phospholipase A_2, liberating arachidonic acid, can also result in the production of prostaglandins and leukotrienes which themselves may exert many biologic activities

(vi) Exposure of cells to PDGF can cause rapid activation of certain cellular proto-oncogenes

(vii) Gene activation is involved in the action of most growth factors

PROGESSION OF TUMORS

(i) A cell once becomes a tumor cell, tendency for malignancy is increased This is signified by increasing rates of growth, and increasing tendency to invade and metastasize

(ii) Cells having faster rates of growth will have a selective advantage

(iii) The biochemical affairs of highly malignant cells may be very different from that of normal cells Some changes are secondary to rapid growth rates and others are due to chromosomal instability

(iv) The fast-growing cells tend to increase the anabolic processes involved in growth (e.g., DNA and RNA synthesis), cut down on catabolic functions (e.g., catabolism of pyrimidines), and dispense with the differentiated functions shown by their normal ancestors. In fact, they are mostly concentrated upon growth. They also show biochemical changes that reflect altered gene regulation, such as the synthesis of certain fetal proteins.

METASTASIS

Metastasis is a complex process to analyze in humans. It is the spread of cancer cells from a primary site of origin to other tissues where they grow as secondary tumors, it is the major problem presented by the disease. Biochemical knowledge regarding this is quite restricted.

Much attention has been put to the comparisons of the biochemistry of the surfaces of normal and malignant cells.

At present, metastasis has been studied by considerable research work on developing suitable animal model systems. Much studies are being carried out to identify the possible roles of certain proteases (e.g., type 4 collagenase) and of certain glycoproteins and glycosphingolipids of the cell surface in the process of metastasis. For instance, it may be possible that changes in the oligosaccharide chains of cell glycoproteins may be effective in permitting metastasis to occur. The biochemical mechanisms involved in metastasis may provide a basis for the rational development of more effective anticancer therapies.

APPENDIX

CHAPTER – 1
BIOPHYSICS

Write the correct answer number of the followings :

1 The pH of gastric juice of infants
(a) 2 0
(b) 4 0
(c) 4 5
(d) 5 0

2. The pH of water is 7·0 at the temperature in centigrade
(a) 22.
(b) 30
(c) 37
(d) 40

3 The pH of blood is 7 4 when the ratio between [$NaHCo_3$] and [H_2Co_3] is
(a) 10 f
(b) 20 1
(c) 25 1
(d) 30 1

4 The difference in pH between arterial and venous blood is rarely more than
(a) 0 02
(b) 0 03
(c) 0 04
(d) 0 06

5 The pH of buffer is determined by $pH = PKa + Log \frac{[Salt]}{[acid]}$ which is also known as the equation of
(a) Henderson-Joules
(b) Henderson-Smith
(c) Henderson-Harris
(d) Henderson-Hasselbalch

6 The chief buffering system in the blood
(a) K_2HPO_4 and KH_2PO_4
(b) B Protein and H. Protein
(c) $NaHCo_3$ and H_2Co_3
(d) B Hemoglobin and H. Hemoglobin

Answers

1) d.

2) a.

3) b

4) c

5) d.

6) c

7 During severe muscular exercise when the blood lactic acid content rises over 100 mg. per 100 mL the p^H of blood
(a) Slightly increases.
(b) Highly increases.
(c) Slightly decreases.
(d) Markedly decreases.

8 In case of non homogeneous solution, diffusion is expressed as $\frac{ds}{dt} = DA\frac{dc}{dx}$ by
(a) Fick s Law
(b) Peterson's Law
(c) Faraday s Law
(d) Dick s Law

9 The average p^H of urine is
(a) 5 6
(b) 6 0
(c) 6 4
(d) 6 8

10 The osmotic pressure of a solution increases with the rise in
(a) Temperature
(b) Cold.
(c) Humidity
(d) Rancidity

11 The osmotic pressure of a solution relating to solute molecules depends on the
(a) Size
(b) Shape
(c) Number
(d) Volume.

12. If a cell is immersed in a concentrated solution, it follows the phenomenon
(a) Turgor
(b) Plasmolysis
(c) Hemolysis.
(d) Paralysis

13 Osmosis is opposite to
(a) Effusion.
(b) Affusion
(c) Confusion.
(d) Diffusion.

Answers

7) d.

8) a.

9) b

10) a.

11) c.

12) b

13) d.

14. The intracellular fluid of red cells and the red cell membrane in 0 92% NaCl solution maintains a relation
    (a) Hypertonic
    (b) Hypotonic
    (c) Isotonic.
    (d) None of the above

15 The Purgative action of Epsom($MgSO_4$)salt follows
    (a) Osmosis
    (b) Diffusion
    (c) Adsorption
    (d) Absorption

16. Hemolysis is caused by the dilution of RBC by
    (a) Diffusion
    (b) Osmosis
    (c) Effusion
    (d) Imbibation

17 The surface tension of a solution in decreased by
    (a) Calcium sulphate.
    (b) Barium sulphate
    (c) Magnesium phosphate
    (d) Potassium permanganate

18 The surface tension of a solution is lowered by
    (a) Ammonia
    (b) Sodium hydroxide
    (c) Potassium hydroxide.
    (d) Aluminium hydroxide

19 The surface tension of a solution is increased by
    (a) Bile salts
    (b) Bile acids
    (c) Concentrated sulphuric acid
    (d) Acetic acid.

20 Lipids and proteins which are both effective in lowering surface ten sion are found concentrated in the cell wall following the principle of
    (a) Johnson John
    (b) Gibbs Thomson
    (c) Peterson Pollen
    (d) None of the above

Answers.

14) c

15) a

16) b

17) d.

18) a

19) c

20) b

21 Adsorption decreases with the rise in
(a) Cold
(b) Humidity
(c) Dryness.
(d) Temperature

22. The process of adsorption is applied in the purification of
(a) Enzymes
(b) Hormones.
(c) Vitamins.
(d) Coenzymes

23 The following is the hydrotropic substance
(a) Hydrochloric acid.
(b) Nitric acid.
(c) Hippuric acid.
(d) Salicylic acid.

24 Bile salts make emulsification with fat for the action of
(a) Amylase
(b) Lipase.
(c) Pepsin.
(d) Trypsin.

25 The viscosity of a liquid increases due to the presence of
(a) Suspended particles.
(b) Soluble particles
(c) Small sized particles.
(d) None of the above.

26. The size of each colloidal particle in nm
(a) 4 to 40
(b) 6 to 60
(c) 8 to 80
(d) 10 to 100

27 The serum colloidal protein particles may be precipitated by th addition of large amount of
(a) Calcium sulphate.
(b) Ammonium sulphate
(c) Barium sulphate
(d) Magnesium sulphate.

28 Some of the calcium phosphate of blood is held in colloidal suspension by the protective action of
(a) Lipids.
(b) Carbohydrate
(c) Proteins.
(d) Minerals

29 Water is not expelled by squeezing in
(a) Imbibition
(b) Precipitation
(c) Combination
(d) Dilution

30 In simple diffusion $\frac{ds}{dt} = PA\ (C_0 - C_1)$ can be expressed by the modification of
(a) Rontgent s Law
(b) Fabry s Law
(c) Pollinger s Law
(d) Fick's Law

31 Fatty acids can be transported into and out of mitochondria through
(a) Active transport
(b) Facilitated transfer
(c) Non facilitated transfer
(d) None of the above

32 The absorption of intact protein from the gut in the foetal and newborn animals takes place by
(a) Pinocytosis
(b) Passive diffusion
(c) Simple diffusion
(d) Active transport

33 Thyroid function is determined by the use of the isotopes
(a) Na^{24}
(b) K^{42}
(c) Ca^{45}
(d) I^{131}

34 Pernicious anemia is diagnosed by the radio-active substance
(a) CL^{36}
(b) P^{32}
(c) Co^{60}
(d) Fe^{59}

Answers

28) c
29) a
30) d
31) b
32) a
33) d
34) c

Answers

35 The protein biosynthesis has been studied by using
(a) N^{15}
(b) Ca^{45}
(c) Na^{24}
(d) Au^{198}

35) a

Fill up the blanks of the followings :

1 Solution of mixed indicators having a number of colour changes over a wide range of pH are called _____ indicators — 1) Universal.

2. The logarithm of the reciprocal of hydrogen ion concentration is said to be _____ — 2) pH

3 Temperature can not alter the pH of a solution of _____ — 3) 0 1 N HCl

4 The mixture of weak acids and their salts of strong bases are said to be _____ — 4) Buffers.

5 The spreading of solute molecules throughout the water molecules is called _____ — 5) Diffusion

6 Diffusion takes place against _____ — 6) Gravity

7 The most selective artificial membrane is _____ — 7) $Cu_2Fe(CN)_6$

8 Soaps have _____ osmotic pressure — 8) Lower

9 If a cell is kept in a hypotonic solution, the cell is called _____ — 9) Turgid.

10 The force with which the surface molecules are held together is called _____ — 10) Surface tension.

11 Surface tension is involved in the process of _____ — 11) Digestion.

12. The process by which water-insoluble substances are made water soluble by hydrotropic substances is called _____ — 12) Hydrotropy

13 The resistance experienced by one layer of a liquid in moving over another is called _____ — 13) Viscosity

14 The sparingly soluble cholesterol and the calcium salt of bilirubin is kept in colloidal solution by the protective colloids _____ — 14) Bile Salts.

15 The osmotic pressure of serum protein is about _____ — 15) 30 mm. Hg.

16 The process of separation of crystalloids from colloids by diffusion through a membrane by osmotic force is called _____ — 16) Dialysis.

17 Dialysis is applied in medicine in the _____ — 17) Artificial Kidney

18 An emulsoid may be changed into a suspensoid by _____ — 18) Dehydration

19 The cell membrane consists of small water filled pores of radius about _____ nm. — 19) 0 4

		Answers
20	The atoms having the same atomic number but different atomic weights are said to be _____	20) Isotopes.
21	Absorption of fat is studied by using _____	21) I^{131} oleic acid
22.	The life span of RBC has been determined by tagging _____ to RBC	22) Cr^{51}
23	The absorption, mobilization and transport of iron have been studied by the use of _____	23) Fe^{59}
24	Plasma volume is measured by using _____ labelled serum albumin	24) I^{131}
25	The formation of antibody in the reticulo-endothelial system has been studied by _____	25) P^{32}
26.	_____ is the end product of Au^{198} after emiting β and γ- rays	26) Hg^{198}

Indicate "True" or "False" of the followings :	Answers
1 The pH range of solutions is 0 to 14 only	1) True
2 The buffer acts almost as if it is absorbing the added free hydrogen or hydroxyl ions	2) True
3 The buffering system of lymph cerebrospinal fluid etc are similar to that of blood	3) True
4 In cases of gas, diffusion is slow	4) False
5 *Absorption from gastrointestinal tract does not follow the principles of osmosis.	5) False
6 Adsorption is a surface phenomenon	6) True
7 Heat is not given off in all adsorpuon	7) False
8 Adsorption decreases with the rise in temperature	8) True
9 Surface adsorption helps to combine enzymes with substrates to give reaction product	9) True
10 Phospholipids are hydrotropic substances	10) False
11 The unit of Viscosity is the Poise"	11) True
12. Brownian movement is quite regular	12) False
13 In the body equilibrium may be established owing to the removal of excreted ions in other reactions.	13) False
14 Emulsoids are easily precipitated by salts.	14) False
15 Formaldehyde can remove water from the gel	15) True
16 The mitochondrial membrane is permeable to citrate	16) True
17 The transport of most ions occur more slowly than the non electrolytes.	17) True
18 Active transport is involved in the absorption of lactose from the small intestine	18) False
19 Mineral metabolism is studied by S^{35}	19) False
20 The half life of I^{131} is 8 04 days.	20) True

Match the followings .

Solution	*pH*	*Answer*
(a) Saliva	(i) 7 1	(a) (ii)
(b) Milk.	(ii) 6 8	(b) .. (i)
(c) Tears	(iii) 12 0	(c) (iv)
(d) Pancreatic Juice	(iv) 7·2	(d) .. (v)
(e) Gastric Juice (adult)	(v) 8 0	(e) .. (vi)
(f) N/100 NaOH	(vi) 1 4	(f) .. (iii)

CHAPTER – 2
CARBOHYDRATES

Write the correct answer number of the following :

1 The heptose ketose sugar formed as a result of chemical reaction in HMP shunt.
(a) Glucoheptose
(b) Galactoheptose
(c) Sedoheptulose
(d) Mannoheptose

2. The general formula for polysacchande
(a) $(C_6 H_{10} O_5)\ \eta$
(b) $(C_6 H_{12} 0_6)\ \eta$
(c) $(C_6 H_{12} 0_5)\ \eta$
(d) $(C_5 H_{10} O_5)\ \eta$

3 The number of isomers of glucose.
(a) 4
(b) 8
(c) 12
(d) 16

4 The epimers of glucose
(a) Fructose
(b) Galactose
(c) Ribose
(d) Deoxyribose

5 Human heart muscle contains
(a) D – Arabinose
(b) D – Ribose
(c) D – Lyxose
(d) D – Xylose

6 The intermediate in hexose monophosphate shunt.
(a) D – Ribulose
(b) D – Arabinose
(c) D – Xylose
(d) D – Lyxose

Answers

1) c

2) a

3) d

4) b

5) c

6) a.

7 Honey contains the hydrolytic Product of
(a) Lactose
(b) Maltose.
(c) Inulin.
(d) Starch

8 On boiling Benedict s solution is not reduced by
(a) Sucrose
(b) Lactose
(c) Maltose
(d) Fructose.

9 Glycosides are found in many
(a) Vitamins
(b) Drugs.
(c) Minerals
(d) Nucleoproteins

10 Erythromycin contains
(a) Diethyl amino sugars
(b) Trimethyl amino sugars
(c) Diethylamino sugars
(d) Triethyl amino sugars

11 Galactose on oxidation with concentrated HNO_3, produces
(a) Gluconic acid
(b) Saccharic acid.
(c) Saccharolactone
(d) Mucic acid.

12. The distinguishing test between monosaccharides and disaccharides.
(a) Bial s test
(b) Seliwanoff's test.
(c) Barfoed s test
(d) Hydrolysis test.

13 Barfoed s solution is not reduced by
(a) Glucose
(b) Mannose
(c) Sucrose
(d) Ribose

14 Cellulose is made up of the molecules of
(a) α Glucose

Answers

7) c

8) a.

9) b

10) a

11) d.

12) c

13) c

14) b

(b) β Glucose
(c) Both of the above
(d) None of the above

15 Iodine solution produces no colour with
(a) Cellulose
(b) Starch
(c) Dextrin
(d) Glycogen

16 Glycogen structure includes a branch in between α glucose units
(a) 4 – 10
(b) 6 – 12
(c) 8 – 14
(d) 12 – 18

17 Amylose contains glucose units
(a) 100 – 200
(b) 200 – 300
(c) 300 – 400
(d) 500 – 600

18 Each branch of amylopectin is at an interval of glucose units
(a) 14 – 20
(b) 24 – 30
(c) 34 – 40
(d) 44 – 50

19 N acetylneuraminic acid is an example of
(a) Sialic acid
(b) Mucic acid
(c) Glucuronic acid.
(d) Hippuric acid.

20 The molecular weight of hyaluronic acid in millions ranges from
(a) 1 – 2
(b) 1 – 4
(c) 1 – 6
(d) 1 – 8

21 In place of glucuronic acid chondroitin sulphate B contains
(a) Gluconic acid.
(b) Gulonic acid

Answers

15) a
16) d
17) c
18) b
19) a
20) b
21) c

(c) Iduronic acid.
(d) Sulphonic acid.

22. Heparin has a molecular weight of about
(a) 14 000
(b) 15 000
(c) 16 000
(d) 17 000

23 Blood group substances consist of
(a) Lactose.
(b) Maltose.
(c) Fucose.
(d) Mucose.

24 The component of cartilage and cornea is
(a) Keratosulphate
(b) Chondroitin sulphate
(c) Cadmium sulphate
(d) Antimony sulphate

25 Benedict's test is less likely to give weakly positive results with concentrated urine due to the action of
(a) Urea.
(b) Uric acid.
(c) Ammonium salts.
(d) Phosphates.

26. Salivary amylase is activated by
(a) Na^+
(b) K^+
(c) HCO^-3
(d) CL.

27 Active transport of sugar is depressed by the agent
(a) Oxaloacetate.
(b) Fumarate.
(c) Malonate.
(d) Succinate.

28. The absorption of glucose is interferred by the deficiency of
(a) Vitamin A
(b) Thiamine.

Answers

22) d.
23) c.
24) a.
25) b.
26) d.
27) c.
28) b.

(c) Magnesium sulphate
(d) Ferrous sulphate.

Answers

29 Glucose absorption may be decreased in
(a) Oedema.
(b) Nephritis.
(c) Rickets.
(d) Osteomelitis

29) a

30 Glycogen synthetase activity is depressed by
(a) Glucose
(b) Insulin
(c) Cyclic AMP
(d) Fructokinase

30) c

31 Glucose is removed from the blood following a meal by
(a) Hexokinase
(b) Glucokinase
(c) Both of the above
(d) None of the above

3f) b

32. The branching enzyme acts on the glycogen when the glycogen chain has been lengthened to between glucose units
(a) 1 and 6
(b) 2 and 7
(c) 3 and 9
(d) 6 and 11

32) d

33. Cyclic AMP is formed from ATP by the enzyme adenylate cyclase which is activated by the hormone.
(a) Insulin
(b) Epinephrine.
(c) Testosterone
(d) Progesterone

33) b

34 The synthesis of adenylate cyclase is increased by
(a) Thyroid hormones
(b) Growth hormones
(c) ACTH
(d) FSH

34) a

35 UDPG is essential for the synthesis of
(a) Lactose

35) a

(b) Maltose.
(c) Sucrose.
(d) Starch.

36 Phosphorylase a in muscle is a tetramer containing 4 mol. of
(a) ATP
(b) NAD^+
(c) Pyridoxal phosphate
(c) CoA

37 Inactive muscle phosphorylase b is activated by active protein kinase which is being stimulated by
(a) ATP
(b) NAD^+
(c) Cyclic GMP
(d) Cyclic AMP

38 Hexokinase has a high affinity for glucose than
(a) Fructokinase.
(b) Galactokinase
(c) Glucokinase
(d) All of the above

39 Fructose–1 6– diphosphate is converted to Fructose–6– phosphate by the enzyme Fructose–1 6– disphosphatase which is stimulated by
(a) Glucagon.
(b) Insulin.
(c) ACTH
(d) None of the above.

40 Dihydroxyacetone phosphate and Glyceraldehyde–3–phosphate are interconverted by
(a) Triose isomerase
(b) Phosphotriose isomerase
(c) Diphosphotriose isomerase.
(d) Dihydroxyacetone phosphorylase.

41 In the liver Glyceraldehyde–3–phosphate is converted to
(a) Glycol.
(b) Formaldehyde
(c) Formic acid.
(d) Glycerol.

Answers

36) c.
37) d.
38) c.
39) a
40) b
41) d

42. Phosphoglycerate kinase responsible for the conversion of 1,3-diphosphoglycerate to 3—phosphoglycerate is inhibited by
    (a) Arsenate
    (b) Fumarate
    (c) Citrate.
    (d) Cyanate.

43. The reduced lipoate is reoxidized by
    (a) NAD^+
    (b) $NADP^+$
    (c) FAD^+
    (d) FMN

44 The approximate number of mols. of Pyruvate dehydrogenase in Pyruvate dehydrogenase complex
    (a) 19
    (b) 29
    (c) 39
    (d) 49

45 Pyruvate is accumulated by the dietary deficiency of vitamin
    (a) B_6
    (b) Folic acid
    (c) B_{12}
    (d) Thiamine.

46 Citrate is converted to isocitrate by aconitase which contains
    (a) Ca^{++}
    (b) Fe^{++}
    (c) Zn^{++}
    (d) Mg^{++}

47 The reaction succinyl-CoA to succinate requires
    (a) CDP
    (b) ADP
    (c) GDP
    (d) $NADP^+$

48 The carrier of the citric acid cycle
    (a) Succinate
    (b) Fumarate
    (c) Malate
    (d) Oxaloacetate

Answers

42) a

43) c

44) b

45) d.

46) b

47) c

48) d

49 Glucose-6-phosphatase is absent from
(a) Adipose tissue
(b) Intestine
(c) Kidney
(d) Heart

50 6-Phosphogluconate is converted to 3-keto-6-phosphogluconate in presence of coenzyme
(a) FAD^+
(b) NAD^+
(c) $NADP^+$
(d) ATP

51 UDPG is oxidized to UDP glucuronic acid by UDP dehydrogenase in presence of
(a) FAD^+
(b) NAD^+
(c) $NADP^+$
(d) ADP

52. Fructokinase is present in
(a) Intestine
(b) Adipose tissue
(c) Heart
(d) Brain.

53 Fructose 1 phosphate is splitted into glyceraldehyde and dihydroxyacetone phosphate by the enzyme
(a) Enolase
(b) Aldolase A
(c) Aldolase A & B
(d) Aldolase B

54 Galactose is phosphorylated by galactokinase to form
(a) Galactose 6-phosphate
(b) Galactose 1 6-diphosphate
(c) Galactose 1 phosphate
(d) All of the above

55 In galactosemic individual UDP Galactose is formed by epimerization from
(a) Glucose
(b) UDP Glucose
(c) CDP Glucose
(d) ITP Glucose

Answers
49) a
50) c
51) b
52) a
53) —
53) d
54) c
55) b

Fill up the blanks of the followings :	*Answers*
1 2 6 monosaccharide units are produced on hydrolysis of _____	1) Oligosaccharides
2 Mucopolysaccharides are _____	2) Heteropolysaccharides
3 Cis trans isomerism occurs in compounds with _____ bonds	3) Double
4 When equal amounts of dextrorotatory and levorotatory isomers are present in a mixture the mixture is said to be _____	4) Racemic
5 In the body epimerization takes place by the enzyme _____	5) Epimerase
6 Sugars forming five membered rings are called _____	6) Furanoses
7 A method for synthesis of monosaccharides was first proposed by _____	7) Kiliani
8 Galactose is not _____ by yeast	8) Fermented
9 The compounds formed by the condensation reaction between a sugar and the hydroxyl group of a aglycone which may or may not be another sugar are called _____	9) Glycosides
10 The inhibitor of the $Na^+ - K^+ - ATPase$ of cell membranes is _____	10) Quabain
11 Sugars containing an amino group are called _____	11) Aminosugars
12 3-amino D ribose is contained in _____	12) Carbomycin
13 Glucose when heated with conc HI is converted into _____	13) Iodohexane
14 Galactose on reduction yields _____	14) Dulcitol
15 Fructose on reduction produces _____ and _____	15) Mannitol & Sorbitol
16 Glucose on treatment with strong mineral acids produces _____	16 Levulinic acid
17 Galactose forms a different osazone owing to _____ in the structure	17 Carbon 4
18 Maltose can exist in _____ or _____ forms	18 α or β
19 Sucrose solution is _____ rotatory but after hydrolysis with dil HCl it shows _____ rotation	19) Dextro Laevo
20 Sucrose is used to be referred to as _____	20) Invert sugar
21 The glycogen content of _____ is more than that of _____	21) Liver Muscle
22. The molecular weight of glycogen ranges from _____ to _____	22) 10^5 , 10^8

		Answers
23	Starch is formed by _____ chain	23) Alpha-glucosidic
24	Each turn of the helix of amylose contains _____ units	24) Six glucose
25	The molecular weight of amylopectin is about _____	25) 5,00 000
26	The amylopectin chains have at least _____ branches	26) 80
27	Dextrins are formed by the partial hydrolysis of _____ by an enzyme _____	27) Starch, Salivary amylase
28	Achrodextrin gives no colour with _____	28) Iodine
29	Inulin is a _____	29) Fructosan
30	Mucosubstances have _____ functions	30) Protective
31	The hyaluronidase present in hyaluronic acid increases the rate of _____	31) Diffusion
32.	The richest source of the hyaluronidase in mammals is the _____	32) Testis
33	Chondroitin sulphates may regulate the flow and _____ of _____ round the cells	33) Concentration, Cations
34	Heparin is used in _____ as an _____	34) Medicine, anticoagulant.
35	The strong _____ of _____ solution can polymerize small quantities of glucose when vigorously boiled	35) Alkali, Fehling's
36	In the stomach some of the sucrose present in the food are hydrolysed by the action of _____	36) HCl
37	The action of _____ ceases due to high _____ in the stomach	37) Amylase, acidity
38	The pancreatic amylase converts starch and glycogen into a mixture of _____ and _____	38) Maltose, Isomaltose
39	Cellulose is not digested in human G I T due to the absence of _____	39) Cellulase.
40	_____ and _____ are absorbed at a faster rate than fructose	40) Galactose, Glucose
41	The free energy required for the active transport is obtained from the hydrolysis of _____ linked to a sodium pump which expels _____ from the cell	41) ATP, Na^+
42	The hydrolysis of lactose proceeds at half the rate for _____	42) Sucrose
43	The patient with _____ damage excrete more disaccharides	43) Intestinal
44	Carbohydrate after absorption into the portal blood passes to the _____ circulation through the _____	44) Systemic; Liver.

		Answers
45	There is synthesis of glucose by the _____ and _____ from non-carbohydrate sources.	45) Liver, Kidney
46	Glucose is converted to _____ when _____ storage is exceeded	46) Fatty acids, Glycogen
47	The process of synthesis of glycogen from _____ in the liver and muscle is called _____	47) Glucose, Glycogenesis
48	The process of breakdown of _____ to glucose in the liver and pyruvate and lactate in the muscle is said to be _____	48) Glycogen, Glycogenolysis
49	The process of breakdown of glucose and _____ to pyruvate and lactate is called _____	49) Glycogen Glycolysis
50	The method of formation of glucose and glycogen from _____ sources is said to be _____	50) Non-carbohydrate, Gluconeogenesis
51	Glucose 1 phosphate reacts with UTP to form _____ catalyzed by the enzyme _____.	51) UDPG, UDPG Pyrophosphorylase
52.	Glucose is converted to Glucose-6-phosphate by the enzyme glucokinase being stimulated by _____ with the help of the coenzyme _____	52) Insulin ATP
53	In muscle glycogen synthetase exists in two forms _____ and _____	53) Synthetase D, Synthetase I
54	Glycogen synthetase is stimulated by _____ and _____	54) Insulin, Glucose.
55	Cyclic AMP is destroyed by _____	55) Phosphodiesterase
56	In liver _____ increases the activity of cyclic AMP	56) Insuline
57	The debranching enzyme causes the hydrolytic splitting of _____ linkages of _____	57) 1 6 ; Glycogen
58.	Glucose-6-Phosphate is converted to glucose in the liver by the enzyme _____ with the release of _____	58) Glucose-6-phosphatase Phosphate
59	In liver, phosphorylase exists in both _____ and _____ form	59) Active, Inactive
60	In muscle, phosphorylase is present in two forms _____ and _____	60) Phosphorylase a, Phosphorylase b
61	Conversion of phosphorylase b to _____ signifies the mechanism for increasing _____	61) Phosphorylase a, Glycogenolysis
62	A muscle when contracts under anaerobic condition _____ and _____ become the principal end product	62) Pyruvate Lactate
63	Pyruvate and Lactate disappear if the muscle contracts under _____	63) Aerobic condition
64	When _____ is in short supply, _____ is reoxidized by being coupled to the reduction of pyruvate to lactate	64) Oxygen NADH

		Answers
65	The enzyme _____ splits fructose—1, 6-di phosphate into two _____ phosphates	65) Aldolase; Triose.
66	2 phosphoglycerate is catalyzed by ——— to produce phosphoenolpyruvate in presence of _____	66) Enolase, Mg^{++}
67	Pyruvate is reduced to lactate by _____ in presence of _____	67) Lactate dehydrogenase Reduced NAD
68	Glyceraldehyde 3-phosphate dehydrogenase responsible for the conversion of _____ to 1 3-diphosphoglycerate is inhibited by _____	68) Glyceraldehyde-3-phos phate, Iodoacetate.
69	_____ inhibits enolase involved in the con version of 2 phosphoglycerate to _____.	69) Fluoride; Phosphoeno-lpyruvate.
70	The reduced NAD enters the _____ chain producing _____ ATP	70) Respiratory, 3
71	Pyruvate dehydrogenase is inhibited by _____	71) Arsenite
72.	The pyruvate dehydrogenase complex consists of about _____ mol. of pyruvate dehydroge-nase	72) 29
73	Kreb s cycle consists of a series of reactions in mitochondria which catabolyzes the oxidation of _____ to _____ and H_2O in aerobic condi tion	73) Acetyl-CoA CO_2
74	α Ketoglutarate dehydrogenase is inhibited by _____ and causes _____ to accumulate.	74) Arsenite, α Ketoglutarate.
75	Gluconeogenesis is a reversal of _____.	75) Glycolysis.
76	Propionyl-CoA undergoes _____ fixation to form D methylmalonyl -CoA with the help of the coenzyme _____	76) CO_2, Biotin
77	The conversion of muscle _____ to glucose in liver and its re-entry into muscle is called _____	77) Lactate, Cori cycle.
78	Transketolase takes the help of the coenzyme _____ and the cofactor _____.	78) TPP, Mg^{++}
79	The deficiency of _____ in erythrocytes is associated with a tendency to _____ by primaquine.	79) Glucose-6-phosphate dehydrogenase Haemolysis
80	Ascorbic acid cannot be synthesized in _____.	80) Man
81	The Km for fructokinase in _____ is very _____.	81) Liver; Low
82.	Galactosemia is an _____ metabolic _____.	82) Inherited, Disease
83	Glycogenosis Type I occurs due to the deficien cy of _____ for which _____ cannot be broken down	83) Glucose-6-phosphatase, Glycogen
84	Type VII glycogenosis is a disease due to the deficiency of _____.	84) Phosphofructokinase

		Answers
85	Lactosuria occurs in women during the period of _____ and appears more frequently in the _____	85) Lactation, Afternoon
86	Insulin is destroyed by _____ and also by _____	86) Insulinase, Peptidase
87	Thyroxine accelerates hepatic _____ with the rise in blood _____	87) Glycogenolysis, Sugar
88	Glucagon stimulates gluconeogenesis in ____	88) Liver
89	The renal tubular reabsorption capacity is at the rate of _____ mg/_____	89) 350, minute
90	The venous blood sugar level when exceeds 160-180 mg% is called _____ for glucose	90) Renal threshold
91	Glucocorticoids are the _____ to insulin	91) Antagonists
92	In juvenile diabetes the _____ of pancreas are exhausted	92) β-cells
93	Normal urine contains _____ of glucose which cannot be detected by _____ test	93) Traces Benedict's
94	Glycosuria results in the use of ether due to hypersecretion of _____	94) Epinephrine
95	In renal glycosuria carbohydrate utilization is _____	95) Normal
96	GTT gives the assessment of the degree of severity of _____	96) Diabetes Mellitus
97	HMP shunt is closely associated with _____ due to the supply of reduced _____	97) Lipogenesis, NADP
98	In mild diabetes the blood sugar returns to the fasting level after _____ hours	98) 4
99	_____ and _____ are present in the urine of a person suffering from diabetic coma	99) Ketone bodies, Glucose
100	In diabetes mellitus _____ occurs due to increased blood _____ level	100) Glycosuria glucose

Indicate "True" or "False" of the following :

		Answers
1.	Cis-trans isomerism occurs in compounds with double bonds	1) True.
2.	When the OH group around the carbon atom adjacent to the terminal primary alcohol carbon is on the right the sugar is a member of L-series.	2) False
3	The separation of optically active isomers from a racemic mixture is called resolution	3) True
4	Isomers formed as a result of interchange of the —OH and —H on carbon atoms 2, 3 and 4 of glucose are known as epimers	4) True
5	Ribose and Deoxyribose are important constituents of many coenzymes	5) True

		Answers
6	The aglycone may be —COOH containing substances	6) False.
7	The amino sugars are formed by the replacement of hydroxyl group attached to carbon atom 3 of the sugar by an amino group.	7) False.
8.	Mannosamine is an important constituent of mucoprotein.	8) True.
9	Glucose on reduction yields mannitol.	9) False.
10	Gluconic acid on heating produces lactones.	10) True.
11	The osazone is formed by phenylhydrazine only	11) False
12.	In maltose molecule there is one β—1, 2 glucosidic linkage.	12) False.
13.	Sucrose is also said to be "invert sugar"	13) True.
14	Each glycogen molecule contains 5000 to 10000 glucose molecule.	14) True.
15	Starch is a fructosan.	15) False.
16.	In amylopectin each branch is at an interval of 24-30 glucose units.	16) True.
17	Inulin produces colour with iodine.	17) False.
18.	Hyaluronic acid does not occur in the skin.	18) False.
19	Chondroitin sulphates have a marked capacity to hold water	19) False.
20	Galactose and fucose are also present in blood group substances.	20) True.
21	Disaccharides are absorbed and used in the body	21) False
22.	Fructose is actively transported.	22) False
23	The absorption of glucose is retarded in hypothyroidism.	23) True.
24	Disaccharidurla results in the absence of disaccharidase.	24) True.
25	Glucuronic acid is not involved in detoxication reactions.	25) False.
26.	Cyclic AMP depresses phosphorylase activity	26) False.
27	Thyroid hormones may decrease the synthesis of adenylate cyclase.	27) False.
28	PGE, at low concentration stimulates cGMP accumulation.	28) False.
29	Conversion of phosphorylase b to phosphorylase a signifies the mechanism for increasing glycogenolysis.	29) True.
30	Muscle phosphorylase is affected by glucagon.	30) False.
31	Glucose-6-phosphate is not an important compound in the metabolic pathway	31) False.
32.	Aldolase requires a coenzyme for its activity	32) False
33	Glycolysis in erythrocytes even under aerobic condition forms lactate.	33) True.
34	Insulin stimulates phosphofructokinase.	34) True.
35	S — acetyl lipoate reacts with coenzyme A in presence of dihydrolipoyl transacetylase.	35) True.
36	Fluoride inhibits pyruvate dehydrogenase	36) False.
37	Citrate synthetase forms citrate in the presence of the coenzyme CoA.	37) False.
38.	Under anaerobic conditions, one molecule of glucose produces 8 ATP	38) False.

		Answers
39	One molecule of acetyl CoA in TCA cycle for complete oxidation forms 12 ATP	39) True
40	Succinate dehydrogenase takes the help of the coenzyme NAD^+	40) False
41	Pyruvate is converted to oxaloacetate by pyruvate decarboxylase	41) False
42.	The citric acid cycle is *amphibolic in nature.*	42) True
43	The enzymes of the TCA cycle are located at the cytosol	43) False
44	Oxaloacetate inhibits succinate dehydrogenase	44) True
45	In mammals, the liver and the muscles are the principal organs for gluconeogenesis	45) False
46	The glycolytic activity is low when there is active gluconeogenesis.	46) True
47	Dihydroxy acetone phosphate is also converted to glucose in the liver	47) True
48	Acetyl-CoA is permeable to pass through the mitochondrial membrane	48) False
49	The glucogenic amino acids are converted to the intermediates of TCA cycle.	49) True
50	CO_2 formed in HMP shunt is utilized for the synthesis of fatty acids and purine bases etc	50) True
51	The reduced NADP produced in HMP shunt is required for ketone bodies formation	51) False
52	In essential pentosuria large quantities of L-xylulose appear in the urine	52) True

Match the following :

		Answers
(i) Fructokinase.	(a) occurs due to the deficiency of branching enzyme in the liver.	(i) .....(j)
(ii) Hexokinase	(b) are the stimulators of insulin release.	(ii) .....(l)
(iii) Galactose.	(c) occurs due to the deficiency of lysosomal enzyme.	(iii) .....(h)
(iv) Galactosemia.	(d) occurs due to the lack of galactose-1-phosphate uridyl transferase in the liver and red blood cells	(iv) ..... (d)
(v) Von Gierke's disease.	(e) has no clinical significance except the wrong idea for glycosuria.	(v) .....(g)
(vi) Pompe's disease.	(f) accelerates the conversion of glucose to fat.	(vi) .....(c)
(vii) Amylopectinosis.	(g) children tend to develop hypoglycemia.	(vii) .....(a)
(viii) Pentosuria.	(h) is also required as a constituent of glycolipids and mucoproteins.	(viii) .....(e)
(ix) Insulin.	(i) has less affinity for fructose.	(ix) .....(f)
(x) Sulfonylureas.	(j) the activity is not affected by fasting or by insulin.	(x) .....(b)
(xi) Adrenocortical hormones.	(k) muscle glycogen does not serve directly as a source of glucose.	(xi) .....(r)
(xii) Epinephrine.	(l) the symptoms are sweating, headache, semiconsciousness.	(xii) .....(p)
(xiii) In the liver.	(m) Normal blood sugar but sugar is present in urine.	(xiii) .....(n)
(xiv) During hypoglycemia.	(n) galactose and fructose are converted to glucose.	(xiv) .....(k)
(xv) Administration of diabenese.	(o) the symptoms are dehydration, abdominal pain and vomiting.	(xv) .....(q)
(xvi) In hypoglycemic coma	(p) accelerates glycogenolysis in the liver and muscles.	(xvi) .....(i)
(xvii) In diabetic coma.	(q) in moderate diabetes mellitus.	(xvii) .....(o)
(xviii) Emotional glycosuria.	(r) stimulates gluconeogenesis.	(xviii) .....(s)
(xix) In renal glycosuria.	(s) occurs in worried athletes and candidates for competitive examinations.	(xix) .....(m)

				Answers	
(xx)	Phlorrhizin	(t)	is incapable of synthesizing ribose	(xx)	(v)
(xxi)	Skeletal muscle	(u)	is said to be the common metabolic pathway	(xxi)	(t)
(xxii)	Malate	(v)	poisons the kidney and prevents reabsorption of glucose from the tubules	(xxii)	(w)
(xxiii)	TCA cycle	(w)	is readily diffused from mitochondria	(xxiii)	(u)
(xxiv)	Adenylate cyclase	(x)	depresses active transport of glucose	(xxiv)	(z)
(xxv)	A mobile carrier	(y)	is needed for *transport of glucose*	(xxv)	(y)
(xxvi)	Cyanide	(z)	occurs in cell membrane	(xxvi)	(x)

CHAPTER – 3
LIPIDS

Write the correct answer number of the followings :

Answers

1 Fats are solids at
(a) 10°c
(b) 20°c.
(c) 30°c
(d) 40°c.

1) b.

2. Esters of fatty acids with higher alcohols other than glycerol are side to be.
(a) Waxes.
(b) Fats.
(c) Both of the above.
(d) None of the above.

2) a.

3 The combination of an aminoalcohol, fatty acid and sialic acid form
(a) Phospholipids.
(b) Sulpholipids.
(c) Glycolipids.
(d) Aminolipids.

3) c.

4 Hydrolysis of fat by alkali is called
(a) Saponification number
(b) Saponification.
(c) Both of the above.
(d) None of the above.

4) b.

5 The number of millilitres of 0 1 N KOH required to neutralize the insoluble fatty acids from 5 grams of fat is called
(a) Acid number
(b) Acetyl number
(c) Halogenation.
(d) Polenske number

5) d.

6 The rate of fatty acid oxidation is increased by
(a) Phospholipids.
(b) Glycolipids.
(c) Amino lipids.
(d) All of the above

6) a.

7 Cardiolipin found in mitochondria is formed from
(a) Lipositol
(b) Phosphatidyl ethanolamine
(c) Phosphatidyl glycerol
(d) None of the above

8 Lecithin contains a nitrogenous base named as
(a) Ethanolamine.
(b) Choline.
(c) *Inositol*
(d) All of the above

9 Lecithins contain an unsaturated fatty acid at position
(a) α–
(b) α– and β–
(c) β–
(d) None of the above

10 Lecithins are soluble in ordinary fat solvents except
(a) Benzene.
(b) Ethyl alcohol.
(c) Methyl alcohol
(d) Acetone.

11 When lecithins exposed to air become
(a) Black.
(b) Brown
(c) Red
(d) Yellow

12 Lecithins combine with protein to form
(a) Phosphoprotein.
(b) Mucoprotein
(c) Lipoprotein
(d) Glycoprotein

13 Phosphatidyl inositol is found in
(a) Cabbages
(b) Soyabeans
(c) Cauliflowers.
(d) Apples

Answers

7) c

8) b

9) c

10) d

11) b

12) c

13) b

14. Instead of ester link plasmalogens possess an ether link in position
    (a) α–.
    (b) β–.
    (c) γ-.
    (d) None of the above.

15. The alkyl radical in plasmalogen is an alcohol.
    (a) Saturated.
    (b) Unsaturated.
    (c) Both of the above.
    (d) None of the above.

16. The concentrations of sphingomyelins are increased in
    (a) Gaucher's disease.
    (b) Fabry's disease.
    (c) Fabrile disease.
    (d) Niemann-Pick disease.

17. Sphingomyelins contain a complex amino alcohol named as
    (a) Serine.
    (b) Lycolecithin.
    (c) Sphingosine.
    (d) Glycol.

18. The types of sphingomyelins are
    (a) 2.
    (b) 3.
    (c) 4.
    (d) 5.

19. Glycolipids contain an amino alcohol
    (a) Sphingosine.
    (b) Iso-sphingosine.
    (c) Both of the above.
    (d) None of the above.

20. Cerebrosides may also be classified as
    (a) Sphingolipids.
    (b) Sulpholipids.
    (c) Aminolipids.
    (d) Glycolipids.

Answers

14) a.

15) b.

16) d.

17) c.

18) b.

19) c.

20) a.

Answers

21 Kerasin contains
(a) Nervonic acid.
(b) Hydroxynervonic acid
(c) Cerebronic acid.
(d) Lignoceric acid

21) d

22. Oxynervon contains
(a) Nervonic acid.
(b) Hydroxynervonic acid
(c) Lignoceric acid
(d) Hydroxylignoceric acid

22) b

23 Gaucher's disease is characterized specially by the increase in
(a) Lignoceric acid.
(b) Nervonic acid
(c) Cerebronic acid.
(d) Hydroxynervonic acid

23) c.

24 Gangliosides are the glycolipids occurring in
(a) Liver
(b) Brain.
(c) Kidney
(d) Muscle

24) b

25 Some gangliosides also contain
(a) Sphingosine
(b) Dihydrosphingosine.
(c) Both of the above
(d) None of the above.

25) b

26 Most of the gangliosides contain sialic acid upto the molecule number
(a) 1
(b) 2
(c) 3
(d) 4

26) c

27 The percentage of triacylglycerol of lipoproteins
(a) 30
(b) 35
(c) 40
(d) 45

27) d

28 Cholesterol and cholesteryl esters in per cent present in lipoproteins
(a) 15
(b) 20
(c) 25
(d) 28

29 Lipoprotein present in cell membrane is by nature
(a) Hydropholic.
(b) Hydrophillic.
(c) Both of the above.
(d) None of the above.

30 The density of lipoproteins increases as the protein content
(a) Rises.
(b) Decreases.
(c) Highly decreases
(d) Slightly and promptly decreases.

31 Lipoproteins may be identified more accurately by means of
(a) Electrophoresis.
(b) Centrifugation
(c) Immuno-electrophoresis.
(d) Ultra centrifugation

32. Very low density lipoproteins are also known as
(a) β–Lipoproteins.
(b) Pre β–lipoproteins.
(c) α–lipoproteins
(d) None of the above

33 The protein moiety of lipoprotein is known as
(a) Apoprotein
(b) Pre-protein
(c) Post protein.
(d) Pseudoprotein

34 Apoprotein constitutes chylomicrons in per cent.
(a) 0 4
(b) 0 6
(c) 0 8
(d) 1 0

Answers

28) a.

29) b

30) a.

31) c

32) b

33) a

34) d.

35 The β lipoprotein fraction increases in severe
(a) Diabetes Mellitus
(b) Uremia
(c) Nephritis.
(d) Muscular dystrophy

Answers
35) a

36 Sulpholipids have been isolated from
(a) Heart
(b) Liver
(c) Brain
(d) Intestine

36) c

37 Δ^9 indicates a double bond between carbon atoms of the fatty acids
(a) 8 and 9
(b) 9 and 10
(c) 9 and 11
(d) 9 and 12

37) b

38. The number of carbon atoms in decanoic acid present in butter
(a) 6
(b) 8
(c) 10
(d) 12

38) c

39 Lignoceric acid present in peanut oil contains carbon atoms
(a) 18
(b) 20
(c) 22
(d) 24

39) d

40 Arachidonic acid contains the number of double bonds
(a) 2
(b) 3
(c) 4
(d) 5

40) c

41 The prostaglandins are synthesized from
(a) Arachidonic acid
(b) Oleic acid
(c) Linoleic acid
(d) Linolenic acid

41) a

42 Chaulmoogric acid was used many years ago in the treatment of
(a) Bronchitis

42) b

(b) Leprosy
(c) Nephritis
(d) Oedema.

43 The iodine number of essential fatty acids of vegetable oils
(a) High
(b) Very high
(c) Very low
(d) Low

44 The essential fatty acids retard
(a) Atherosclerosis.
(b) Diabetes Mellitus
(c) Nephritis
(d) Oedema.

45 The shape of arachidonic acid
(a) L
(b) M
(c) U
(d) V

46 Waxes contain higher alcohols named as
(a) Methyl
(b) Ethyl
(c) Phytyl
(d) Cetyl

47 The example of cardiac glycosides
(a) Digitonin
(b) Stropanthin
(c) Lycopyll
(d) Digitalis

48 The reduction product of cholesterol by bacteria in the intestine occurs in feces
(a) Ergosterol
(b) Demosterol.
(c) Coprosterol.
(d) Lanosterol

49 Lieberman Burchard reaction in performed to detect
(a) Cholesterol

Answers

43) d.

44) a.

45) c.

46) d

47) b

48) c

49) a

(b) Glycerol
(c) Fatty acid
(d) Vitamin D

50 Lipase present in the stomach cannot hydrolyze fats owing to
(a) Alkalinity — 50) c
(b) Acidity
(c) High acidity
(d) Neutrality

51 Before the action of lipase the fat is emulsified by
(a) Lipoproteins — 51) b
(b) Phospholipids.
(c) Ergosterols.
(d) Digitonin

52 The free glycerol of the total amount of triacylglycerol in the intestinal lumen is present in per cent about.
(a) 16 — 52) d
(b) 18
(c) 20
(d) 22

53 The great majority of absorbed fat appears in the form of
(a) HDL
(b) Chylomicrons — 53) b
(c) VLDL.
(d) LDL.

54 More metabolic water is available on oxidation of
(a) Fatty acids — 54) a
(b) Glycerol
(c) Both of the above
(d) None of the above

55 Fatty acids are oxidized by
(a) α– — 55) d
(b) β–
(c) ω–
(d) All of the above

56 The fatty acids containing even number and odd number of carbon atoms as well as the unsaturated fatty acids are oxidized by

Answers

(a) α– oxidation
(b) β– oxidation
(c) ω– oxidation
(d) All of the above

56) b

57 Long chain fatty acids are first activated to acyl-CoA in the
(a) Cytosol
(b) Mitochondria
(c) Microsomes
(d) Lysosomes

57) a

58 Activation of lower fatty acids occur within the
(a) Cytosol
(b) Mitochondria.
(c) Ribosomes
(d) Microsome

58) b

59 Long chain acyl-CoA penetrates mitochondria in the presence of
(a) Palmitate
(b) Carnitine
(c) Sorbitol
(d) DNP

59) b

60 Acyl-CoA dehydrogenase converts Acyl-CoA to α, β-unsaturated acyl-CoA in presence of the coenzyme
(a) NAD^+
(b) $NADP^+$
(c) ATP
(d) FAD^+

60) d

61 For the activation of long chain fatty acids the enzyme thiokinase requires the cofactor
(a) Mg^{++}
(b) Ca^{++}
(c) Mn^{++}
(d) K^+

61) a

62 ω– oxidation takes place by the hydroxylase in microsomes involving
(a) Cytochrome b
(b) Cytochrome c
(c) Cytochrome P–450
(d) Cytochrome a_3

62) c

63 Carboxylation of acetyl-CoA to malonyl CoA takes place in presence of
(a) FAD^+
(b) Biotin
(c) NAD^+
(d) $NADP^+$

64 Malonyl-CoA reacts with the central
(a) – SH group
(b) $-NH_2$ group
(c) – COOH group
(d) $-CH_2OH$ group

65 Fatty acid synthesis takes place in presence of the coenzyme
(a) NAD^+
(b) Reduced NAD
(c) $NADP^+$
(d) Reduced NADP

66 Fatty acids are activated to acyl CoA by the enzyme thiokinase using
(a) NAD^+
(b) $NADP^+$
(c) CoA
(d) FAD^+

67 Phospholipase A_1 attacks the ester bond of phospholipids in position
(a) 1
(b) 2
(c) 3
(d) All of the above

68 Phospholipase C release, 1, 2-diacylglycerol and a phosphoryl base attacking the ester bond in position
(a) 1
(b) 2
(c) 3
(d) 4

69 Phospholipids help the oxidation of
(a) Glycerol
(b) Fatty acids
(c) Glycerophosphates
(d) None of the above

Answers

63) b

64) a

65) d

66) c

67) a

68) c

69) b

70 The desaturation and chain elongation system of polyunsaturated fatty acids are greatly diminished in the absence of
(a) Insulin
(b) Glucagon
(c) Epinephrine
(d) Thyroxine

71 Prostaglandins are liberated in the circulation by the stimulation of
(a) Anterior pituitary glands
(b) Posterior pituitary gland
(c) Adrenal gland
(d) Thyroid gland

72 Prostaglandins have a common structure based on prostanoic acid which contains carbon atoms
(a) 12
(b) 16
(c) 18
(d) 20

73 The carbon chains of prostanoic acid are bonded at the middle of the chain by a
(a) 5 membered ring
(b) 6-membered ring
(c) 8 membered ring
(d) None of the above

74 All active prostaglandins have at least one double bond between positions
(a) 7 and 8
(b) 9 and 10
(c) 11 and 12
(d) 13 and 14

75 The synthesis of prostaglandins is inhibited by
(a) Aspirin
(b) Arsenite
(c) Fluoride
(d) Cyanide

76 The synthesis of prostaglandins requires the consumption of two molecules of oxygen and two molecules of reduced
(a) NAD
(b) NADP

Answers

70) a

71) c

72) d

73) a

74) d

75) a

76) c

(c) Glutathione
(d) Liopoate

Answers

7 Prostaglandins lower cyclic AMP in
(a) Thyroid
(b) Adipose tissue
(c) Platelets
(d) Lung

77) b

78 The inhibitory activity of prostaglandins in liver is effective on
(a) Glycogen synthetase
(b) Hexokinase
(c) Phosphorylase
(d) Glucose-6-phosphatase

78) a

79 In adipose tissue prostaglandins decrease.
(a) Lipogenesis
(b) Ketogenesis.
(c) Lipolysis
(d) Ketolysis

78) a

80 Prostaglandins increase intestinal motility and cause
(a) Constipation
(b) Loose motion
(c) Diarrhoea
(d) Dysentery

80) b

81 The enzyme responsible for the metabolism of Prostaglands is blocked by the introduction of a methyl group at the
(a) C_9 position
(b) C_{11} position
(c) C13 position
(d) C15 position

81) d

82. In adipose tissue less glycerol 3 phosphate is formed in
(a) Diabetes Mellitus
(b) Nephritis
(c) Coronary thrombosis
(d) Heart failure

82) a

83 LDL contains the apoprotein
(a) C — I
(b) C — II

83) d

(c) C – III
(d) B

84 The normal concentration of β–lipoproteins in mg per cent in plasma
(a) 100
(b) 200
(c) 300
(d) 400

85 The concentration of triacylglycerol in Pre-β -lipoproteins in per cent
(a) 40
(b) 45
(c) 50
(d) 55

86 Chylomicrons and VLDL both are released from the intestine or hepatic cell by reverse
(a) Pinocytosis.
(b) Diffusion
(c) Osmosis
(d) Passive diffusion

87 HDL is synthesized and secreted from
(a) Pancreas
(b) Liver
(c) Kidney
(d) Muscle

88 Serum LDL has been found to be increased in
(a) Obstructive jaundice
(b) Hepatic jaundice
(c) Hemolytic jaundice.
(d) Septicemia.

89 The enzyme systems for lengthening and shortening, for saturating and desaturating of fatty acids occur in
(a) Intestine.
(b) Muscle
(c) Kidney
(d) Liver

Answers

84) c
85) c.
86) a.
87) b
88) a.
89) d.

90 The lowered glucokinase leading to diminished fatty acid synthesis in the liver is caused by the effect of
(a) Feeding
(b) Overfeeding
(c) Starvation
(d) Diarrhoea

91 The increased levels of plasma free fatty acids resulting from mobilization of fat from
(a) Muscle
(b) Adipose tissue
(c) Kidney
(d) Intestine

92 Fatty appearance and enlargement of the liver are caused by
(a) Pregnancy
(b) Nephritis
(c) Abortion
(d) Toxaemia of pregnancy

93 Triacylglycerol is accumulated even in normal rate of fatty acid synthesis by the deficiency of
(a) Lipotropic factor
(b) Vitamin A
(c) Calcium
(d) Vitamin C

94 The lipotropic activity is possessed by
(a) Casein
(b) Cellulose
(c) Phospholipids
(d) Glycolipids

95 Fatty liver is caused by
(a) CH_3Cl
(b) CCL_4
(c) $MgSO_4$
(d) CH_3COOH

96 Fatty liver results in the deficiency of
(a) Stearic acid
(b) Caproic acid
(c) Vitamin A
(d) Pantothenic acid

Answers

90) c

91) b

92) d

93) a

94) a

95) b

96) d

97 The normal concentration of ketone bodies in mg. per cent in blood not exceeding.
(a) 1.0
(b) 1.5
(c) 2.0
(d) 2.5

98 Ketosis generally occurs in
(a) Nephritis.
(b) Oedema.
(c) Infective hepatic disease
(d) Coronary thrombosis.

99 Ketone bodies are utilized in
(a) Mitochondria.
(b) Extrahepatic tissues
(c) Nuclei
(d) Chromosomes.

100 The excretion of ketone body in the urine involves the deficiency of
(a) Na^+
(b) Fe^{++}
(c) Ca^{++}
(d) Mg^{++}

101 The total amount of cholesterol in grams in the body of man weighing 70 kg is about
(a) 200
(b) 180
(c) 160
(d) 140

102. The blood cholesterol level is increased in the deficiency of
(a) Vitamin D
(b) Vitamin B_1
(c) Pyridoxine.
(d) Inositol.

Answers

97) a.
98) c.
99) b
100) a
101) d.
102) c

Fill up the blanks of the followings

1 The lipids are a _____ group of compounds
2. The lipids produce metabolites through _____ in the tissues.

Answers

1 Heterogeneous.
2 Oxidation

3 Glycerides cholesterol and cholesteryl esters are ___ lipids because they are _____

4 Fats are esters of _____ with _____

5 The spreading effect of fat is to lower _____

6 The number of _____ of KOH required to neutralize 1gm of fat is called _____

7 The number of _____ of iodine absorbed by _____ gms of fat in called iodine number

8 The number of milligrams of KOH required to neutralize the _____ obtained by saponification of 1gm of fat after it has been _____ is said to be acetyl number

9 Waxes are esters of _____ with _____

10 In the human body the commonest waxes are esters of _____

11 Phospholipids act as carriers of _____ across the membranes

12 Lecithin can exist in _____ or _____ forms

13 Acetylcholine formed from choline has an important role in the _____ of nerve impulses across _____

14 Choline is the most important _____ agent

15 Phosphatidyl inositol are more _____ than the other phospholipids

16 Plasmalogens possess an _____ link in α-position instead of ester link

17 _____ are in much higher concentration in medullated nerve fibers

18 In chylomicrons the predominant lipid is _____

19 In HDL the predominant lipid is _____

20 Aminolipids are _____ and _____

21 The end methyl carbon of fatty acids is known as the _____ carbon

22 In the structure of linoleic acid the unsaturated carbon atoms are at _____ & _____

23 Linolenic and arachidonic acids are formed from ___

Answers

3 Neutral, Uncharged

4 Fatty acids, Glycerol

5 Surface tension

6 Milligrams Saponification number

7 Grams 100

8 Acetic acid, acetylated

9 Fatty acids higher alcohols

10 Cholesterol

11 Inorganic ions

12 α, β

13 Transmission Synapses

14 Lipotropic

15 Acidic

16 Ether

17 Cerebrosides

18 Triacylglycerol

19 Phospholipid

20 Phosphatidyl ethanolamine, Serine

21 ω-

22 Δ^9, Δ^{12}

23 Linoleic acids

		Answers
24	The poly unsaturated fatty acids which are not synthesized in the body but are taken from natural sources are called _____	24 Essential fatty acids.
25	The essential fatty acids of vegetable oils have low ___ and _____	25 Melting points iodine number
26.	The example of saponins is _____	26 Digitonin
27	Cholesterol in ester form is often referred to as _____ cholesterol esters which are normally rich in _____	27 Bound Linoleic acid.
28.	_____ is essential for cholesterol absorption	28 Bile.
29	Blood cholesterol level increases with _____ and also during _____	29 Ages Pregnancy
30	Cholesterol controls the red cells from being easily _____	30 Hemolysed.
31	Cholesterol acts as an antagonist to _____	31 Phospholipids.
32.	Plasma cholesterol level is not changed by the high intake of _____	32. Protein.
33	_____ in large amounts causes lowering of plasma cholesterol.	33 Nicotinic acid.
34	The conversion of acetate to cholesterol is depressed by _____ salts and increased by _____ salt.	34 Iron Manganese
35	Physical exercise _____ the serum cholesterol level.	35 Lowers.
36	The principal organ for cholesterol synthesis is _____	36 Liver
37	The HMG CoA. reductase activity is not _____ in diabetes mellitus.	37 Reduced
38.	The activity of HMG—COA reductase is _____ by cholesterol feeding.	38 Inhibited.
39	HMG—COA reductase activity is increased by the administration of the hormone _____ or _____	39 Insulin Thyroxin.
40	Cholesterol synthesis is _____ by cyclic AMP	40 Inhibited.
41	Atherosclerosis is characterised by the deposition of _____ and _____ in the connective tissue of the arterial walls.	41 Cholesterylester, other lipids.
42.	The severe atherosclerosis is accompanied by the prolonged elevated levels of _____ and _____ in the blood.	42. LDL, VLDL.
43	Polyunsaturated fatty acids can _____ serum cholesterol level.	43 Decrease.
44	Drugs like _____ and _____ can cause the increased fecal excretion of cholesterol and bile acids.	44 Cholorin Neomycin.
45	The total carbon numbers in linolenic acid and arachidonic acids are _____ and _____ respectively	45 18, 20

46. Acetoacetyl CoA is splitted to _____ by the enzyme _____

47 Acetoacetate and β–hydroxyburate are readily oxidized by _____ tissues but acetone utilization is very _____

48 Ketonemia is due to increased production of _____ by the _____

49 Acetoacetate reacts with _____ to form acetoacetyl CoA by the enzyme _____

50 The enzyme responsible for the activation of acetoacetate to ____ is absent from _____ for which these are utilized in extrahepatic tissues

51 HMG-CoA is splitted into acetoacetic acid and _____ by _____ present in mitochondria

52. The condition in which there is a high concentration of ketone bodies in tissues and _____ is called _____

53 The substances that prevent the accumulation of fat in the _____ are known as _____

54 Vitamin _____ and _____ which are important in hematopoiesis are also able to produce lipotropic effect

55 Inositol exerts a limited _____ effect in _____ free diet.

56 Choline is synthesized using labile _____ groups donated by _____ in the process of transmethylation

57 Fatty liver causes the metabolic block in the synthesis of lipoproteins from _____ and _____

58 The hypoglycemia stimulates _____ hormone production which stimulates _____

59 LDL is formed from _____ and chylomicrons

60 HDL is synthesized and secreted from _____ and _____

Answers

46 Acetyl-CoA Thiolase

47 Extrahepatic Slow

48 Ketone bodies Liver

49 Succinyl-CoA, Thiophorase

50 Acetoacetyl CoA Liver

51 Acetyl-CoA HMG-CoA Lyase

52 Blood Ketosis

53 Liver Lipotropic factor

54 B_{12} folic acid

55 Lipotropic fat.

56 Methyl Methionine

57 Lipid Apoprotein

58 Growth Lipolysis.

59 VLDL

60 Liver Intestine

Indicate 'True or "False of the followings

1 Some lipoproteins are also glycoproteins.

2 The apoproteins present in chylomicrons are B C—I C—II C—III

3 α lipoproteins occupy the β globolin region after electrophoresis

Answers

1 True

2 True

3 False

		Answers
4	Free fatty acids are classified with the other plasma lipoproteins	4 False.
5	Apoprotein B is synthesized by ribosomes in the rough endoplasmic reticulum	5 True
6	Lipolysis is controlled by the amount of cAMP present in the tissue	6 True.
7	Nicotinic acid and Prostaglandin E_1 stimulate the synthesis of cAMP	7 False
8	Insulin inhibits the release of free fatty acids from adipose tissue and enhances lipogenesis	8 True
9	Glucocorticoids and Thyroid hormones increase lipolysis	9 False
10	Administration of caffeine causes no change in plasma free fatty acids in humans	10 False
11	When glucose utilization in adipose tissue is reduced the minimum portion of it is utilized to form acylglycerol	11 False
12.	Triacylglycerol is hydrolyzed by a hormone sensitive lipase to form free fatty acids and glycerol	12 True
13	Prostaglandins are used to control inflammation	13 True
14	Prostaglandins are also used as contraceptives to prevent conception	14 True
15	PGE_1 does not irritate the mucosa lining of the throat.	15 False
16.	The direct inhibitory effect is produced on glycogen synthetase in liver by prostaglandins.	16 True
17	Since the prostaglandins are synthesized from essential fatty acids they relieve symptoms of essential fatty acid deficiency	17 False
18	Fourteen prostaglandins have been isolated from male reproductive tract	18 True
19	Phospholipase A_2 catalyzes the hydrolysis of the ester bond in position 2 of glycerophospholipids to form sphingosine	19 False
20	Phospholipids do not facilitate lipid transport between tissues	20 False
21	Cardiolipin in formed from phosphatidyl glycerol.	21 True
22.	Choline is directly activated to form phosphatidyl choline	22 False
23	Butyryl CoA is the primer molecule in mammalian liver and mammary gland	23 True
24	In mammalian systems free palmitate is liberated from the enzyme complex by hydrolysis	24 True

		Answers
25	If biotin is bound to the protein avidin of egg white, acetyl CoA carboxylase is inhibited.	25 True
26.	Fatty acid oxidation captures high-energy phosphate as 48 per cent.	26 False
27	Unsaturated fatty acids are not oxidized by β–oxidation	27 False
28	The reduced NAD is required for the conversion of β–hydroxyacyl - CoA to β–Keto acyl - CoA	28 False
29	Fatty acids with odd number of carbon atoms are not oxidized by β–oxidation	29 False
30	"Fatty acid oxidase" are found in the mitochondrial matrix adjacent to the respiratory chain	30 True
31	Thiokinases are found both inside and outside the mitochondria	31 True
32	The lipids occur in animal kingdom only	32 False
33	Fat serves as an efficient source of energy when stored in liver only	33 False
34	The phosphatides of blood platelets are involved in the production of thromboplastin activity in the early stages of blood clotting	34 True.
35	Lipids produce metabolites through oxidation in tissues	35 True
36	Oils are liquids at 30°C	36 False
37	Phospholipids are esters of fatty acid and nitrogen containing base	37 False
38	Fats act as insulator for the gain of body heat	38 False
39	The number of milligrams of KOH required to neutralize the free fatty acids of one gram of fat is called acid number	39 True
40	Peroxides are fromed at the double bounds of unsaturated fatty acids by oxidation when exposed to air	40 True
41	Waxes resemble the fat and are usually solid	41 True
42	Phospholipids act as prosthetic group to all enzymes	42 False
43	Phosphatidic is important in the synthesis of prostaglandins	43 False
44	Lecithins constitute valuable agents for the emulsification of fats and oils.	44 True
45	Lecithins form complexes with many different substances especially with other lipids proteins, carbohydrates	45 True
46	Choline is not the lipotropic agent	46 False

		Answers
47	Phosphatidyl inositol contains no base but has inositol in its place	47 True.
48	Plasmalogens give a negative reaction when tested for aldehydes with schiff's reagent	48 False
49	Cerebrosides are chief constituents of skeletal muscle	49 False
50	Gangliosides contain ceramide and glucose only	50 False
51	Lipoproteins may be identified more accurately by means of immunoelectrophoresis.	51 True
52.	The structural integrity of cell surface and subcellular particles are maintained by lipoproteins.	52 True.
53	Stearic acid contains 16 carbon atoms	53 False
54	Palmitoleic acid is a saturated fatty acid	54 False
55	Essential fatty acids effect the prolongation of clotting time and increase fibrinolytic activity	55 True
56	The lipids of gonads also contain a high concentration of polyunsaturated fatty acids	56 True
57	Cholesterol occurs in animal fats as well as in plant fats.	57 False
58	Ergosterol is the precursor of vitamin D	58 True.

Match the followings :

				Answers	
1	Glycerol	(a)	allows long chain acyl groups to penetrate the mitochondria	1	(e)
2.	Long chain fatty acids.	(b)	formed from the β–oxidation of fatty acids with odd number of carbon atoms are converted to Succinyl CoA	2.	(c)
3	Carnitine-Palmitylacyl transferase	(c)	are oxidized in liver heart, kidney, testis, brain and adipose tissue	3	(a)
4	Crotonase	(d)	yields 129 ATP by β–oxidation	4	(f)
5	Propionyl-CoA.	(e)	is utilized by the tissues where glycerokinase is present abundantly	5	(b)
6	α oxidation	(f)	catalyzes the formation β–hydroxy acyl-CoA from α, β unsatura ted acyl CoA by the addition of water	6	(g)
7	Palmitic acid	(g)	does not require CoA intermediates.	7	(d)
8	Stearic acid	(h)	undergoes 8 times β–oxidation and produces 9 molecules of acetyl – CoA	8	(h)
9	Reduced NADP	(i)	is required in the initial carboxylation of acetyl CoA to malonyl CoA	9	(k)
10	Carbon dioxide	(j)	is present in maternal venous blood immediately before uterine contractions in normal spontaneous labor	10	(i)
11	ATP and CoA.	(k)	required for the synthesis of fatty acids in the extra mitochondria	11	(m)
12	Reduced NAD	(l)	is an effective nasal decongestant	12	(o)
13	Prostaglandins	(m)	activates fatty acids to acyl CoA by thiokinase	13	(n)
14	Prostaglandin F	(n)	are a group of naturally occurring substances synthesized primarily in the prostrate	14	(p)
15	PGF 2α	(o)	is required for the catalytic conversion of stearyl CoA to oleonyl CoA by an enzyme system	15	(j)
16	PG E_1	(p)	has a hydroxyl group at position 9	16	(l)

				Answers	
17	15 hydroxy prostaglandin dehydrogenase	(q)	performs important functions in the metabolism of lipids and it is under nervous control	17	(t)
18	Adipose tissue	(r)	does not contain apoprotein C but only apoprotein A and it is formed by the liver	18	(q)
19	Theophylline	(s)	in the daily excretion of normal person is less than one milligram	19	(z)
20	Thyroid hormone	(t)	metabolize prostaglandin quickly and is present in most mammalian tissues	20	(y)
21	Chylomicrons	(u)	is converted to lanosterol by ring closures	21	(x)
22	Nascent HDL	(v)	is increased by the consumption of excessive amounts of sucrose	22	(r)
23	Liver	(w)	is the site for the synthesis of bile acids from cholesterol	23	(w)
24	Ketone bodies	(x)	derived from intestinal absorption of triacylglycerol	24	(s)
25	Serum cholesterol level	(y)	inhibits phosphodiesterase activity	25	(v)
26	Squalene	(z)	inhibit cyclic 3 , 5 - nucleotide phosphodiesterase	26	(u)

CHAPTER – 4
PROTEINS

Write the correct number of the followings :

		Answers
1	Out of 200 different amino acids found in nature the number of amino acids present in protein	
	(a) 20	1) a
	(b) 25	
	(c) 30	
	(d) 35	
2.	Enzyme catalyzed hydrolysis of proteins produces amino acids of the form	
	(a) D–	2) c
	(b) DL–	
	(c) L–	
	(d) All of the above	
3	The ionizable groups of amino acids are at least	
	(a) 1	3) b
	(b) 2	
	(c) 3	
	(d) 4	
4	The carboxyl groups of amino acids exist almost entirely as the conjugated base at p^H	
	(a) 6 6	4) d
	(b) 6 8	
	(c) 7 2	
	(d) 7 4	
5	The p^I of Arginine is	
	(a) 10 5	5) c
	(b) 10 6	
	(c) 10 8	
	(d) 11 0	
6	The neutral amino acid is	
	(a) Leucine	6) a
	(b) Lysine	
	(c) Proline	
	(d) Histidine	

Answers

7 The amino acid containing hydroxyl group
(a) Alanine
(b) Isoleucine
(c) Arginine
(d) Threonine

7) d.

8 The sulphur containing amino acid
(a) DOPA
(b) Homoserine
(c) Methionine
(d) Valine

8) c.

9 The basic amino acid
(a) Glycine.
(b) Histidine
(c) Proline
(d) Serine

9) b

10 All amino acids are optically active except
(a) Glycine
(b) Serine
(c) Threonine
(d) Tryptophan

10) a.

11 The amino acid which synthesizes many hormones
(a) Valine
(b) Phenylalanine
(c) Alanine
(d) Histidine

11) b

12. Amino acids are insoluble in
(a) Acetic acid
(b) Chloroform
(c) Ethanol
(d) Benzene

12) d.

13 The melting point of amino acid is above
(a) 100°C
(b) 180°C
(c) 200°C
(d) 220°C

13) c

14 From two amino acids peptide bond formation involves removal of one mole of
(a) Water
(b) Ammonia
(c) Carbondioxide
(d) Carboxylic acid

15 Polymers of more than 100 amino acids are termed
(a) Proteins
(b) Polypeptides.
(c) Both of the above
(d) None of the above

16 Insulin degradation or disulfied bond formation is effected by
(a) Pyruvate dehydrogenase
(b) Xylitol reductase
(c) Glutathione reductase
(d) Xanthine oxidase

17 The example of globulins
(a) Leucosin
(b) Tuberin
(c) Oryzenin
(d) Legunelin

18 The example of scleroproteins
(a) Glutenin
(b) Gliadin
(c) Salmine
(d) Elastin

19 The example of phosphoprotein
(a) Mucin
(b) Ovovitellin
(c) Ovomucoid
(d) Tendomucoid

20 The example of metalloprotein
(a) Siderophilin
(b) Osseomucoid
(c) Elastin
(d) All of the above

Answers

14) a

15) a

16) c

17) b

18) d

19) b

20) a

21 The example of chromoprotein
(a) Salmine
(b) Catalase
(c) Zein.
(d) Gliadin.

22 Protein structures are confirmed by weak bonds the example of which is
(a) Hydropbobic
(b) Disulpbide
(c) Peptide
(d) All of tbe above

23 Proteases produce polypeptides from proteins by
(a) Oxidizing.
(b) Reducing
(c) Hydrolyzing
(d) None of the above

24 Proteins react with biuret reagent which is suggestive of 2 or more
(a) Hydrogen bonds
(b) Peptide bonds
(c) Disulphide bonds
(d) Hydrophobic bonds.

25 Insulin is oxidized to separate tbe protein molecule into its constituent polypeptide chains without affecting the other part of the molecule by tbe use of
(a) Performic acid
(b) Oxalic acid
(c) Citric acid.
(d) Malic acid

26 The disulphide bond is not broken under the usual conditions of
(a) Filtration.
(b) Reduction.
(c) Oxidation
(d) Denaturation

27 Each hydrogen bond is quite
(a) Strong
(b) Weak
(c) Both of the above
(c) None of the above

Answers

21) b

22) a.

23) c.

24) b

25) a.

26) d.

27) b

28 A coiled structure in which peptide bonds are folded in a regular manner by
(a) Globular proteins
(b) Fibrous proteins
(c) Both of the above
(d) None of the above

29 In many proteins, the hydrogen bonding produees a regular coiled arrangement called
(a) β—helix
(b) α—helix
(c) Both of the above
(d) None of the above

30 Many globular proteins are stable in solution although they lack in
(a) Hydrogen bonds
(b) Salt bonds.
(c) Non polar bonds
(d) Disulphide bonds

31 Each turn of α-helix contains the number of amino acids
(a) 2 8
(b) 3 2
(c) 3 4
(d) 3 6

32 The distanee travelled per turn of α-helix in nm is
(a) 0 34
(b) 0 44
(c) 0 54
(d) 0 64

33 The space covered by eacb amino acid residue of α-helix in nm is
(a) 0 09
(b) 0 12
(c) 0 15
(d) 0 18

34 α-helix is disrupted by certain amino acid like
(a) Proline
(b) Arginine
(c) Histidine
(d) Lysine

Answers

28) a

29) b

30) d

31) d

32) c

33) c.

34) a.

Answers

35 α–helix is stabilized by
(a) Hydrogen bonds
(b) Disulphide bonds
(c) Salt bonds.
(d) Non polar bonds

35) a.

36 Glutamic dehydrogenase is a
(a) Monomer
(b) Tetramer
(c) Dimer
(d) None of the above

36) b

37 Aldolase molecule is a
(a) Dimer
(b) Trimer
(c) Both of the above
(d) None of the above

37) b

38 Foetal haemoglobin contains
(a) Two α and two γ chains.
(b) Two α and two β chains
(c) Both of the above
(d) None of the above.

38) a.

39 When haemoglobin takes up oxygen there is a change in the structure due to the moving closer together of
(a) β chains.
(b) α chains.
(c) γ chains
(d) α and γ chains.

39) a.

40 The hydrogen bonds between peptide linkages are interfered by
(a) Guanidine
(b) Uric acid
(c) Salicylic acid
(d) Oxalic acid.

40) a.

41 The hydrogen bonds in the secondary and tertiary structure of proteins are directly attacked by
(a) Salts.
(b) Alkalis.
(c) Detergents.
(d) All of the above

41) b

42 The digestability of certain denatured proteins by proteolyuc enzymes is
(a) Decreased
(b) Increased
(c) Normal
(d) None of the above

43 The antigenic or antibody functions of proteins by denaturation are frequently
(a) Not changed
(b) Changed
(c) Both of the above
(d) None of the above

44 In case of severe denaturation of protein there is
(a) Reversible denaturation
(b) Moderate reversible denaturation
(c) Irreversible denaturation
(d) None of the above

45 Bovine ribonuclease of single polypeptide chain of 124 amino acid residues with small molecular weight contains the number of disul phide bonds.
(a) 2
(b) 3
(c) 4
(d) 6

46 When eggalbumin is heated till it is coagulated the secondary and tertiary structures of the proteins are completely lost resulting in a mixture of randomly arranged
(a) Dipeptide chains
(b) Tripeptide chains.
(c) Polypeptide chains
(d) All of the above

47 In glycoproteins the carbohydrate is in the form of disaccharide units the number of units are
(a) 50 — 100
(b) 200 — 300
(c) 400 — 500
(d) 600 — 700

Answers

42) b

43) b

44) c

45) c

46) c

47) d

48 The disaccharide units of glycoproteins are attached to the peptide chain, one per
(a) 3 4 amino acid residues.
(b) 4 4 amino acid residues.
(c) 5 4 amino acid residues.
(d) 6 4 amino acid residues.

Answers
48) d.

49 The milk protein in the stomach of the infants is digested by
(a) Pepsin
(b) Trypsin
(c) Chymotrypsin
(d) Rennin

49) d.

50 Achylia gastrica is said to be when absence of
(a) Pepsin only
(b) Both pepsin and HCl.
(c) HCl only
(d) All of the above

50) b

51 The P^H of gastric juice become low in
(a) Hemolytic anemia
(b) Pernicious anemia.
(c) Both of the above
(d) None of the above.

51) b

52. In the small intestine trypsin hydrolyzes peptide linkages containing
(a) Arginine
(b) Histidine.
(c) Serine
(d) Aspartate

52) a.

53 Chymotrypsin in the small intestine hydrolyzes peptide linkage con taining
(a) Phenylalanine
(b) Alanine.
(c) Methionine
(d) Valine

53) a

54 Carboxypeptidase B in the small intestine hydrolyzes peptides con taining
(a) Leucine
(b) Isoleucine.
(c) Arginine
(d) Cysteine

54) c.

55 The transport of amino acids is regulated by active processes of di fferent numbers —
(a) 1
(b) 2
(c) 3
(d) 4

56 The third active process for amino acids transport involves
(a) Basic amino acids
(b) Neutral amino acids.
(c) Acidic amino acids.
(d) Sulphur containing amino acids.

57 The neutral amino acids for absorption need
(a) TPP
(b) B_6 PO_4
(c) NAD^+
(d) $NADP^+$

58 If one amino acid is fed in excess, the absorption of another is
(a) Slightly accelerated
(b) Moderately accelerated
(c) Highly accelerated
(d) Retarded

59 Under normal conditions, food proteins are generally readily diges ted upto the per cent.
(a) 67 to 73
(b) 74 to 81
(c) 82 to 89
(d) 90 to 97

60 By overheating the nutritional value of cereal proteins is
(a) Increased
(b) Lowered
(c) Unchanged
(d) None of the above

61 More than half of the protein of the liver and intestinal mucosa are broken down and resynthesized in
(a) 10 days.
(b) 12 days
(c) 15 days
(d) 18 days

Answers
55) c
56) b
57) b
58) d
59) d
60) b
61) a

62 The half life of antibody protein is about
(a) 4 weeks
(b) 3 weeks
(c) 2 weeks
(d) 1 week.

63 Protein anabolism is stimulated by
(a) ACTH
(b) Testosterone
(c) Glucagon
(d) Epinephrine

64 The metabolism of protein is integrated with that of carbohydrate and fat through
(a) Oxaloacetate
(b) Citrate
(c) Isocitrate
(d) Malate.

65 The building up and breaking down of protoplasm are concerned with the metabolism of
(a) Carbohydrate
(b) Fat
(c) Protein
(d) Minerals

66 The amino acids abstracted from the liver are not utilized for repair or special synthesis but are broken down to
(a) Keto acids
(b) Sulphur dioxide
(c) Water
(d) Ammonia.

67 The unwanted amino acids abstracted from the tissues are either used up by the tissue or in the liver converted into
(a) Ammonia
(b) Urea
(c) Ammonium salts.
(d) Uric acid

68 Amino acids provide the nitrogen for the synthesis of
(a) The bases of the phospholipids
(b) Uric acid
(c) Glycolipids.
(d) Chondroitin sulphates.

Answers
62) c
63) b
64) a.
65) c
66) d
67) b
68) a.

69 The metabolism of all proteins ingested over and above the essential requirements is called
(a) Endogenous metabolism
(b) Exogenous metabolism
(c) Both of the above
(d) None of the above

70 Sulphur containing amino acids after catabolism produces a substance which is excreted
(a) SO_2
(b) HNO_3
(c) H_2SO_4
(d) H_3PO_4

71 Ethereal sulphate is synthesized from the amino acid
(a) Neutral
(b) Acidic
(c) Basic
(d) Sulphur containing

72. Keratin the protein of hair is synthesized from the amino acid
(a) Glycine
(b) Serine
(c) Proline
(d) Methionine

73 In human and other ureotelic organisms the end product of amino acid nitrogen metabolism is
(a) Bile acids
(b) Ketone bodies
(c) Urea
(d) Barium sulphate

74 The end product of amino acid nitrogen metabolism in uricotelic organisms (eg reptiles and birds) is
(a) Bilirubin
(b) Urea
(c) Uric acid
(d) Biliverdin

75 The transaminase activity needs the coenzyme
(a) ATP
(b) B_6 Po_4
(c) FAD^+
(d) NAD^+

Answers
69) b
70) c
71) d
72) d
73) c
74) c
75) b

76 Transamination is a
(a) Irreversible process.
(b) Reversible process
(c) Both of the above
(d) None of the above.

77 Most amino acids are substrates for transamination except
(a) Alanine.
(b) Threonine
(c) Serine.
(d) Valine

78 Oxidative conversion of many amino acids to their corresponding α-keto acids occurs in mammalian
(a) Liver and kindey
(b) Adipose tissue
(c) Pancreas.
(d) Intestine

79 The α-keto acid is decarboxylated by H_2O_2 forming a carboxylic acid with one carbon atomless in absence of the enzyme
(a) Catalase
(b) Decarboxylase.
(c) Deaminase
(d) Phosphatase

80 The activity of mammalian L-amino acid oxidase, an FMN flavoprotein, is quite
(a) Slow
(b) Rapid
(c) Both of the above.
(d) None of the above

81 From dietary protein as well as from the urea present in fluids secreted into the gastrointestinal tract intestinal bacteria produce
(a) Carbondioxide
(b) Ammonia.
(c) Ammino sulpbate
(d) Creatine

82. The symptom of ammonia intoxication includes
(a) Blurring of vision
(b) Constipation
(c) Mental confusion
(d) Diarrhoea

Answers
76) b.
77) b.
78) a.
79) a.
80) a.
81) b.
82) a.

83. Ammonia intoxication symptoms occur when brain ammonia levels are
(a) Slightly diminished.
(b) Highly diminished.
(c) Increased.
(d) All of the above.

Answers
83) c.

84. Ammonia production by the kidney is depressed in
(a) Acidosis.
(b) Alkalosis.
(c) Both of the above.
(d) None of the above.

84) b.

85. Ammonia is excreted as ammonium salts during metabolic acidosis but the majority is excreted as
(a) Phosphates.
(b) Creatine.
(c) Uric acid.
(d) Urea.

85) d.

86. Synthesis of glutamine is accompanied by the hydrolysis of
(a) ATP.
(b) ADP.
(c) TPP.
(d) Creatine phosphate.

86) a.

87. In brain, the major mechanism for removal of ammonia is the formation of
(a) Glutamate.
(b) Aspartate.
(c) Asparagine.
(d) Glutamine.

87) d.

88. Carbamoyl phosphate synthetase structure is markedly changed in the presence of
(a) N-Acetyl glutamate.
(b) N-Acetyl Aspartate.
(c) Neuraminic acid.
(d) Oxalate.

89. In bacteria, the synthesis of carbamoyl phosp
the substrate.
(a) Ammonium salts.
(b) Ammonia.
(c) Glutamine.
(d) Aspartate.

76 Transamination is a
(a) Irreversible process.
(b) Reversible process
(c) Both of the above
(d) None of the above.

77 Most amino acids are substrates for transamination except
(a) Alanine.
(b) Threonine
(c) Serine
(d) Valine

78 Oxidative conversion of many amino acids to their corresponding α-keto acids occurs in mammalian
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(c) Deaminase
(d) Phosphatase

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(a) Slow
(b) Rapid.
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(d) None of the above

81 From dietary protein as well as from the urea present in fluids secreted into the gastrointestinal tract intestinal bacteria produce
(a) Carbondioxide
(b) Ammonia.
(c) Ammino sulphate
(d) Creatine

82. The symptom of ammonia intoxication includes
(a) Blurring of vision.
(b) Constipation
(c) Mental confusion
(d) Diarrhoea

Answers
76) b
77) b.
78) a.
79) a.
80) a.
81) b
82) a.

83 Ammonia intoxication symptoms occur when brain ammonia levels are
(a) Slightly diminished
(b) Highly diminished
(c) Increased
(d) All of the above

Answer
83) c

84 Ammonia production by the kidney is depressed in
(a) Acidosis
(b) Alkalosis.
(c) Both of the above
(d) None of the above

84) b

85 Ammonia is excreted as ammonium salts during metabolic acidosis but the majority is excreted as
(a) Phosphates.
(b) Creatine
(c) Uric acid
(d) Urea

85) d

86 Synthesis of glutamine is accompanied by the hydrolysis of
(a) ATP
(b) ADP
(c) TPP
(d) Creatine phosphate

86) a

87 In bram the major mechanism for removal of ammonia is the formation of
(a) Glutamate
(b) Aspartate
(c) Asparagine
(d) Glutamine

87) d.

88 Carbamoyl phosphate synthetase structure is markedly changed in the presence of
(a) N Acetyl glutamate
(b) N Acetyl Aspartate
(c) Neuraminic acid
(d) Oxalate

88) a

89 In bacteria the synthesis of carbamoyl phosphate takes place from the substrate.
(a) Ammonium salts.
(b) Ammonia.
(c) Glutamine
(d) Aspartate

89) c

90 The competitive inhibitor of arginine is
(a) Citrulline
(b) Malate
(c) Lysine.
(d) Serine

91 The biosynthesis of urea occurs mainly in the liver
(a) Cytosol
(b) Mitochondria.
(c) Microsomes.
(d) Nuclei

92. One mol. of urea is synthesized at the expense of the mols. of ATP
(a) 2.
(b) 3
(c) 4
(d) 5

93 Urea biosynthesis occurs mainly in the liver involving the number of amino acids
(a) 3
(b) 4
(c) 5
(d) 6

94 The normal daily output of urea through urine in grams
(a) 10 to 20
(b) 15 to 25
(c) 20 to 30
(d) 25 to 35

95 In severe acidosis the output of urea is
(a) Decreased
(b) Slightly increased.
(c) Highly increased
(d) Moderately increased

96 Uremia occurs in
(a) Cirrhosis of the liver
(b) Nephritis.
(c) Diabetes Mellitus.
(d) Coronary thrombosis.

Answers
90) c.
91) b
92) b
93) d.
94) c.
95) a.
96) b

97. Clinical symptom in urea cycle disorder is
(a) Mental retardation.
(b) Drowsiness.
(c) Diarrhoea.
(d) Oedema.

98. The sparing action of methionine is
(a) Tyrosine.
(b) Cystine.
(c) Arginine.
(d) Tryptophan.

99. NH_4^+ aminates glutamate to form glutamine requiring ATP and
(a) Mg^+.
(b) Ca^{++}.
(c) Na^+.
(d) K^+.

100. Glutathione is a
(a) Dipeptide.
(b) Tripeptide.
(c) Polypeptide.
(d) None of the above.

Answers
97) a.
98) b.
99) a.
100) b.

Fill up the blanks of the followings :

1. The amino acids which can not be synthesized by the organism from substances ordinarily present in the diet according to physiological requirements are called _____.
2. Two amino acids _____ and _____, which are required for animals, are _____ for humans.
3. The omission of an essential amino acid from the diet results in the _____.
4. Alanine is formed from pyruvate by _____ in presence of the coenzyme _____.
5. Glutamate is synthesized in animals by _____ in presence of the coenzyme _____.
6. Asparagine is synthesized from aspartate by _____ with the help of coenzyme _____ and Mg^{++}.
7. Glycine is synthesized from _____ as well as _____.

Answers
1. Essential amino acids.
2. Arginine; Histidine; Nutritionally semi-essential.
3. Negative nitrogen balance.
4. Transamination; B_6~PO_4.
5. Glutamate dehydrogenase; Reduced NADP.
6. Asparagine synthetase; ATP.
7. Serine; Choline

		Answers
8	Cysteine is formed from _____	8 Methionine
9	Phenylalanine is covered to tyrosine by ------- with the incorporation of one atom of oxygen the para position of phenylalanine and the other atom is reduced forming water, the reducing power supplied by ---------- is immediately provided as tetrahydrobiopterine.	9 Phenylalanine hydroxylase com plex, Reduced NADP
10	The only ketogenic amino acid ----------.	10 Leucine
11	Arginine is converted to _____ by arginase with the removal of _____	11 Ornithine, Urea
12.	Histidine on deamination produces ---------- which is converted to 4-imidazolone-5 propionate by -------	12 Urocanic acid;— Urocanase
13	Serine forms _____ by _____ rich in liver of rats.	13 Pyruvate, Serine dehydratase
14	Cystine is converted to cysteine by an _____ depen dent _____	14 NADH, Cystine reductase
15	Threonine aldolase cleaves threonine to _____ and _____	15 Acetaldehyde, Glycine
16	Hydroxyproline is converted to _____ and _____	16 Pyruvate, Glyoxa late
17	P hydroxyphenylpyruvate hydroxylase converts P-hydroxyphenyl pyruvate to ------- with cofactor -------.	17 Homogentisie acid, Ascorbic acid
18	Homogentisate oxidase, an _____ metalloprotein of mammalian liver forms maleylacetoacetate, the rea ction is inhibited by a _____ agent that binds iron	18 Iron, Chelating
19	_____, the black pigment present in the skin, hair and retina of the eye is formed from phenylalanine in the _____ present in the skin	19 Melanin, Melano-blasts
20	DOPA is converted to dopamine by a _____ with _____ as cofacter	20 Decarboxylase, B_6—PO_4
21	The hydroxylation of dopamine forms norepinephrin in the presence of _____ and molecular _____	21 Ascorbic acid, Oxygen
22	Epinephrine is formed from norepinephrine by _____ from _____	22 Methylation, Me-thionine
23	Two peptides are liberated during the conversion of fibrinogen into _____ One of the peptides contains _____	23 Fibrin, Tyrosine -o-sulphate.
24	Tryptophan oxygenase enzyme is an _____ porphyrin metalloprotein which is present in the _____ of ma mmals.	24 Iron, Liver

		Answers
25	The chief inducing agent _____ of tryptophan oxygenase is blocked by _____	25 Adrenal corticosteroids, Puromycin
26	Kynurenine is hydroxylated with molecular _____ in presence of ----------.	26 Oxygen, Reduced NADP
27	In the deficiency of vitamin _____ kynurenine derivatives reach the extrahepatic tissues where they are converted to _____	27 B_6, Xanthurenic acid
28	In the deficiency of vitamin B_6 the synthesis of _____, and ---------- in the tissues is impaired owing to the non-conversion of tryptophan to niacin	28 NAD^+, $NADP^+$
29	_____ mg of tryptophan produce _____ mg of niacin	29 60, 1
30	The vitamins _____ and _____ are required for the formation of niacin from tryptophan in the body	30 B_6, B_2
31	5 hydroxytryptophan on _____ produces _____, a vasoconstrictor and is stored in platelets	31 Decarboxylation, Serotonin
32	Serotonin on acetylation and _____ produces the hormone _____	32 Methylation, Melatonin
33	Methionine is first activated by _____ forming S-adenosylmethionine which is also termed as _____	33 ATP, Active methionine
34	L-methylmalonyl-CoA is converted to ---------- by methylmalony-CoA isomerase with ---- ------- as coenzyme	34 Succinyl-CoA; B_{12}
35	Leucine on catabolism yields _____ and _____	35 Acetoacetate, Acetyl-CoA
36	Both valine and isoleucine on catabolism produce _____	36 Succinyl CoA
37	Imidazolone—3—propionic acid is converted into _____ by _____	37 FIGLU, Hydrolase
38	In human beings and animals deficiency of _____ or _____ causes an excessive excretion of FIGLU	38 Folic acid, Vitamin B_{12}
39	FIGLU is converted into _____ resulting in the transfer of C_1 carbon atom to _____	39 Glutamate, Tera hydrofolic acid
40	Histidine is converted into _____ and _____ by minor pathways	40 Carnosine, Anserine
41	Carnosine is a peptide of _____ and _____	41 Histidine, β–alanine
42	Ergothionine is present in high concentration in human _____	42 Erythrocytes
43	_____ acts as a coenzyme in the formation of prostaglandin, PGE_2 from _____	43 Glutathione, Arachidonic acid

		Answers
44	Skeletal muscle contains about ——— per cent creatine and heart muscle about ——— that amount.	44 0 5, Half
45	Creatinine is formed largely in the _____ by the irreversible and nonenzymic removal of water from ____.	45 Muscle Creatine phosphate.
46	The synthesis of creatine is completed in the liver by the methylation of _____ in which _____ methionine is the methyl donor	46 Glycocyamine, Active
47	In the methylation of creatine _____ and _____ are required	47 ATP, Oxygen
48	The activation of methionine occurs requiring ATP, _____ and _____ as well as methionine activating enzyme	48 Mg^{++}, Gluta thione
49	In the methylation of glycocyamin. guanidoacetate methyl ferase found in the ——— of mammals requires ——— for its optimal activity	49 Liver; Gluta thione
50	The rate of creatine biosynthesis is dependent on kidney _____ activity	50 Transamidinase
51	Creatinine is the _____ of creatine and is formed by the _____ means in muscle	51 Anhydride, Nonenzymatic
52.	Creatinine is reabsorbed in the _____ at _____ concentrations	52 Renal tubules, Lower
53	The number of milligrams of creatinine (plus creatine) nitrogen excreted per kg body wt in 24 hours is called _____	53 Creatinine coefficient.
54	The creatinine coefficient is an _____ of the amount _____ muscle tissue in the body	54 Index, Active
55	Positive nitrogen balance exists when _____ exceeds _____	55 Intake, Output.
56	All body proteins continually undergo _____ and _____	56 Degradation, Synthesis
57	Amino acids have at least two ionizable groups _____ and _____	57 $-CooH$ NH_3^+
58	The pH at which the amino acid has no net charge and hence does not move in an electric field is called ____	58 Isoelectric pH (PI)
59	The ion at the isoelectric point which carries + and — charges internally neutralized is called ———	59 Zwitterion
60	Owing to the opposite reactions depending on the acidity or alkalinity of the solution the amino acids are called _____	60 Ampholytes
61	Albumins are also widely distributed in plants especially in the _____ and _____	61 Seeds, Fruits.
62.	Scleroproteins are similar to ——— and ———	62. Albumins Globulins

		Answers
63	Degradation of protein in one tissue is accompanied by _____ in others	63 Synthesis
64	Antibody protein induced by immunization also undergo continual _____ and _____	64 Breakdown Synthesis
65	The metabolic pool is contributed by substances derived from catabolism of protein in the tissues by stimulations of the excessive amounts of _____ hormones and _____ hormones	65 Thyroid 11-oxygenated adreno-cortical
66	The protein contains *nitrogen* _____ per cent.	66 6 25

Indicate "True" or "False" of the followings :

		Answers
1	The free amino acids after hydrolysis of peptides are separated and identified by chromatography or by electrophoresis	1 True
2.	Taurine is an amino acid but not a constituent of protein Therefore it is not present in the classification of amino acids	2 True
3	Ninhydrin is a powerful oxidizing agent	3 True
4	The neutral amino acids absorb ultraviolet light	4 False
5	D amino acids are also present in proteins	5 False
6	Substitution of a single amino acid for another in a sequence of 100 or more amino acids may abolish biologic activity with serious consequences	6 True
7	All hormones are proteins	7 False
8	The proteins are involved in blood clotting	8 True
9	The proteins act as the defence against infections by means of protein antibodies	9 True
10	Proteins may be defined as the high molecular weight mixed polymers of α–amino acids joined together with peptide linkage	10 True
11	The denatured protein is highly increased in solubility at its iso electric point	11 False
12	The native proteins are said to be occurring in plants only	12 False
13	The native proteins possess no sedimentation rate	13 False
14	Denaturation is used to know the enzyme catalyzed reaction of an extract at the loss of the enzyme activity when boiled or acidified	14 True
15	Denatured ribonuclease when freed from urea and β–mercaptoethanol by dialysis slowly regains enzymic activity	15 True
16	In severe malnutrition blood albumin level is unchanged	16 False
17	Albumin has no role in fatty acid transport	17 False
18	The cause of hypoalbuminemia is impaired synthesis	18 True
19	Pathological proteinuria may occur as a result of increased glomerular permeability	19 True

		Answers
20	Glycoproteins are secreted by the sublingual glands of some animals.	20 False
21	The glycine content of many albumins is high	21 Flase
22	Some glycoproteins have many identical dissaccharide units attached to the polypeptide chain.	22. True
23	Visual purple of the retina a chromoprotein in which the prosthetic group is a carotenoid pigment.	23 True
24	Chromoproteins of certain animal fibres in which the prosthetic group is melatonin	24 False
25	Scleroproteins are readily attacked and dissolved by alkali	25 False
26	Scleroproteins can not form the supporting structures of animals.	26 False
27	Nitroprusside test is positive for the amino acid cysteine	27 True
28	Millon s test is negative for the amino acid tryosine.	28 False
29	Arginine is an aromatic amino acid	29 False
30	Tryptophan is a basic amino acid	30 False
31	The intestinal mucosa also contains tripeptidase dipeptidase etc	31 True
32	The polypeptides formed in the stomach are digested there by trypsin	32. False
33	If one amino acid is fed in excess it favours the absorption of an other	33 False
34	D amino acids are absorbed by simple diffusion	34 True
35	The amino acid associates with the carrier and Na^+ in the microville	35 True
36	Sometimes the whole protein is absorbed into the blood	36 True
37	The insoluble fibrous protein is also hydrolysed by enzymes of the human digestive tract	37 False
38	Endogenous metabolism is involved in the formation of tissue and the other nitrogenous substances and also the normal break down of tissue proteins	38 True
39	Aromatic amino acids form glutathione	39 False
40	In ammonotelic organisms, uric acid is the end product of amino acid nitrogen metabolism	40 False
41	δ–amino group of ornithine undergo transamination	41 True
42.	Both L- and D amino acid oxidase activities occur in mammalian brain also	42. False
43	The reduced FMN or FAD is reoxidized directly by molecular oxygen forming H 0 without the help of electron carriers.	43 False
44	If catalase is present the α–keto acid is decarboxylated by H_2O forming a carboxylic acid with one carbon atom less	44 False

		Answers
45	Certain hormones also depress glutamate dehydrogenase activity	45 False
46	Ammonia is produced in the kidney from glutamine catalyzed by renal glutaminase	46 True
47	Glutamate dehydrogenase uses reduced NAD or NADP as cosubstrate	47 False
48	The normal blood concentration of ammonia is 10-20 mg/100 ml	48 False
49	Formation of glutamine is catalyzed by glutamine oxidase a mitochondrial enzyme	49 False
50	Asparaginase and glutaminase are employed as antitumor agents	50 True
51	The amino acids ornithine and citrulline occur in protein	51 False
52	Small quantities of arginase also occur in brain mammary gland testicular tissue and skin	52 True
53	High erythrocyte level of arginase causes hyperargininemia.	53 False
54	Creatine can not be synthesized in the pancreas of mammals	54 False
55	Dietary creatine or high blood creatine has no effect on the rate of synthesis of creatine in the liver	55 True
56	Hypothyroidism is associated with redueed kidney transamidinase activity	56 False
57	Excessive creatinuria has been observed in hyperthyroidism during pregnancy and the menstrual period	57 True
58	Normal values of creatinine coefficient range from 30 to 36 mg creatinine for men	58 False
59	Less creatinuria may occur in starvation	59 False
60	Glutamate aspartate and ammonia are utilized for purine and pyrimidine synthesis	60 True

Match the followings :

				Answers	
1	Edema	(a)	are the active agents in the processes of deamination and amination through the participation in transamination reactions	1	(f)
2	Cardiac edema	(b)	Produces a prompt excretion of water sodium and chloride	2	(i)
3	Edema of the brain	(c)	may occur as a result of increased glomerular permeability	3	(g)
4	Furosemide	(d)	may occur in fever after intense physical exertion and after minor emotional stimuli	4	(b)
5	SGOT activity is significantly increased	(e)	is defined as the quantitative difference between the nitrogen intake and the nitrogen output	5	(j)
6	Acidic amino acids	(f)	is recognized by the increase in the volume of interstitial fluid	6	(a)
7	Methionine	(g)	occurs in acute glomerulonephritis	7	(m)
8	In nephrotic syndrome	(h)	urinary albumin Globulin ratios are high	8	(h)
9	In amyloid diseases of the kidneys	(i)	Is produced by increased venous pressure	9	(o)
10	Transitory proteinuria	(j)	in acute myocardial infarction	10	(d)
11	Nitrogen balance	(k)	splits urea into ammonia and carbon dioxide at the optimum temperature and pressure	11	(e)
12	Creatine synthesis	(l)	is participated by glycine arginine and methionine	12	(l)
13	Serum albumin level is decreased	(m)	is involved in the detoxification of nicotinic acid for the formation of the excretory product trigonelline	13	(n)
14	Pathological proteinuria	(n)	In sudden haemodilution	14	(e)
15	Plant urease	(o)	urinary albumin Globulin ratios are low	15	(k)
16	Ammonia intoxication	(p)	a stimulant of the central nervous system and also promotes peristalsis.	16	(t)

		Answers
17. Uses of antibiotics.	(q) if introduced into the blood stream, appear in the urine.	17. ..... (w)
18. In hyperammonemia type 11.	(r) takes part as a coenzyme in the prevention of peroxidation of poly-unsaturated fatty acids in tissues.	18. ..... (y)
19. 5-hydroxytryptamine	(s) Found in daily excretion of creatine in urine of a normal male on a balanced diet.	19. ..... (p)
20. Aniserine.	(t) is caused by all disorders of urea synthesis.	20. ..... (v)
21. Glutathione.	(u) found in the daily excretion of creatinine in urine of a normal adult female.	21. ..... (r)
22. Blood normal level of creatine.	(v) is a peptide of methylhistidine and β–alanine.	22. ..... (z)
23. Nil.	(w) are to decrease the absorption of ammonia formed by bacterial decomposition in the intestine.	23. ..... (s)
24. 0.8 to 1.8 grams.	(x) may occur in febrile and wasting diseases, diabetes mellitus, congenital muscle dystrophies.	24. ..... (u)
25. Excessive creatinuria.	(y) patients suffer from a deficiency of ornithine transcarbamoylase.	25. ..... (x)
26. Egg albumin and other	(z) 2.6 — 7.6 mg/100 ml.	26. ..... (q)

CHAPTER – 5
NUCLEIC ACIDS

Write the correct answer number of the followings

Answers

1 The best role of purine and pyrimidine nucleotides is to serve as the monomeric precursors of
- (a) RNA
- (b) DNA
- (c) Both of the above
- (d) None of the above

1) c

2 The Purine nucleotides act as the components of
- (a) FAD^+
- (b) NAD^+
- (c) $NADP^+$
- (d) All of the above

2) d

3 The pyrimidine nucleotides act as the high energy intermediates
- (a) UDPG
- (b) ATP
- (c) ADP
- (d) AMP

3) a

4 The chemical name of thymine
- (a) 2 – oxy – 4 – amioopyrimidine
- (b) 2 4 – dioxy – 5 – methylpyrimidine
- (c) 2 4 – dioxypyrimidine
- (d) None of the above

4) b

5 The chemical name 2–amino–6–oxypurine is said to be
- (a) Adenine
- (b) Xaothine
- (c) Guanine
- (d) Hypoxanthioe

5) c.

6 The lactam form is the predominant tautomer of
- (a) Uracil
- (b) Cytosine
- (c) Adenine
- (d) Xanthine

6) a

7 N^1 — methylguanine has been found more recently in the nucleic acids of the cells of
(a) Bacteria
(b) Yeast
(c) Plant
(d) Mammals

8 Hypoxanthine and ribose constitute
(a) Adenosine
(b) Inosine
(c) Guanosine
(d) Cytidine

9 Thymine and deoxyribose form
(a) Deoxycytidine
(b) Deoxyadenosine
(c) Deoxythymidine
(d) Deoxyuridine

10 The most abundant intracellular free nucleotide
(a) ATP
(b) FAD^+
(c) NAD^+
(d) $NADP^+$

11 The concentration of ATP in living mammalian cells in mM is near.
(a) 0·2
(b) 0·4
(c) 0·6
(d) 1·0

12 The intracellular cAMP concentrations in μM are near
(a) 3·0
(b) 2·0
(c) 1·0
(d) 0·5

13 The epimerization of galactose to glucose and vice versa takes place by
(a) UTP
(b) CTP
(c) GTP
(d) ATP

Answers

7) d

8) b

9) c

10) a

11) d

12) c

13) a

14 The biosynthesis of phosphoglycerides in animal tissue requires
(a) ATP
(b) CTP
(c) GTP
(d) TPP

15 The chemical name 4 hydroxypyrazole pyrimidine is used for
(a) Thioguanine
(b) Mercaptopurine
(c) Azathioprine
(d) Allopurinol

16 Transforming factor is used for the name of
(a) RNA
(b) DNA
(c) tRNA
(d) None of the above

17 Guanosine nucleotide is held by the cytosine nucleotide by the number of hydrogen bonds
(a) 1
(b) 2
(c) 3
(d) 4

18 Within the single turn of DNA the number of base pair exists
(a) 4
(b) 6
(c) 8
(d) 10

19 Each turn of DNA structure has a pitch in nm of
(a) 1 4
(b) 2 4
(c) 3 4
(d) 4 4

20 The double stranded DNA molecule loses its viscosity upon
(a) Denaturation
(b) Filtration
(c) Sedimentation
(d) Concentration

Answers
14) b
15) d
16) b
17) c.
18) d
19) c
20) a

21 DNA molecule contains the number of nucleotides
(a) 800 to 4000
(b) 1000 to 6000
(c) 1200 to 8000
(d) 1600 to 9000

22 DNA is denatured by
(a) Heat
(b) Acid
(c) Alkali
(d) All of the above

23 Chromatin contains the number of repeating units in nm
(a) 10
(b) 15
(c) 20
(d) 25

24 In RNA structure, its guanine content does not necessarily equal its cytosine content and its adenine content does not necessarily equal its uracil content since it is a
(a) Double- stranded molecule.
(b) Single-stranded molecule
(c) Stable molecule
(d) Unstable molecule

25 Each RNA molecule contains the number of nucleotides
(a) 40 to 4000
(b) 50 to 5000
(c) 60 to 6000
(d) 70 to 7000

26 Messenger RNA has a molecular weight of
(a) 15000 to 30000
(b) 20000 to 35000
(c) 25000 to 40000
(d) 30000 to 50000

27 Of the total cellular RNA molecules the Pc of transfer RNA molecules amount to
(a) 5 to 10
(b) 8 to 16
(c) 10 to 20
(d) 15 to 30

Answers
21) d
22) d
23) a
14) b
25) c
26) d
27) c

28. Each transfer RNA molecule contains the number of nucleotides
    (a) 70
    (b) 75
    (c) 80
    (d) 85

29 In every cell the number of tRNA molecules are at least
    (a) 10
    (b) 15
    (c) 20
    (d) 25

30 All tRNA molecules have a common CCA sequence at the
    (a) 3' termini
    (b) 5 termini.
    (c) 3' 5 termini
    (d) All of the above

31 In nearly all tRNA molecules, there is a loop containing the nucleotides of
    (a) Uridine.
    (b) Thymidine
    (c) Ribothymine and Pseudouridine
    (d) Cytidine

32. In tRNA molecules, there is another loop containing the minor base
    (a) Uracil.
    (b) Dihydrouracil.
    (c) Cytosine
    (d) Dihydrocytosine

33 The PC. of the ribosomal RNA of the RNA within the cell
    (a) 60
    (b) 70
    (c) 80
    (d) 90

34 The mammalian ribosome contains the number of major nucleoprotein subunits
    (a) 2
    (b) 3
    (c) 4
    (d) 5

Answers

28) b

29) c.

30) a.

30) a.

31) c.

32) b

33) c.

35 Gene is a segment of the DNA molecule containing base pairs about
(a) 300
(b) 400
(c) 500
(d) 600

36 The fragments of DNA attached to an RNA initiator component were discovered by
(a) Watson and Crick
(b) Okazaki
(c) Peterson
(d) Nelson

37 Synthesis of RNA molecule is terminated by a signal which is recog nized by
(a) ρ (Rho) factor
(b) δ– factor
(c) α– factor
(d) None of the above

38 The promoter site at which the synthesis of new RNA molecule begins with the help of another factor
(a) σ
(b) γ
(c) β
(d) α

39 The binding of Prokaryotic DNA — dependent RNA polymerase to promoter sites of genes is inhibited by the antibiotic
(a) Septran
(b) Streptomycin
(c) Aureomycin
(d) Rifampin

40 The N atoms at positions 3 and 9 of Purine base are derived from the amide nitrogen of
(a) Glutamate
(b) Glutamine
(c) Asparagine
(d) Asparate

41 The carbon atoms at positions 4 and 5 and the N atom at position 7 of purine base are supplied from
(a) Valine

Answers
35) d
36) b
37) a
38) a
39) d
40) b
41) c

(b) Alanine
(c) Glycine
(d) Serine

41 5 phosphoribosylamine reacts with glycine to produce glycinamide ribosylphosphate by glycinamide kinosynthetase in presence of
(a) ATP
(b) GTP
(c) UTP
(d) CTP

43 The carbon atoms at position 4 5 and 6 and the N atom at position 3 of pyrimidine base are derived from.
(a) Glutamic acid
(b) Aspartic acid
(c) Glycine
(d) Serine

44 The end product of purine catabolism in other mammals except man
(a) Uric acid
(b) Allantoin
(c) Ammonia
(d) Creatinine

45 The net excretion of total uric acid in normal men in mg in 24 hours
(a) 100 — 300
(b) 200 — 400
(c) 300 — 500
(d) 400 — 600

46 A portion of uric acid in converted to urea and ammonia by intestinal
(a) Urogenolysis
(b) Ureolysis
(c) Uricolysis
(d) Ureotolysis.

Answers

42) a.
43) b
44) b
45) d.
46) c.

Fill up the blanks of the followings :

1 Xanthine is oxidized to uric acid by ————

Answers

1 Xanthine oxidase

		Answers
2.	Xanthine oxidase is inhibited by _____ for which uric acid cannot be formed	2 Allopurinol
3	The chief end product of purine catabolism in man _____	3 Uric acid
4	Urinary Uric acid is both _____ and _____ in origin	4 Endogenous Exogenous
5	Birds and reptiles do not possess _____ activity	5 Uricase
6	_____ and _____ also increase the urinary excretion of uric acid by inhibiting renal tubular reabsorption	6 ACTH Adrenocortical oxysteroids
7	Blood uric acid level also _____ in hemolytic anemia and thalassemia	7 Increases.
8	Sodium urate crystals are deposited in soft tissues and these urate deposits are referred to as _____	8 Tophi
9	The synthesis of pyrimidin starts with the formation of carbamoyl phosphate from _____, _____ and CO_2 being catalyzed by carbamoyl phosphate synthetase present in the cytosol of the cell	9 Glutamine ATP
10	Dihydroorotic acid on dehydrogenation by dihydroorotate dehydrogenase utilizing _____ as coenzyme is converted into _____	10 NAD^+ Orotic acid
11	The main site for the catabolism of pyrimidines _____	11 Liver
12.	The major end products of cytosine uracil and thymine are _____ and _____ respectively	12 β– alanine β–aminoisobutyric acid
13	For each gene in the DNA molecule there is a _____ strand and its complimentary _____ strand	13 Sense Antisense
14	The strand which is transcribed into an RNA molecule is referred to as the _____ of the DNA	14 Sense strand
15	RNA and DNA chain elongation is inhibited by _____	15 Actinomycin D
16	DNA and RNA synthesis is nhibited by _____	16 Anthramycin
17	Tea contains the purine base containing substituent _____	17 Theophylline
18	Cocoa contains the purine base containing substituent _____	18 Theobromine
19	The base thymine of DNA is replaced by the base _____ in _____	19 Uracil RNA
20	Cyclic AMP is destroyed in tissues by its conversion to _____ by the enzyme _____	20 AMP cAMP phosphodiesterase
21	S adenosylmethionine serves as a form of _____ methionine	21

		Answers
22	cAMP is formed from _____ by the enzyme _____	22 ATP, Adenylate cyclase
23	cGMP is catabolized by _____ to produce _____	23 Phosphodiesterase, 5' monophosphate
24	cGMP acts antagonistically to _____	24 cAMP
25	Uridine diphosphoglucuronic acid serves as the _____ glucuronide for the formation of _____ in the liver	25 Active, Bilirubin glucuronide
26	CTP acts as the precursor for the polymerization of _____ into _____	26 CMP, Nucleic acids.
27	Ceramide and CDP— choline are responsible for the formation of _____ and other substituted _____	27 Sphingomyelin, Sphingosine.
28	_____ and _____ are used in the chemotheraphy of cancer and viral infections	28 Cytarabine, Vidarabine
29	Azathioprine is useful in organ _____	29 Transplantation
30	Both _____ and _____ are used clinically to inhibit the catabolism of intracellular cAMP	30 Aminophylline, Theophylline
31	DNA is referred as _____	31 Transforming factor
32.	Two strands of double helical molecule are _____	32 Anti parallel
33	DNA molecules are long and its length is _____ times greater than its breadth	33 250
34	Chromatin consists of a long double stranded _____ molecules	34 DNA
35	The messenger RNA is the most _____ in size and stability	35 Heterogeneous
36	The heterogeneous nuclear RNA (HnRNA) molecules are processed to generate the _____ molecules which then enter the cytoplasm to serve as templates for _____ synthesis.	36 mRNA, Protein
37	One tRNA corresponds to each of the _____ amino acids required for _____ synthesis.	37 20 Protein
38	The _____ loop at the end of a base paired stem of tRNA recognizes the codon of the template _____	38 Anticodon mRNA
39	Ribosomes are _____ particles and reticular granules of _____ in diameter	39 Nucleoprotein, 100 — 150 Å.
40	The 60S subunits contain a 5S rRNA, a _____ rRNA and a _____ rRNA	40 5 8S, 28S
41	The 40S subunit contains _____ r RNA and about _____ polypeptide chains	41 18S 30
42.	The unit of genetic information is the _____ or _____	42 Gene Cistron

		Answers
43	RNA can be synthesized by _____ which is dependent on the presence of _____ acting as a template	43 RNA polymer ase, DNA
44	DNA produces a _____ which helps in placing _____ in the code for protein synthesis	44 mRNA, Amino acids
45	The formation of RNA template is directed by _____	45 Nuclear DNA
46	Nucleoproteins are conjugated proteins containing _____ and a basic protein like _____ or _____	46 Nucleic acids Protamine, Histones

CHAPTER – 6
PROTEIN SYNTHESIS AND GENETIC CODE

Fill up the blanks of the followings :

		Answers
1	In the nucleotide sequenee of the mRNA molecule, code words exist for each amino acid. This is referred to as the _____	1 Genetic code
2.	The adapter molecules translating the code words into the amino acid sequence are the _____ molecules.	2 Transfer RNA (- tRNA)
3	The process by which the cell translate information by the machinery from the nucleotide sequence of an mRNA into the sequence of amino aeids of the corresponding specific _____ is said to be _____	3 Protein, Translation
4	The rough endoplasmic reticulum is the faetory of _____	4 Polyribosomes.
5	Code words eonsting of 2 nueleotides eaeb can provide for only _____ specific code words	5 16
6.	Each eode word termed a _____ consists of a sequenee of _____ nucleotides	6 Codon, 3
7	64 triplet codes in mRNA with 4 nueleotides are to provide for _____ amino acids and three codons do not code for specifie amino acids and these have been termed _____	7 20, Nonsense codon
8	Only a single amino acid is indicated for any specific codon the genetic code is _____	8 Unambiguous.
9	Once the reading is started at a specific codon there is no _____ between codons.	9 Punctuation.
10	Amino acids are activated by the enzyme _____ in presence of the coenzyme _____	10 Aminoacyl-tRNA synthetase, ATP.
11	The binding of the mRNA to the 40S ribosomal subunit requires the presence of _____.	11 IF-3
12.	The initiation factor is a _____ factor	12. Protein.
13	The aminoacyl tRNA interacts with _____ and _____ to form a complex.	13 GTP, IF-2.
14	The complex formed by anticodon of tRNA and IF I attaches the 60 S ribosomal subunits with the release of _____, _____, _____.	14 IF-I, IF-2, IF-3
15	The complete ribosome contains two sites _____ site and _____ site on the mRNA	15 P, A

	Answers
16. During the process of initiation the complete _____ ribosome is formed.	16. 80S.
17. _____ forms a complex with _____ and the entering aminoacyl — tRNA.	17. EF-1; GTP.
18. The alpha amino group of the new aminoacyl-TRNA in the A site combines with the carboxyl group of the peptidyl — tRNA occupying the P site in presence of ------ of the ------- ribosomal subunits.	18. PeptidyL transferase; 60S.
19. The newly formed peptidyl-tRNA at the A site is translocated into the vacated P site by _____ and _____.	19. EF-2; GTP.
20. After multiple cycles of elongation the _____ codon of mRNA appears in the ____ site.	20. Nonsense; A.
21. The releasing factor hydrolyzes the bond between the peptide and the _____ occupying the ____ site.	21. tRNA; P.
22. On hydrolysis and release the 80S ribosome dissociates into its ____ and ____ subunits which are then recycled.	22. 40S; 60S.
23. The releasing factors are _____.	23. Proteins.
24. The antibiotic _____ has structural similarity with that of tyrosinyl — tRNA is incorporated via the A site on the ribosome.	24. Puromycin.
25. Since EF-2 is inactivated by _____ and thereby inhibits mammalian protein synthesis.	25. Diptheria toxin.
26. A single ribosome is capable of translating about ____ codons in _____ seconds into a protein with a molecular weight of 40,000.	26. 400; 10
27. There are only two types of gene regulation _____ regulation and _____ regulation.	27. Positive; Negative
28. A double negative has the effect of acting as a _____.	28. Positive.
29. The gene is the smallest segment of the _____ molecule containing about _____ base pairs.	29. DNA; 600.
30. When a pair carries genes with the same characteristics, say tallness, then the individual is said to be _____.	30. Homozygous.
31. When one of the pair tallness and the other gene shortness, the individual is _____.	31. Heterozygous.
32. Lac operon is nothing but _____ and _____.	32. Structural gene; operator gene.
33. The regulator gene induces the synthesis of protein macromolecules called _____.	33. Repressors.
34. The operon becomes active because the repressor system is itself inactivated. The pbenomenon is said to be _____.	34. Derepression.

		Answers
35	The operator locus is a region of double-stranded DNA of _____ base pairs long with a 2 fold rotational symmetry in a region that is _____ base pairs long	35 27 21
36	The operator locus lies between the _____ at which the DNA-dependent RNA polymerase attaches to commence transcription and the beginning of the _____ gene	36 Promoter site Z.
37	Normally _____ repressor molecules are present and one or two operator loci per cell are also present	37 20—40
38	The binding of the inductor to the repressor molecule involves the amino acid residues in positions _____ and _____.	38 74 75

CHAPTER – 7
HEMOGLOBIN

Write the correct answer number of the followings | *Answer*

1 Hemoglobin contains the number of gram atoms of iron per mole in the ferrous state –
(a) 1
(b) 2
(c) 3
(d) 4 — 1) d

2 The molecular weight of hemoglobin
(a) 44 450
(b) 54 450
(c) 64 450
(d) 74 450 — 2) c

3 The porphyrins are cyclic compounds formed through methylene bridges by the linkages of pyrrole rings number
(a) 4
(b) 3
(c) 2
(d) 1 — 3) a

4 The porphyrins are found in nature in which the various side chains are substituted for the hydrogen atoms number
(a) 2
(b) 4
(c) 6
(d) 8 — 4) d

5 A porphyrin with a completely symmetrical arrangement of the sub stituents is classified as a porphyrin of
(a) Type I
(b) Type II
(c) Type III
(d) All of the above — 5) a

6 The more abundance found in nature is
(a) Type I series.
(b) Type II series
(c) Type III series.
(d) None of the above — 6) c

7 In the biosynthesis of porphyrins the activation of glycine needs the coenzyme
(a) NAD^+
(b) $B_6 - PO_4$
(c) FAD^+
(d) ATP

8 The anemia has been observed in the deficiency of vitamin
(a) Biotin
(b) Inositol.
(c) Niacin
(d) Pantothenic acid.

9 The dipyrryl compounds are of two types
(a) A and B
(b) B and C
(c) A and C.
(d) B and D

10 A type III porphyrin results with the condensation of the components
(a) Two of the (A)
(b) One (A) and one (B)
(c) Two of the (B)
(d) One (A) and one (C)

11 The enzyme coproporphyrinogen oxidase is able to act on coproporphyrinogen
(a) Type I
(b) Type II.
(c) Type III
(d) All of the above

12. In mammalian liver the reaction of conversion of corproporphyrinogen to protoporphyrin requires
(a) Molecular oxygen
(b) Water
(c) ATP
(d) $B_6 - PO_4$

13 Heme is synthesized by the incorporation of ferrous ion (Fe^{++}) into protoporphyrin III being catalyzed by the enzyme
(a) Ferroxidase
(b) Ferroreductase

Answers

7) b

8) d.

9) a.

10) b.

11) c.

12) a.

13) c.

(c) Ferrochelatase.
(d) None of the above.

14. The globin of the hemoglobin is a protein composed of closely packed polypeptide chains of
(a) 6 parallel layers
(b) 4 parallel layers.
(c) 3 parallel layers.
(d) 2 parallel layers.

15. Two α-chains of globin have identical amino acid composition of
(a) 111.
(b) 121.
(c) 131.
(d) 141.

16 The total number of amino acid in globin
(a) 544.
(b) 554.
(c) 564.
(d) 574.

17. Hemoglobin takes up the number of molecules of oxygen
(a) 1.
(b) 2.
(c) 4.
(d) 6.

18. Carboxyhemoglobin is formed by
(a) CO.
(b) CO_2.
(c) HCO_3.
(d) HCN.

19. One mol. of hemoglobin contains histidine residues
(a) 5.
(b) 15.
(c) 25.
(d) 35

20. Methemoglobin is formed as a result of the oxidation of hemoglobin by oxidizing agent
(a) Oxygen of air.
(b) Hydrogen peroxide.

Answers

14) b.
15) d.
16) d.
18) a.
19) d.
20) c.

(c) Potassium Ferricyanide
(d) Potassium permanganate

21 Methemoglobin can be reduced to hemoglobin by
(a) Removal of hydrogen
(b) Vitamin C
(c) Glutathione
(d) Creatinine

22 The colour of cyanomethemoglobin
(a) Yellow
(b) Pink
(c) Brown
(d) Bright red

23 The iron of heme is coordinated in β-chains at positions
(a) 43 and 72
(b) 53 and 82
(c) 63 and 92
(d) 73 and 102

24 Myoglobin contains the number of gram atom of iron per mole
(a) 1
(b) 2
(c) 3
(d) 4

25 Catalases contain the number of gram atoms of iron per mole
(a) 1
(b) 2
(c) 3
(d) 4

Answers

21) b

22) d

23) c

24) a.

25) d

Fill up the blanks of the followings :

1 Myoglobins are the _____ pigments occurring in the _____ cells of vertebrates and invertebrates.

2. The molecular weight of catalases is about _____

3 Tryptopban pyrrolase catalyzes the oxidation of _____ to _____

4 The fundamental role of cytochromes is in _____

Answers

1 Respiratory, Muscle

2 225000

3 Tryptophan, Formyl Kynurenine

4 Cellular respiration.

		Answers
5	Cytochrome C has a molecular weight of about ____ and contains ____ iron	5 13000 0 43 per cent.
6	The peptide chain of human heart cytochrome C contains ____ amino acids	6 104
7	Cytochrome oxidases can carry three general types of reactions e.g oxygen transfer ____ ____	7 Mixed function oxidation Electron transfer
8	Hemoglobin A has a molecular weight of ____ and contains ____ pairs of peptide chains	8 64 456 Two
9	The α-chains of hemoglobin A contains ____ and β-chain contains ____ amino acids.	9 141 146
10	Fetal hemoglobin comprises ____ per cent of the total hemoglobin in the new born	10 50 to 90
11	Fetal hemoglobin takes up ____ more readily at low oxygen tension and releases ____ more readily than adult hemoglobin (A)	11 O_2 CO_2
12	Hemoglobin F is gradually replaced by ____ during the first 6 months of extrauterine life	12 Hemoglobin A
13	Fetal hemoglobin is more resistant to denaturation by ____ and is more susceptible to conversion to methemoglobin by ____	13 Alkali Nitrites
14	Some of the abnormal hemoglobins are easily differentiated by their electrophoretic mobilities and have given rise to the concept of ____	14 Molecular disease
15	Acidic amino acid is replaced by a ____ or ____ amino acid for the formation of abnormal hemoglobin from normal hemoglobin	15 Basic Neutral
16	The abnormality of hemoglobin C is found in the β-chain at position ___, the amino acid glutamic acid is replaced by ____	16 6 Lysine
17	Hemoglobin C is characterized by the mild ____ with a tendency to ____	17 Anemia Infarction
18	In hemoglobin S ____ anemia develops and the ____ becomes long and boat shaped	18 Sickle cell RBC
19	The abnormality in hemoglobin S occurs in ____ chain glutamic acid at position 6 is replaced by ____	19 β Valine
20	If HbF is present in large amounts in the blood of adults it gets ____ rapidly producing ____	20 Hemolysed Thalassemia major
21	The abnormality of hemoglobin M is found in the α-chain the histidine residues in ____ and ____ positions are replaced by ____	21 58 87 Tyrosine

		Answers
22.	Methemoglobin is not reduced to _____ by _____ agents	22 Hemoglobin, Reducing
23	Trypsin splits the peptides in hemoglobin at points where only _____ and _____ occur	23 Lysine, Arginine
24	Methemoglobin is an _____ hemoglobin _____ is in the firm combination	24 Oxidized, Oxygen
25	When the concentration of methemoglobin reaches _____ per 100 ml of blood, _____ usually develops.	25 3 grams, Cyanosis
26	Methemoglobin is present in normal _____ about _____ per cent of the total hemoglobin.	26 Erythrocytes, 0 4
27	_____ hemoglobin combines with _____ to form sulfhemoglobin.	27 Reduced, H_2S
28	Sulfhemoglobin is also observed in subjects with marked _____ in the presence of _____ producing bacteria in the intestine	28 Constipation, Nitrite
29	Carboxyhemoglobin is formed by the combination of _____ with the _____ in the hemoglobin molecule	29 CO, Iron
30	Carboxyhemoglobin is produced by the excessive exposure to artificial illuminating _____ and to automobile _____ in closed or poorly ventilated rooms	30 Gas, Exhaust gases
31	The condition by which the excretion of both coproporphyrin and uroporphyrin increases is said to be _____	31 Porphyria.
32.	Erythropoietic porphyria has got tendency to _____ and defective _____	32 Hemolysis; Erythropoiesis.
33	In erythropoietic protoporphyria, there is increased _____ and _____ in the circulating erythrocytes, plasma and the feces	33 Protoporphyrin, Uroporphyrin.
34	Acute intermittent porphyria is due to a marked increase of hepatic _____	34 ALA synthase
35	Increased serum PBI and hepercholestemia occur in acute _____ porphyria	35 Intermittent.
36	In porphyria variegata there is increased hepatic _____	36 ALA synthase.
37	Hereditary coproporphyria causes increased urinary output of _____ and _____ during acute attacks	37 Porphobilinogen, ALA
38	Acquired porphyria is caused by severe _____ diseases and ingestion of certain _____	38 Liver; Toxins
39	In acquired porphyria, there is increased excretion of _____ in urine	39 Uroporphyrin.
40	Porphyria cutanea tarda shows frequent rise in serum _____	40 Iron

Indicate "True" or "False" of the followings :		Answers
1	Chlorophyll is magnesium containing porphyrin and the photosynthetic pigment of plants	1 True
2	Chlorophyll and heme of hemoglobin are synthesized in living cells by different pathways	2 False
3	The condensation of active succinate and glycine is catalyzed by the enzyme Amlev synthetase	3 True
4	Two mols of Amlev condense to form porphobilinogen catalyzed by the enzyme Amlev dehydrase	4 True
5	Tripyrrylmethane is formed by the condensation of 2 mols of porphobilinogen	5 False
6.	In low oxygen tension oxyhemoglobin takes up more oxygen	6. False
7	Histidine contained in hemoglobin exerts its buffering action through its basic imidazole ring for which hemoglobin plays an important role in regulating the acid base balance of blood	7 True
8	Methemoglobin can carry oxygen in blood	8 False
9	Sulphemoglobin formed by the administration of certain drugs continues to remain in the blood and can be converted into hemoglobin	9 False
10	Reduced hemoglobin in the absorption spectra shows one single broad band in the green region	10 True
11	Oxyhemoglobin in the absorption spectra shows three bands	11 False
12.	The biosynthesis of hemoglobin takes place in the α-cells of pancreas	12 False.
13	Iron in the ferrous state is incorporated into protoporphyrin to form heme	13 True
14	The iron of heme is coordinated to 2 imidazole nitrogen of histidine at positions 36 and 85	14 False
15	The function of erythrocruorins is dissimilar to hemoglobin	15 False
16	Catalases are zinc porphyrin enzymes	16 False
17	In plants, the activities of catalases are significant	17 False
18	The cytochromes are iron porphyrins and act as electron transfer agent in oxidation — reduction reactions.	18 True
19	The reduced form of cytochrome C is autooxidizable	19 False
20	Cytochrome q3 is found in kidney	20 False
21	Methemoglobin also functions as oxygen carrier	21 False
22.	Porphyrias are of two types — congenital and acquired.	22. True
23	Erythropoietic porphyria causes highly increased excretion of uroporphyrin I and to a lesser extent, coproporphyrin I in both urine and feces	23 True
24	Erythropoietic coproporphyria shows large amounts of coproporphysin II in the erythrocytes	24 False
25	Acute intermittent porphyria causes periodic attacks of abdominal pain which is associated with fever and leukocytosis	25 True

Match the followings

				Answers	
1	Catabolism of hemoglobin means	(A)	in the reticuloendothelial cells of the liver spleen and bone marrow	1	(G)
2	About 8 grams of hemoglobin	(B)	yields about 300 mg of bilirubin	2	(I)
3	The protoporphyrin	(c)	formed by the oxidation of hemoglobin by oxygen in presence of ascorbic acid is said to be choleglobin	3	(B)
4	When hemoglobin is catabolized in the body	(D)	is formed by the reduction of biliverdin by the enzyme bilirubin reductase requiring NAD^+ or $NADP^+$	4	(F)
5	The porphyrin portion is broken down mainly	(E)	35 mg. of bilirubin which is carried in loose association with plasma protein	5	(A)
6.	The green conjugated protein	(F)	the iron enters ferritin pool for reuse	6	(C)
7	Biliverdin the chief pigment of the bile in birds	(G)	conversion of hemoglobin to bile pigments and metabolism of bile pigments	7	(K)
8	Bilirubin the chief pigment in human bile	(H)	0 1 to 1 5 mg per cent	8	(D)
9	Biliverdin jaundice is largely limited to	(I)	contains 27 mg. of iron	9	(J)
10	One gram of hemoglobin yields	(J)	biliary obstruction as a result of carcinoma and severe parenchymal liver disease	10	(E)
11	Normal serum bilirubin level	(K)	is formed after removal of iron and cleavage of the porphyrin ring of heme	11	(H)
12	The feces acquires a green tinge	(L)	appears in the urine in the concentration of 0–4 mg per day	12	(P)

				Answers	
13	L stercobilinogen	(M)	when the concentration of the bile pigment in blood is more and diffuses into the tissues producing a yellow pigmentation	13	(Q)
14	L Stercobilin	(N)	is caused by the milder defect in the bilirubin conjugated system	14	(O)
15	Some of the urobilinogen	(O)	is an orange yellow pigment which gives the normal colour of the feces and is strongly levorotatory	15	(L)
16	The unabsorbed urobilinogen	(P)	when the intestinal flora is diminished by administration of antibiotics the bilirubin is auto-oxidized to biliverdin in contact with air	16	(R)
17	Jaundice occurs	(Q)	is the reduction product of bilirubin	17	(M)
18	Crigler Najjar syndrome Type II	(R)	is excreted in the stool in the concentration of 40—280 mg per day	18	(N)
19	Gilbert s disease	(S)	urobilinogen increased and bilirubin present in urine and urobilinogen decreased in feces	19	(U)
20	In hepatic jaundice	(T)	urobilinogen absent in urine and urobilinogen trace to absent in feces.	20	(S)
21	In hemolytic jaundice	(U)	is caused by the defect in the hepatic clearance of bilirubin	21	(W)
22	In obstructive jaundice	(V)	is owing to a primary metabolic defect in the conjugation of bilirubin	22	(T)
23	Crigler-Najjar syndrome Type I	(W)	urobilinogen increased and bilirubin absent in urine and urobilinogen increased in feces.	23	(V)
24	Unconjugated hyperbilirubinemia	(X)	results from liver dysfunction caused by chloroform, CCL_4 hepatitis virus and cirrhosis.	24	(X)

CHAPTER – 8
ENZYMES AND COENZYMES

Write the correct answer number of the followings

	Answer
1 The example of extracellular enzymes (a) Glucose-6-phosphatase (b) Hexokinase (c) Glucokinase (d) Pancreatic amylase	1) d.
2. Enzymes are usually named by adding the suffix ase to the main part of the name of the substrate on which they act except (a) Erepsin (b) Maltase (c) Lactase (d) Sucrase	2) a.
3 Some enzymes are named by their functions only e.g. (a) Ptyalin. (b) Pepsin. (c) Reductases (d) Trypsin	3) c.
4 Some enzymes acting on the substrates are freely described by the adjectives e g (a) Lactate dehydrogenase (b) Lipolytic (c) Cytochrome oxidase (d) Phosphatase	4) b
5 The enzyme which uses oxygen as hydrogen accepter e g (a) Tyrosinase (b) Succinate dehydrogenase (c) Aconitase. (d) Carboxylase	5) a
6 The enzyme which uses H_2O_2 as substrate e g (a) Catalase (b) Malate dehydrogenase (c) Ascorbic oxidase (d) Phosphorylase	6) a.

7. The enzyme which acts on single hydrogen donor with incorporation of oxygen e.g.
   (a) Succinate thiokinase.
   (b) Glycogen synthetase.
   (c) Tryptophan oxygenase.
   (d) Uricase.

8. The enzyme which acts on paired donors with incorporation of oxygen into one donor e.g.
   (a) Pyruvatekinase.
   (b) Enolase.
   (c) Steroid hydroxylase.
   (d) Glycerokinase.

9. The example of transglycosidase
   (a) Hexokinase.
   (b) Phosphorylase.
   (c) GPT.
   (d) Amino acid transacetylase.

10. Enzyme acting on peptide bonds e.g.
   (a) Hexokinase.
   (b) Chymotrypsin
   (c) GOT.
   (d) Glucose-6-phosphatase.

11. The peroxidase has the coenzyme
   (a) FAD^+.
   (b) NAD^+.
   (c) $NADP^+$.
   (d) None of the above.

12. Enzyme exergonic reaction means the system undergoes a loss of free energy e.g.
   (a) Synthetase.
   (b) Phosphatase
   (c) Hexokinase.
   (d) Urease.

13. The optimum temperature of plant urease in centigrade
   (a) 40
   (b) 37.
   (c) 45.
   (d) 60.

Answers

7) c.

8) c.

9) b

10) b

11) d.

12) d

13) d.

14 The maximum activity of the most of the enzymes is at the optimum pH
(a) Between 2 and 3
(b) Between 4 and 5
(c) Between 5 and 9
(d) Between 7 and 12.

15 Oxidases are generally inhibited by
(a) Cyanides
(b) Fluorides
(c) Salts of mercury
(d) Salts of silver

16 The group transferring coenzyme
(a) COA
(b) NAD^+
(c) $NADP^+$
(d) FAD^+

17 The coenzyme containing an aromatic heteroring in the structure
(a) Biotin
(b) TPP
(c) Sugar phosphate
(d) Coenzyme Q

18 The example of hydrogen transferring coenzyme
(a) $B_6 - PO_4$
(b) $NADP^+$
(c) TPP
(d) ATP

Answers
14) c.
15) a.
16) a.
17) b
18) b

Fill up the blanks of the followings .

1 Enzymes which are used in the cells which make them are said to be _____ enzymes.
2. An extracellular enzyme which is secreted ready for action is called a _____ secretion
3 Trypsinogen is activated by _____ to give active trypsin.
4 Prothrombin is activated by _____ to give active thrombin.
5 A dipeptide can be attacked by _____ enzymes

Answers
1 Intracellular
2 Zymase
3 Enterokinase.
4 Thromboplastin.
5 Three

		Answers
6	Enzymes catalyzing hydrolysis of ester peptide by the addition of water are called _____	6 Hydrolases
7	Enzymes catalyzing the linking together of two compounds couple to the breaking of a pyrophosphate bond in _____ or a similar compound are called _____	7 ATP Ligases
8	Enzymes that catalyze removal of groups from substrates without addition or removal of water are called _____	8 Lyases
9	The imidazole ring and the alcoholic group of serine are responsible for the union between substrate and enzyme These groups are termed _____ and the region of the protein surface at which they are located is termed _____	9 Active groups Active centre
10	Many enzymes are _____ proteins	10 Conjugated
11	Enzymes, the organic catalysts differ from inorganic catalysts in their extraordinary _____	11 Specificity
12	Many substrates form _____ with enzymes	12 3 bonds
13	Oxidoreductases function in biosynthetic processes in mammalian system tend to use _____ as reductant but those function in degradative processes tend to use _____ as oxidant	13 Reduced NADP NAD^+
14	In liver about _____ of the _____ enzyme occurs extramitochondrially	14 90% NADP specific
15	The _____ enzyme of mitochondria is specifically activated by _____	15 NAD - specific ADP
16	Trypsin of pancreatic juice is a mixture of _____ _____ and _____	16 Trypsin Chymotrypsin A Chymotrypsin B
17	Most active enzyme preparations are made by various processes of _____ involving _____ to remove inorganic impurities.	17 Purification Dialysis
18	The insoluble substrates must be made soluble by the help of _____ substances whenever required for good mixing with _____	18 Hydrotropic Enzymes
19	The substrate concentration that produces _____ velocity is called _____ or _____	19 Half maximal Michaelis constant Km value
20	At the temperature O°C most enzymes are practically _____	20 Inactive
21	Pepsin works only in _____ medium and is inactivated by making the medium _____	21 Acid Alkaline
22	At low pH values E will be _____ and lose its _____ charge	22 Protonated Negative

		Answers
23	The loss of enzyme activity by oxidation may be regained by _____ or _____	23 Cystein, Glutathione
24	High energy radiation forms _____ which causes _____ of the enzyme resulting loss in enzyme activity	24 Peroxides Oxidation
25	Some enzymes which are activators are called _____	25 Kinases
26	The best preservative for enzyme solution is _____	26 Toluene
27	Enzymes are destroyed by _____	27 Formaldehyde.
28	The enzymes depending for their activity on the presence of free — SH groups can be inactivated by _____ which reacts with the free — SH groups.	28 Mercuric chloride
29	Sulphanilamide competes _____	29 P aminobenzoic acid.
30	Enzymes which show allosteric inhibition have two sites namely _____ and _____	30 Isosteric site Allosteric site
31	Inhibition of enzymes may also be reversed by removal of the inhibitor by the treatment with _____	31 H_2S
32.	It is believed that the mucus membrane contains suitable _____ for which the alimentary canal is not digested by its own secretions	32. Anti-enzymes.
33	The non protein organic molecules and the prosthetic groups of enzymes are called _____	33 Coenzymes.
34	The chief function of CoA is to carry _____ groups.	34 Acyl.
35	The coenzyme TPP is also called _____ which carries _____ group	35 Co-carboxylase Active aldehyde
36	The chief function of tetrahydrofolic acid as a carrier of _____ and it is used in the synthesis of _____ and _____	36 Formate Purines, Pyrimidines
37	In dehydrogenation reactions _____ and _____ coenzymes function as hydrogen acceptors.	37 NAD^+ $NADP^+$
38	Lysosomes can be regarded as a type of _____	38 Ground substance.
39	Lysosomes are responsible for post mortem _____	39 Autolysis.
40	Lysozyme has a molecular weight of about _____ and consists of a single polypeptide chain of _____ amino acid residues having no _____ or _____ cofactors	40 15000 129 Coenzymes Metal ion
41	Lysozyme has a central _____ site with _____ subunits.	41 Catalytic 6
42.	The enzymes that occur in a number of different forms and differ each other chemically electrophoretically and immunologically are called _____	42. Isoenzymes.
43	Lactate dehydrogenase exists in _____ isozymic forms	43 Five

Indicate "True" or "False" of the followings :

		Answers
1	Lysosomes can be regarded as a type of Ground substance	1 True
2	The enzyme systems present in lysosomes are for oxidation - reduction processes	2 False
3	Lysozyme destroys the cell wall of many airborne gram positive bacteria in tears and nasal mucus	3 True
4	Isozymes are present in the serum and tissues of mammals birds insects, plants and unicellular organisms	4 True
5	The isozymic forms of lactate dehydrogenase are trimers	5 False
6	Splitting and reconstitution of lactate dehydrogenase I_5 produces new isozymes	6 False
7	LD5 is usually predominant in the tumors	7 True
8	The kinetic or collision theory states that the molecules to react must collide and must possess sufficient energy to overcome the energy barrier for reaction	8 True
9	If some molecules have insufficient energy to react increased temperature which increases their kinetic energy will decrease the rate of the reaction	9 False
10	Hydrophobic groups and charged groups both are not involved in substrate binding	10 False
11	In the absence of substrate the catalytic and the substrate-binding groups are several bond distances removed from one another	11 True
12.	The catalytic activity of certain key enzymes can be irreversibly decreased or increased by small molecules	12 False
13	Many enzymes catalyze a reaction between two or more substrates producing one product	13 False
14	Reactions whose rates vary as regards to changes in H^+ or H_3O^+ concentration but are independent on the concentration of other acids or bases present in the solution are said to be specific acid or specific base catalysis	14 True
15	More than 25 per cent of the enzymes contain tightly bound metal ions for their activity	15 True
16	Most kinases form substrate - bridge complexes of the type Enz nucleotide — M	16 True
17	The metal ion in pyruvate kinase also holds one substrate (ATP) to activate it	17 True
18	Enzymes whose concentration in a cell is dependent of an added inducer are termed constitutive enzymes	18 False
19	Permeases have many properties in common with enzymes and perform functions like cytochromes in electron transport.	19 True

		Answers
20	Protein synthesis takes place in the nuclei	20 False
21	Increase in the enzyme activities in C S F is reflected in the blood	21 False
22	Lactate dehydrogenase activity in C S F is increased in meningitis	22 True
23	The serum acid phosphatase concentration is in metastatic prostatic carcinoma	23 True
24	Serum amylase concentration is decreased in liver disease	24 True
25	Creatine phosphokinase level in serum is increased in muscular dystrophy	25 True

Match the followings :

			Answers	
1	Serum Aldolase level	(A) in normal pregnancy from fourth month	1	(G)
2.	The normal level of serum lipase	(B) 59 — 153 4 μ kat/l (5 — 13 units K.A)	2.	(D)
3	CoA	(C) is seen in untreated pernicious anemia	3	(I)
4	Serum oxytocinase level is increased	(D) 0 93 — 6 96 μ Kat/l (0 2 — 1 5 units/dl)	4	(A)
5	The normal level of SGOT	(E) in Wilson s disease	5	(J)
6	Serum ceruloplasmin (Ferroxidase activity) level is decreased	(F) in cirrhosis and hemolytic jaundice	6	(E)
7	The normal level of alka line phosphatase	(G) is increased in hemolytic anemia	7	(B)
8	A great rise in serum LDH	(H) 1 50 — 3 34 μ Kat/l (90 — 200 I U /liter)	8	(C)
9	SGOT is higher than SGPT	(I) is involved in ketone body formation	9	(F)
10	SGPT is always higher than SGOT	(J) 40 1 — 320 8 n Kat/l (6—25 I U./liter)	10	(N)
11	Normal level of serum LDH	(K) in nephrotic syndrome	11	(H)
12	Serum Lipase level is decreased	(L) in muscle trauma, polymyositis MC Ardle s syndrome etc	12	(Q)
13	Serum cholinesterase level is increased	(M) occur in parietal cells of the stomach	13	(K)
14	Serum Glucose-6 phos phate dehydrogenase level is decreased	(N) in viral hepatitis	14	(X)

			Answers
15. Serum Isocitrate dehydrogenase level is increased	(O)	2.48 — 5.58 μ Kat/l. (0 8 — 32 I.U./liter).	15. ..... (Y)
16. Normal serum amylase level	(P)	the enzymes for hydroxylation are present.	16. ..... (O)
17. Normal serum CPK level	(Q)	in vitamin A deficiency and diabetes mellitus.	17. ..... (W)
18. Serum CPK level is increased	(R)	in liver and kidney as well as in erythrocytes. Hence serum levels are not affected by hemolysis.	18. ..... (L)
19. Much larger quantities of carbonic anhydrase	(S)	zinc-protein complex.	19. ..... (M)
20. CPK is apparently not present	(T)	during myocardial injury.	20. ..... (R)
21. Transaminases exist	(U)	in acute diseases of the pancreas.	21. ..... (V)
22. Carbonic anhydrase is a	(V)	in isozyme forms.	22. ..... (S)
23. Carbonic anhydrase is involved	(W)	12 — 99 U/l for males (By Rosalki method).	23. ..... (Z)
24. LDH is liberated to the blood stream	(X)	congenital deficiency causes hemolytic anemia.	24. ..... (T)
25. Serum trypsin level is increased	(Y)	in liver diseases.	25. ..... (U)
26. In microsomes	(Z)	in hydrogen ion secretion.	26. ..... (P)

CHAPTER – 9
BIOLOGIC OXIDATION

Write the correct answer number of the followings :

	Answers
1 Oxidases are conjugated proteins having the prosthetic group (a) Magnesium (b) Manganese (c) Copper (d) Iron	1) c
2 Cytochrome oxidase is (a) a_3 (b) aa_3 (c) a (d) None	2) b
3 Cytochrome oxidase is poisoned by (a) Cyanide (b) Sulphide (c) Sulphite (d) Sulphate	3) a
4 Phenolase is an enzyme containing (a) Cobalt (b) Iron (c) Zinc (d) Copper	4) d
5 Monoamine oxidase oxidizes (a) Epinephrine (b) Norepinephrine (c) Glucagon (d) Glutathione	5) a
6 Aerobic dehydrogenases have the prosthetic group (a) ATP (b) NAD^+ (c) FAD^+ (d) $NADP^+$	6) c

7 Uricase catalyzes the oxidation of uric acid to
(a) Carbon dioxide
(b) Ammonia.
(c) Glyoxal
(d) Allantoin

8 Xanthine dehydrogenase converts purine bases to
(a) Uric acid.
(b) Hypoxanthine
(c) Xanthine
(d) Urea.

9 Alcohol dehydrogenase from liver contains
(a) Copper
(b) Zinc
(c) Sodium
(d) Potassium

10 NADP linked dehydrogenases in the extramitochondria are found to synthesize
(a) Urea
(b) Steroid
(c) Ascorbic acid.
(d) Niacin

11 The endoplasmic reticulum cytochrome
(a) b
(b) c
(c) a_1
(d) P—450

12 Mono-oxygenases are found in the
(a) Mitochondria
(b) Microsomes.
(c) Nuclei
(d) Cytosol.

13 The mitochondrial superoxide dismutase contains
(a) Mg^{++}
(b) Zn^{++}
(c) Mn^{++}
(d) CO^{++}

Answers

7) d

8) a.

9) b

10) b

11) d

12) b

13) c

14 When substrates are oxidized through an NAD linked dehydrogenase the P O ratio is
(a) 1
(b) 2
(c) 3
(d) 4

15 The antibiotic piericidin A inhibits the site of the respiratory chain
(a) I
(b) II
(c) III
(d) All of the above

16 The uncoupling agent of oxidative phosphorylation
(a) Antimycin A
(b) Dicoumarol
(c) Barbiturates
(d) Penicillin

17 The oxidation and phosphorylation in intact mitochondria is completely blocked by
(a) Streptomycin
(b) Gentamycin
(c) Puromycin
(d) Oligomycin

18 The high energy compound is
(a) UDPG
(b) ATP
(c) ADP
(d) Arginine phosphate

Answers

14) c

15) a

16) b

17) d

18) a

Fill up the blanks of the followings

1 Mitochondria are generally impermeable to _____ and other _____

2 Uncouplers like DNP increase the permeability of mitochondria to _____ reducing the electrochemical potential for the generation of _____

3 The P/H^+ (transported out) quotient of the ATP _____ is _____

4 The electrochemical potential difference is utilized to drive a membrane-located _____ which in the presence of _____ forms ATP

Answers

1 Protons ions

2 Protons ATP

3 Synthetase ½

4 ATP synthetase ADP + Pi

		Answers
5	The respiratory chain is folded into _____ oxidation/ reduction loops in the membrane	5 Three
6	A single loop consists of a _____ carrier and an _____ carrier	6 Hydrogen, Electron.
7	The phosphorylating subunits consist of several proteins collectively known as an _____ subunit which projects into the matrix containing the _____	7 Fi ATP sythetase
8	For every proton pair passing through the _____ complex, one _____ molecule is formed from ADP + Pi	8 Fo — Fi, ATP
9	A proton pair attacks one oxygen of Pi to form _____	9 H_2O
10	Actively respiring mitochondria accumulate _____	10 Cations.
11	Coenzyme Q has a structure very similar to vitamin _____ and vitamin _____	11 K, E.
12.	Coenzyme Q is the constituent of _____ lipids	12 Mitochondrial.
13	Thyroxine is an uncoupling agent and causes _____ of the mitochondria	13 Swelling
14	The process by which ADP is phosphorylated by Pi to _____ in the respiratory chain is called _____	14 ATP; Oxidative phosphorylation.
15	Various oxidations occur in other parts of the cell except mitochondria and liberate _____ instead of _____	15 Heat, Energy
16	Site III of respiratory chain is inhibited by _____ and _____	16 Dimercaprol Antimycin A
17	There must be a redox potential of about _____ volts between components of the respiratory chain if that particular site is to support the coupled formation of 1 mole of _____	17 O 2 ATP
18	The toxicity of oxygen is due to its conversion to _____	18 Superoxide
19	Superoxide is formed when _____ flavins are reoxidized by molecular _____ in the respiratory chain	19 Reduced Oxygen.
20	Superoxide can be removed by the specific enzyme _____ in the presence of _____	20 Superoxide dismutase, protons.
21	The sequence of enzymes and carriers responsible for the transport of reducing equivalents from substrate to molecular oxygen is known as _____	21 Respiratory chain
22.	In oxidation and reduction reactions, the free energy exchange is proportionate to the tendency of reactants to donate or accept electrons is called _____	22. Redox potential.

CHAPTER – 10
INBORN ERRORS OF METABOLISM

Fill up the blanks of the followings

		Answers
1	Inborn errors of metabolism are a group of metabolic _____ which can be formed as a result of the lack of a single _____ in a single metabolic pathway	1 Diseases Enzyme
2	The hereditary disorder in which acid mucopolysaccharides are excessively deposited in tissues is called _____ in which there is corneal opacity	2 Hurler's syndrome
3	Gaucher s disease is caused by the deficiency of____	3 Glucocerebrosidase
4	In Gaucher's disease, the spleen is significantly _____ and there are signs of leukopenia and _____	4 Increased Thrombocytopenia
5	The deficiency of sphingomyelinase causes _____ which occurs in _____	5 Niemann Pick disease Infancy
6	In Niemann Pick disease the liver and spleen are _____	6 Enlarged
7	Tay sach s disease caused by the deficiency of the enzyme _____ in tissues is characterized by the increased accumulation of _____ in brain and spleen	7 Hexosaminidase A Gangliosides.
8	In Tay sach s disease, there is repeated respiratory tract _____ of the patient and the patient expires at the third and fourth _____	8 Infections Years
9	The deficiency of the enzyme ceramide trihexosidase causes _____ in which large amounts of _____ are accumulated in the kidney	9 Fabry s disease Ceramide trihexoside
10	The deficiency of phytanic acid oxidase causes _____ in which large amounts _____ are accumulated	10 Refsum s disease Phytanic acid
11	In Refsum s disease there is night blindness and narrowing of the _____ fields	11 Visual
12.	Krabbe sdisease results in the deficiency of the enzyme _____ which catalyzes the hydrolysis of _____ to form ceramide and galactose	12 Galactocerebrosidase Galactocerebroside
13	Galactocerebroside is the important component of _____	13 Myelin
14	Albinism appears in the total absence of _____ inside the _____ in the skin	14 Tyrosinase Melanocytes
15	Tyrosinosis is due to the absence either of hepatic _____ or of _____ activities	15 P hydroxyphenyl pyruvate hydroxylase Tyrosine transaminase

		Answers
16.	Hereditary tyrosinemia is caused by the deficiency of _____ and this disorder is not controlled by the administration of _____	16 P hydroxyphenylpyruvic acid oxidase, Ascorbic acid.
17	Neonatal tyrosinemia occurs in the _____ and the urine contains _____, _____, P—hydroxyphenyllactic acid and P-hydroxyphenylacetic acid.	17 New born Tyrosine, P hydroxyphenylpyruvic acid
18.	Hereditary tyrosinemia is similar to neonatal tyrosimia, except the large amount of _____ is present in the urine	18 P hydroxyphenyl lactic acid.
19	Phenylketonuria is caused by the absence of the enzyme _____ for which phenylpyruvic acid, phenyllactic acid, phenylacetic acid are formed, the later is converted to _____ which is excreted in the urine.	19 Phenylalanine hydroxylase, Phenylacetylglutamine
20	Patients with phenylketonuria tend to have a deficiency of _____	20 Serotonin.
21	Alkaptonuria is characterized by the excretion of _____ in the urine owing to the lack of _____	21 Homogentisic acid, Homogentisic acid oxidase
22.	In alkaptonuria the urine is _____ due to the oxidation of _____ in air	22 Dark, Homogentisic acid
23	In later life the accumulation of dark piement in cartilages and tendons gives rise to the condition known as _____ which is accompanied by _____ changes.	23 Ochronosis Arthritic
24	Maple syrup urine disease is characterized by the absence of the enzymes required for the _____ of the _____ acids derived from the branched chain amino acid - valine, leucine and isoleucine	24 Oxidative decarboxylation Keto.
25	The urinary excretion of the keto acids derived from branched chain amino acids produces an odour like that of _____ or _____	25 Maple syrup Burnt sugar
26.	The infant suffering from maple syrup urine disease is difficult to _____ and may _____	26 Feed, Vomit
27	Hartnup's disease is characterized by a _____ like skin rash and mental deterioration in the abnormal metabolism of _____	27 Pellagra Tryptophan.
28.	The urine of the patients suffering from hartnup's disease contain highly increased amounts of _____ as well as _____	28 Indole acetic acid, Tryptophan
29	Glycinuria is associated with the excess urinary excretion of _____ with a tendency to form _____ renal stones.	29 Glycine Oxalate
30	The clinical findings _____ are the occurrence of thrombosis, osteoporosis, dislocated lenses in the eyes and frequently mental retardation	30 Homocystinuria

		Answers
31	In homocystinuria a low _____ and a high _____ diet effectively prevent this condition if treated earlier	31 Methionine Cystine
32.	The metabolic block of histidine is due to the insufficient activity of liver _____ which impairs the conversion of histidine to _____	32 Histidase Urocanic acid
33	Histidine excretion is also increased during normal _____ but not in _____ of pregnancy	33 Pregnancy Toxaemia
34	In hypervalinemia the infants suffer from _____ growth _____ wasting and vomiting	34 Stunted Muscle
35	Lesch Nyhan syndrome is chractenzed by the complete deficiency of _____ whieh causes hypoxanthine or guanine to form a nucleotide with _____	35 Phosphoribosyl transfers PRPP
36	Lesch — Nyhan syndrome appears in childhood as a severe neurological syndrome which is sometimes accompanied by _____ Such patients show an increased tendeney to _____	36 Gout Myocardial infarction
37	In hereditary xanthinuria, there is the deficiency of _____. The urinary excretion contains large amounts of _____ with lesser amounts of _____	37 Xanthine oxidase Xanthine Hypoxanthine
38	Orotic aciduria causes the excessive production of _____ due to the deficiency of _____ The urinary excretion consists of large amounts of _____ nucleotide precursor	38 Orotic acid Orotate phosphoribosyl transferase Pyrimidine
39	In orotic aciduria the urine of the patient becomes cloudy on cooling with the deposition of needle-shaped crystals of _____ children affected by this condition develop a severe _____ anemia with physical and mental _____	39 Orotic acid Megaloblastic Retardation
40	Homocystinuria appears due to the lack of _____ in the liver due to which both _____ and _____ are accumulated in blood and urine	40 Cystathionine synthetase Homocystine Methionine
41	Cystine is an _____ amino acid which may precipitate in the kidney tubules to form _____ in cystinuric patients	41 Isoluble Cystine Calculi
42	Cystinuria is a misnomer so that _____ may be preferred as the descriptive term for this disease	42 Cystine Lysinuria
43	The plasma content of glycine is normal in glycinuric patients while the urinary excretion of glycine ranges from _____ /d	43 600 — 1000 mg
44	Maple syrup urine disease is recognized by central nervous system manifestations of _____ and attacks of flaccidity and _____	44 Convulsions Apnea

CHAPTER – 11
WATER AND MINERAL METABOLISM

Write the correct answer number of the followings :

Answers

1 Of the total body weight the average body water in per cent
(a) 55 to 65
(b) 50 to 60
(c) 60 to 70
(d) 70 to 80

1) c.

2. Intracellular fluid contains the per cent of the total body weight
(a) 45
(b) 50
(c) 55
(d) 60

2) b.

3. The per cent of plasma in the extracellular fluid :—
(a) 3.5
(b) 3 8
(c) 4.2.
(d) 4.5

3) d.

4 Transcellular fluid in per cent of extracellular fluid
(a) 1 4
(b) 1.5
(c) 1 6
(d) 1 8

4) b.

5 Organic compounds of small molecular size
(a) Urea.
(b) Uric acid.
(c) Creatinine.
(d) Phosphates.

5) a.

6 Organic substances of large molecular size
(a) Starch.
(b) Inulin.
(c) Lipids.
(d) Proteins.

6) d.

7 100 grams of fat on combustion produces water in ml
(a) 107
(b) 105
(c) 102
(d) 98

8 100 grams of proteins on combustion yields water in ml
(a) 35
(b) 38
(c) 41
(d) 44

9 The daily intake and output of water through different sources in ml
(a) 2500
(b) 2700
(c) 2800
(d) 3000

10 The amount of internal secretion per day in liters
(a) 4 to 7
(b) 5 to 8
(c) 6 to 9
(d) 7 to 10

11 The amount of water excreted per day in feces in ml
(a) 90
(b) 95
(c) 100
(d) 105

12 The regulatory mechanism of body water is influenced by the hormone
(a) Oxytocin
(b) ACTH
(c) FSH
(d) Epinephrine

13 All chemical reactions in the body proceed in presence of
(a) Organic salts.
(b) Inorganic salts
(c) Water
(d) Alkali

Answers

7) a.

8) c

9) b

10) d

11) c

12) a

13) c

14 The symptom of water intoxication
(a) Muscular weakness.
(b) Anemia.
(c) Paralysis.
(d) Fever

Answers
14) a.

15 Debydration may be ordinanly corrected by parenteral ingestion of the solution of
(a) Zncl
(b) Mgcl.
(c) $Cacl_2$
(d) Nacl

15) d.

16. A mixture of two-thirds isotonic saline solution and one-third lactate solution should be administered intravenously in case of
(a) Constipation.
(b) Prolonged diarrhea.
(c) Malaria.
(d) Jaundice.

16) b

17 Dehydration is a problem as regards to
(a) Vomiting.
(b) Typboid.
(c) Uremia.
(d) Hot climates.

17) c.

18. The principal mineral elements are also said to be
(a) Macronutrients.
(b) Micronutrients.
(c) Semi micronutrients.
(d) None of the above.

18) a.

19 The trace elements are subdivided into
(a) Two groups.
(b) Three groups.
(c) Four groups.
(d) None of the above.

19) b

20 The essential trace elements include
(a) Lead.
(b) Nickel
(c) Boron.
(d) Cobalt.

20) d.

Answers

21 The number of principal mineral elements
(a) Five
(b) Six
(c) Seven
(d) Eight

21) c

22. Zinc is a constituent of
(a) Carbonic anhydrase
(b) Malate dehydrogenase
(c) Aldolase
(d) Amylase

22) a

23 Hemoglobin formation needs both
(a) Iron and zinc
(b) Iron and calcium
(c) Iron and copper
(d) Iron and magnesium

23) c

24 Cobalt is a constituent of
(a) Folic acid
(b) Vitamin B_{12}
(c) Niacin
(d) Biotin

24) b

25 Calcium is required for the activation of the enzyme
(a) Isocitrate dehydrogenase
(b) Fumarase
(c) Succinate thiokinase
(d) ATPase

25) d

26 The absorption of calcium is increased by the dietary higher levels of
(a) Fats.
(b) Proteins.
(c) Cereals.
(d) *Vitamin A*

26) b

27 Calcium absorption is interfered by
(a) Fatty acids
(b) Amino acids
(c) Vitamin D
(d) Vitamin B_{12}

27) a.

28 Calcium in the complex form is about
(a) 8 mg%
(b) 6 mg%
(c) 4 mg%
(d) 2 mg%

29 The percentage of calcium in milligram in nonionized form is about
(a) 3
(b) 4
(c) 5
(d) 6

30 In man the number of grams of calcium filtered in 24 hours by the renal glomeruli is about
(a) 8
(b) 10
(c) 12.
(d) 14

31 The percentage of calcium excreted in the feces
(a) 40 to 60
(b) 50 to 70
(c) 60 to 80
(d) 70 to 90

32. The daily loss of calcium in mg in sweat is about
(a) 12.
(b) 13
(c) 14
(d) 15

33 The concentration of serum calcium may drop below 7 mg/100 ml in
(a) Hyperparathyroidism
(b) Hypoparathyroidism.
(c) Tetany
(d) Rickets.

34 Serum phosphate concentration is 1 to 2 mg/100 mL in
(a) Rickets.
(b) Tetany
(c) Osteoporosis.
(d) Hyperthyroidism.

Answers

28) d.
29) c.
30) b
31) d.
32) d.
33) b
34) a.

Fill up the blanks of the followings .

		Answers
1	There is virtually no calcium in _____	1 Erthrocytes.
2	Calcium exists in the plasma in _____, _____, _____ forms	2 Diffusible Nondi ffusible Complexed
3	The active transport process of calcium is regulated by _____ a metabolite of vitamin _____ which is produced in the kidney in response to low plasma _____ concentration	3 1 25 dihydroxy cholecalciferol D Ca^{++}
4	Ionized calcium is required in blood _____ process	4 Coagulation
5	Under optimal conditions the percentage of calcium absorbed is _____	5 75
6	Decreased ionized fraction of serum calcium causes _____	6 Tetany
7	_____ which occurs in cereal grains forms insoluble salts _____ with calcium and magnesium resulting in the impaired absorption of calcium	7 Phytic acid Phy tin
8.	An increase in the _____ calcium levels in the plasma is the stimulus for the production of _____ which then causes a deposition of calcium in bone	8 Ionized Calcito- nin
9	Succinate dehydrogenase requires _____ for its acti vation	9 Calcium
10	In rickets, serum alkaline phosphatase activity is _____	10 Increased
11	Renal rickets are more accurately designated _____ which is an inherited disease	11 Familial hypopho- sphatemic rickets
12	Vitamin D in ordinary doses does not relieve renal ri ckets. Hence it is sometimes referred to as _____	12 Vitamin D resis tant rickets
13	Phosphorus is essential for the formation and develop- ment of bones and _____ along with _____	13 Teeth Calcium
14	Phosphorus is required in the absorption of glucose by _____	14 Phosphorylation
15	High calcium diet and phytic acid _____ phosphorus absorption	15 Decrease
16	The normal inorganic phosphate of plasma is _____ per 100 ml in adults.	16 3 to 4 5
17	The reabsorption of phosphorus is inhibited by _____	17 Parathyroid hor mone
18	Magnesium is the principal _____ of the soft tissue	18 Cation
19	Co-carboxylase is provided with _____	19 Magnesium
20	Parathyro d hormone _____ the absorption of mag nesium	20 Increases.

		Answers
21	In case of Kwashiorkor, the serum magnesium level is _____ causing _____	21 Low Weakness.
22.	In uremia the serum magnesium level is _____	22. Low.
23	Sodium is largely associated with _____ and _____ in regulation of acid-base equilibrium.	23 Chloride Bicarbonate
24	In the absorption of glucose and galactose _____ ion plays an important role	24 Sodium.
25	In man erythrocytes contain _____ or _____ sodium	25 Little No
26	_____ increases plasma sodium level	26 Aldosterone
27	Loss of sodium by excessive sweating causes _____ with the intense and painful contractions of _____ of men working hard in hot humid climates.	27 Heat cramps Skeletal muscle
28.	The metabolism of sodium is regulated by _____	28 Adrenocortical steroids.
29	Hypernatremia occurs in prolonged treatment of _____ and _____ as well as _____ hormones	29 Cortisone ACTH Sex.
30	In certain stages of pregnancy the steroid hormones cause the retention of _____ as well as _____ which results in gain in weight	30 Sodium Water
31	Pyruvate kinase requires _____ for maximal activity	31 K^+
32	Protein biosynthesis by ribosomes requires _____	32. Potassium.
33	_____ decreases serum potassium level.	33 Aldosterone
34	In man, _____ contain large amounts of potassium	34 Erythrocytes.
35	The serum potassium level _____ during increased carbohydrate utilization following administration of _____	35 Decreases Insulin.
36	The symptoms of hyperkalemia are corrected by the administration of _____ which helps the excretion of _____	36 Deoxycorticosterone Potassium.
37	Prolonged hypokalemia causes injury to _____ and _____	37 Myocardium Kidneys.
38	Hypokalemia is exhibited in heart failure treatment with _____	38 Digitalis.
39	Chloride ion is important as an activator of _____	39 Amylase
40	Excessive consumption of _____ causes _____ in protein deficiency	40 NaCl Edema.
41	Sodium concentration in sweat is decreased by _____	41 Aldosterone
42.	Hypochloremic alkalosis may develop in _____ disease or after the administration of _____ or _____	42. Cushing's ACTH, cortisone.

		Answers
43	Sulfur is present primarily in the cell protein in the form of _____ and _____	43 Cysteine Methionine.
44	A small amount of sulfide if absorbed into the blood stream is rapidly oxidized to _____	44 Sulfate
45	An increase in the blood indican concentration may occur in _____	45 Uremia
46	Marked sulfate retention in advanced glomerulonephritis causes the development of _____	46 Acidosis.
47	The nonheme iron is completely _____ which exists in the form of _____ and _____	47 Protein bound, Storage, Transport
48	Iron absorption is enhanced by proteins of _____ digestive products forming _____	48 Low molecular weight, Iron chelate
49	Most of the iron in food occurs in the _____ state	49 Ferric
50	Phytic acid and oxalates _____ with iron absorption	50 Interfere

Indicate "True" or "False" of the followings :

		Answers
1	The total iron content of the normal adult is about 4 to 5 grams	1 True
2	About 60 to 70 per cent of the total iron is present in hemoglobin.	2 True
3	About 1 0 per cent of the total iron is carried in the plasma in combination with β globulin transport protein transferrin	3 False
4	Iron is not involved in the cellular respiration	4 False
5	The richest sources of iron are egg yolk fish figs and apples	5 False
6	A defect in hemoglobin synthesis in anemia is commonly found in copper deficiency	6 True
7	In humans, heme is broken down in the mucosa and iron appears in the plasma transferrin	7 True
8	Vitamin D and alkali increase iron absorption	8 False
9	Apoferritin in the mucosal cells, is the controlling factor for iron absorption	9 False
10	Transferrin or siderophilin is a specific iron binding protein	10 True
11	Only the reticulocytes can utilize the ferric ion bound to transferrin although reticulocytes and mature erythrocytes can take up unbound ferric ion	11 True
12	In hepatic diseases both the bound iron and the total iron binding capacity of the plasma is high	12 False
13	Ferritin is not present in the intestine and also in liver	13 False
14	Iron deficiency anemia is not prevalent among children and adolescent girls	14 False

		Answers
15	Copper is required for melanin formation, phospholipid synthesis and collagen synthesis.	15. True.
16.	80 per cent of the red blood cell copper is present as superoxide dismutase (erythrocuprein).	16. True.
17	The daily biliary excretion of copper is 0.5 to 1.3 mg.	17. True.
18.	Wilson's disease is not a rare hereditary disorder of copper metabolism.	18. False.
19	The absorption of copper from the intestine is very low.	19. False.
20	Excessive deposition of copper in the liver causes hepatic cirrhosis.	20. True.
21	The excess of tissue copper can be removed by administering the copper chelating agent penicillamine.	21. True.
22.	Ceruloplasmin is a copper binding plasma protein.	22. True.
23.	Triodothyronine is less active than thyroxine in many respects.	23. False.
24	The body normally contains about 10 to 20 grams of iodine.	24. False.
25.	All the iodine in saliva is organic.	25. False.
26.	Muscles contain less amount of iodine.	26. False.
27	Erythrocytes contain no inorganic iodine.	27. False.
28	Most tissues contain less amount of iodine in the inorganic form and most of the iodine is present in the organic form.	28. True.
29	90 per cent of the iodine of the thyroid gland is in organic combination	29. True.
30.	The urinary elimination of iodine is largest when the intake is high.	30. False.
31	Urinary iodine is increased by exercise and other metabolic factors	31. True.
32.	In children severe iodine deficiency results in the extreme retardation of growth which is known as cretinism.	32. True.
33.	Fluorine in combination with vitamin C is required for the treatment of osteoporosis.	33. False.
34	Sodium fluoride is a powerful inhibitor of phosphoglycerate kinase.	34. False.
35	Fluoride ions inhibit the metabolism of oral bacterial enzymes and diminish the local production of acids which cause the production of dental caries.	35 True.
36.	The retina contains a zinc metalloenzyme, retinene reductase which is required for the formation of retinene.	36. True.
37	Zinc is not concerned with the healing of wounds.	37. False
38.	High concentration of zinc are present in spermatozoa, prostate, and epididymis.	38. True.
39.	The lowest concentration of copper occurs in the choroid of the eye.	39. False.

		Answers
40	Zinc present in cereals is highly absorbed owing to the presence of phytic acid	40 False
41	The deficiency of zinc causes hepatosplenomegaly delayed closure of the epiphyses of the long bones and anemia	41 True
42.	Methyltetrahydrofolate oxidoreductase and homocysteine methyl transferase require vitamin B_{12} for activity	42 True
43	Cobalt in large doses develops a condition known as polycythemia	43 True
44	Manganese ions inhibit lipid peroxidation reactions	44 True
45	The heart and the brain are the main storage organs for manganese	45 False
46	Manganese toxicity develops hepatolenticular degeneration resembling Parkinson's disease	46 True
47	Liver and kidney contain less amount of molybdenum than other tissues.	47 False
48	Selenium is essential for normal growth and fertility of human beings	48 False
49	Glutathione peroxidase, a selenoprotein catalyzes the peroxidation of glutathione	49 True
50	Selenium is involved in immune mechanism and ubiquinone synthesis	50 True
51	Chromium potentiates the action of insulin in accelerating utilization of glucose in humans	51 True
52	Chromium deficiency is characterized by impaired growth disturbances in glucose, lipid and protein metabolism	52 True

Match the followings

				Answers	
1	Calcium is required for the activation of	(A)	Prolonged vomiting and diarrhea resulting in excessive loss of digestive juices rich in sodium ion	1	(F)
2	The daily requirement of calcium for adults	(B)	Overactivity of adrenal cortex (Cushing s syndrome) which causes increased excretion of potassium in urine	2	(I)
3	Calcium distributed in C S F	(C)	Muscular weakness irritability paralysis, tachycardia and dilatation of the heart	3	(J)
4	The renal threshold for phosphate excretion	(D)	For oxidative phosphorylation	4	(E)
5	The normal level of magnesium in blood	(E)	About 2 mg/100 ml of plasma	5	(O)
6	Magnesium functions as a cofactor	(F)	Succinate dehydrogenase ATPase and certain proteolytic enzymes	6	(D)
7	Magnesium distributed in muscle	(G)	310 — 340 mg/100 ml	7	(R)
8	Sodium ion is involved in	(H)	Initiating and maintaining the heart beat	8	(H)
9	The normal level of sodium in blood	(I)	800 mg	9	(G)
10	The daily requirement of sodium for adults	(J)	4 5 — 5 0 mg/100 ml	10	(U)
11	The normal daily losses of sodium	(K)	Severe dehydration reduced blood pressure decreased blood volume and circulatory failure	11	(W)
12.	The symptom of hyponatremia	(L)	96 — 105 m mol/liter	12	(A)
13	The symptom of hypernatremia	(M)	75 — 175 mcg/100 ml	13	(P)

14	Hypernatremia occurs in	(N)	Prolonged treatment of cortisone and ACTH as well as sex hormones	14	(N)
15	Hyponatremia causes	(O)	1 – 3 mg/100 ml	15	(K)
16	Potassium is distributed in nerves	(P)	Increased retention of water in the body	16	(S)
17	The normal level of potassium in serum	(Q)	Injury to myocardium and kidneys	17	(T)
18	The daily requirement of *potassium*	(R)	21 mg/100 grams	18	(X)
19	The other symptoms of hyperkalemia	(S)	530 mg/100 grams	19	(V)
20	Prolonged hypokalemia causes	(T)	14 – 20 mg/100 ml	20	(Q)
21	The clinical condition of hypokalemia	(U)	5 – 15 grams	21	(B)
22.	The symptoms of hypokalemia are	(V)	Mental confusion numbness weakness of respiratory muscles and flaccid paralysis of the extremities	22	(C)
23	The normal level of chlorine in serum	(W)	40 – 185 mg	23	(L)
24	Sulfur is a constituent of	(X)	About 4 grams	24	(Z)
25	The total sulfate excretion is increased	(Y)	In conditions accompanied by excessive tissue protein breakdown as in high fever and increased metabolism	25	(Y)
26	The normal level of iron in blood	(Z)	Coenzyme A and Lipoic acid	26	(M)
27	The normal level of copper in serum	(Z_1)	4 to 10 µg/100 ml	27	(Z_2)
28	The normal concentration of iodine in serum	(Z_2)	90 µg/100 ml	28	(Z_1)

CHAPTER – 12
DIGESTION AND ABSORPTION FROM THE GASTROINTESTINAL TRACT

Write the correct answer number of the followings :

Answers

1 The amount of saliva secreted each day in ml.
(a) 600 – 800
(b) 700 – 900
(c) 800 – 1000
(d) 1000 – 1500

1 d.

2. The submaxillary secretion contains most of the
(a) Glycoproteins.
(b) Glycolipids
(c) Both of the above
(d) None of the above.

2. a.

3. A saliva contains inorganic material in per cent
(a) 0 1
(b) 0 2
(c) 0.3
(d) 0 4

3 b

4 The amylase is helped to be stabilised by
(a) Ca^{++}
(b) Mg^{++}
(c) Na^{++}
(d) K^{+}

4 a.

5 Salivary amylase is readily inactivated at pH
(a) 6 0
(b) 5 0
(c) 4 0
(d) None of the above.

5 c.

6. The continued gastric secretion is regulated by the hormone
(a) Glucagon
(b) Gastrin.
(c) Epinephrine.
(d) ACTH

6 b.

7 The gastric juice is stimulated to be secreted by
(a) Protamine
(b) Histamine
(c) Prolamine
(d) Albumin

8 Hydrochloric acid secreted by the oxyntic cells contains the pH
(a) 0 5
(b) 0 6
(c) 0 8
(d) 0 9

9 The percentage of organic materials in gastric juice is about
(a) 0 1
(b) 0 2
(c) 0 4
(d) 0 8

10 On ordinary diet the daily amount of secretion of gastric juice by an adult in liters
(a) 1 – 2
(b) 2 – 3
(c) 3 – 4
(d) 4 – 5

11 The intrinsic factor (HCL and mucoproteins) present in the gastric juice helps in the absorption of
(a) Vitamin B_2
(b) Biotin
(c) Folic acid
(d) Vitamin B_{12}

12. Hydrochloric acid stimulates duodenum to liberate
(a) Secretin
(b) Pepsin
(c) Trypsin
(d) Enterocrinin

13 Pepsin contains large amount of amino acids
(a) Neutral
(b) Basic
(c) Acidic
(d) Sulfur containing

Answers

7 b
8 d
9 c
10 b
11 d
12 a
13 c

14. Pepsin has a molecular weight of
    (a) 31.600.
    (b) 32.700.
    (c) 33.300.
    (d) 34.400.

15. Rennin changes the casein of milk to paracasein in presence of
    (a) Mg^{++}.
    (b) Mn^{++}.
    (c) Ca^{++}.
    (d) Ba^{++}.

16. Lipase can act only at the PH
    (a) 3 to 4.5.
    (b) 3.5 to 5.0.
    (c) 4 to 6.
    (d) 5 to 7.

17. Pancreatic juice contains per centage of solids
    (a) 1.6.
    (b) 1.8.
    (c) 2.0.
    (d) 2.2.

18. The amount of pancreatic juice secreted each day in ml
    (a) 300 to 500.
    (b) 400 to 600.
    (c) 500 to 700.
    (d) 600 to 800.

19. Trypsin attacks peptide linkages containing the amino acid residue
    (a) Arginine
    (b) Glycine.
    (b) Tryptophan.
    (c) Glycine.
    (d) Serine.

20. Chymotrypsin attacks peptide linkages containing the amino acid residue
    (a) Leucine.
    (b) Valine.
    (c) Phenylalanine.
    (d) Aspartic acid.

Answers

14. b.

15. c.

16. d.

17. b.

18. d.

19. a.

20. c.

21 Carboxypeptidase contains
(a) Zinc
(b) Copper
(c) Manganese
(d) Magnesium

21 a

22. Carboxypeptidase B hydrolyses terminal peptide linkages containing
(a) Glycine and valine
(b) Serine and tryptophan
(c) Leucine and isoleucine
(d) Lysine and arginine

22 d

23 Collagenase hydrolyses collagen present in
(a) Eggs
(b) Soyabeans
(c) Meat
(d) Milk

23 c

24 RNAase and DNAase are capable of cleaving internal
(a) Phosphodiester bonds.
(b) Diester bonds.
(c) Esterbonds
(d) None

24 a

25 The amount of intestinal juice secreted each day by an adult in litres
(a) 1 to 2
(b) 2 to 3
(c) 3 to 4
(d) 4 to 5

25 b

26 The percentage of solids in intestinal juice is about
(a) 0 5
(b) 1 0
(c) 1 5
(d) 2 0

26 c

27 The inorganic substances present in the solids of intestinal juice is nearly
(a) One fourth
(b) One third
(c) Half
(d) Two third

27 d

28 Bile is produced by the
(a) Liver.
(b) Gall-bladder.
(c) Pancreas
(d) Intestine.

29. Bile formed daily in adult human beings in ml. of about
(a) 200 to 800
(b) 300 to 1200
(c) 700 to 1500
(d) 800 to 1800

30 The pH of hepatic bile
(a) 6 9 — 7 7
(b) 7 1 — 7 3.
(c) 7 4 — 7 8.
(d) 7 6 — 8.1

31 Bile acids are synthesized from cholesterol in the
(a) Duodenum
(b) Intestine
(c) Gall bladder
(d) Liver

32. Bile acids are derived from the parent acid called
(a) Taurocholic acid
(b) Prostanoic acid
(c) Cholanic acid.
(d) Cholic acid.

33 In human bile, sodium glycocholate is greater than sodium taurocholate by
(a) Two times.
(b) Three times
(c) Four times
(d) Five times

34 Bil salts activate
(a) Pancreatic lipase.
(b) Cholesterol esterase.
(c) Both.
(d) None.

Answers

28. a.

29. b.

30 b

31. d.

32. c.

33. b

34. c.

35 In obstructive jaundice, bile salts in the blood are
(a) Decreased
(b) Highly decreased
(c) Increased
(d) Greatly increased

36 The amount of bile salts in mg per day are not absorbed and is eliminated in the feces
(a) 500
(b) 700
(c) 900
(d) 1200

37 Bile is an important source of
(a) Acid
(b) Alkali
(c) Salt.
(d) None of the above

38 Arginine is decarboxylated by the intestinal bacteria into
(a) Agmatine
(b) Arginamine
(c) Arginatine
(d) Argininemine

39 By a series of reactions in the large intestine tryptophan forms
(a) Indole
(b) Methylindole
(c) Both of the above
(d) None of the above

40 In the large intestine cysteine by a series of transformations forms
(a) Ethyl mercaptan
(b) Methyl mercaptan
(c) H_2S
(d) All of the above

41 The quantity of ammonia transported from the large intestine to the blood is reduced by the antibacterial action of
(a) Streptomycin.
(b) Neomycin.
(c) Penicillin
(d) Chloramphenicol

Answers
35 d
36 a.
37 b
38 a
39 c
40 d
41) b

42. The reduction of biliverdin to urobilinogen takes place in the
    (a) Duodenum.
    (b) Small intestine
    (c) Large intestine
    (d) All of the above.

43. Cathepsins occur in the
    (a) Mitochondria
    (b) Cytosol.
    (c) Nuclei
    (d) Lysosomes

44. Enterocrinin is released from the
    (a) Duodenal mucosa
    (b) Pancreas
    (c) Large intestine.
    (d) Small intestine

45. The secretion of enterogastrone by the duodenal mucosa is influenced by the dietary
    (a) Carbohydrate
    (b) Fat
    (c) Protein
    (d) Minerals

Answers
42) c.
43) d.
44) a.
45) b.

Fill up the blanks of the followings :

1. Hepatocrinin liberated from the duodenum stimulates _____ to secrete more _____
2. Enterogastrin inhibits the secretion of _____ and _____
3. Enterocrinin stimulates the secretion of _____
4. CCK PZ is a _____ containing _____ amino acids
5. CCK PZ represents as one of the _____ influencing _____ release during oral administration of glucose
6. CCK PZ is the important stimulator for the _____ enzyme secretion and _____ contraction.
7. CCK PZ performs many of the actions of _____ and _____ on water bicarbonate and acid changes.
8. Gastrin stimulates the secretion of HCl, _____ and _____ from gastric mucosa
9. Gastrin contains _____ amino acids and in Gastrin 11 tyrosine is sulphated in position _____

Answers
1) Liver; Bile.
2) HCl, Pepsin
3) Intestinal juice
4) Polypeptide; 33
5) Gut factors, Insulin
6) Pancreatic, Gall-bladder
7) Gastrin, Secretin.
8) Pepsin, Intrinsic factor
9) 17, 12.

		Answers
10	Secretin is present in _____ and _____ mucosa	10) Duodenal Jejunal
11	Secretin contains _____ amino acids 14 of which are identical to those found in _____	11) 27 Glucagon
12	Secretin stimulates _____ secretion in the stomach and inhibits _____ secretion as well as intestinal _____ activity	12) Pepsin Gastric acid Motor
13	Secretin is administered to assess the pancreatic _____ function	13) Exocrine
14	After death the intracellular enzymes digest the tissu es when kept under _____ condition this self di gestion of tissues are called _____	14) Sterile Autolysis
15	The group of intracellular proteases present in all mammalian tissues is called _____	15) Cathepsin
16	Atropy is a process of _____ in the living animal	16) Autolysis
17	In man intestinal bacteria synthesize certain vitamins, particularly _____ and possibly certain members of the _____	17) Vitamin K B complex
18	In herbivora, the intestinal bacteria also synthesize _____ and _____	18) Essential amino acids Vitamins
19	Intestinal bacteria putrefy nitrogenous substances to form _____ which is absorbed into the portal _____	19) Ammonia Circulation
20	Intestinal bacteria cause the decarboxylation of lysine into _____	20) Cadaverine
21	After reduction cholesterol is converted to _____	21) Coprosterol
22	Lecithin is decomposed to _____ and _____	22) Choline Neurine
23	Urate stones are formed due to _____ and _____	23) Hyperuricemia Gout
24	Renal calculi formation is due to urine _____ and _____	24) Infection Stagnation
25	Mixed gall stones are composed of _____, _____, protein and calcium	25) Cholesterol Bile Pigments.
26.	Gall stones are formed due to defects in the _____ circulation and with the diseases of the terminal ileum as well as in patients with _____	26) Enterohepatic Cirrhosis
27	Cholic acid formed by _____ assists the absor ption of many important _____ compounds.	27) Deoxycholic acid insoluble
28	Bile salts stimulate intestinal _____	28) Peristalsis
29	Many substances such as fatty acids phenols, naphtha lene etc combine with deoxycholic acid to form _____	29) Cholic acid.
30	During digestion the gall bladder contracts by the stimulation of the hormone _____ which is produced by the _____	30) Cholecystokinin Small intestine

		Answers
31	The composition of the bile in the gall bladder is modified by the addition of _____ and other substances and by removal of water, _____ and chloride by reabsorption by the bladder mucosa.	31) Mucin, Bicarbonate.
32.	Enterokinase converts inactive _____ to active _____	32) Trypsinogen, Trypsin
33	Fats proteins and carbohydrates entering the duodenum and upper jejunum stimulate the secretion of _____ which induces the flow of _____.	33) Enterocrinin, Intestinal juice
34	Pancreatic lipase is specific for the hydrolysis of primary _____ linkages which occurs in position _____ of triglycerol	34) Ester, 1 and 3
35	Pancreatic amylase is an _____ and an _____	35) α-amylase, Endoamylase
36	Carboxypeptidase are _____ and hydrolyze only the terminal _____ linkage	36) Exopeptidase, Peptide
37	Carboxypeptidase is a _____ containing enzyme	37) Zinc.
38.	Pancreozymin stimulates the pancreas to produce a viscous fluid low in _____ but high in _____ content.	38) Bicarbonate, Enzyme.
39	The secretion of pancreatic juice is controlled by both _____ and _____ means	39) Nervous, Hormonal.
40	Paracasein is acted on by _____	40) Pepsin.
41	Pepsin converts native proteins into _____ and _____	41) Proteoses Peptones
42.	Hydrochloric acid stimulates duodenum to liberate _____	42) Secretin.
43.	Carbonic anhydrase catalyzes the formation of H_2Co_3 from _____ and _____.	43) H_2O, CO_2.
44	Saliva facilitates swallowing by the lubricating action of the _____	44) Glycoprotein.
45	_____ or _____ mechanisms cause the initiation of gastric secretion	45) Nervous, Reflex.
46	Salivary amylase is activated by _____ ion.	46) Chloride
47	Production of no hydrochloric acid leads to the condition _____.	47) Achlorhydria.
48	Rennin causes the _____ of milk	48) Coagulation

CHAPTER – 13
DETOXIFICATION AND IMMUNOCHEMISTRY

Fill up the blanks of the followings :

		Answers
1	Detoxification converts _____ substances introduced or formed in the body into _____ substances which are easily excreted out by the excretory routes	1) Toxic Non toxic
2	The substances introduced into the body are the _____ used for treatment of diseases and the _____ used for diagnostic purposes	2) Drugs Chemicals
3	The toxic substances are formed in the body by the _____ action in the large intestine	3) Bacterial
4	The other toxic substance _____ is formed in the body by the _____ of hemoglobin	4) Bilirubin Breakdown
5	The detoxification processes take place in the _____	5) Liver
6	Alcohols and aldehydes are detoxified by the process of _____	6) Oxidation
7	Chloral is oxidized to _____	7) Trichloracetic acid
8	Aniline is oxidized to _____	8) P aminophenol
9	Acetanilide is oxidized to _____	9) P acetylamino phenol
10	Benzene is oxidized to _____	10) Phenol
11	Chloral on reduction produces _____	11) Trichlorethylalcohol
12	Picric acid on reduction yields _____	12) Picramic acid
13	Atropine on hydrolysis forms _____ and _____	13) Tropic acid Tropine
14	Aspirin is hydrolyzed to _____ and _____	14) Salicylic acid Acetic acid
15	Procaine on hydrolysis yields _____ and _____	15) P aminobenzoic acid Diethylamino-ethanol
16	The process by which the chemical to be detoxified combines with another chemical formed by the body is called _____	16) Conjugation
17	Bilirubin diglucuronide is formed by the combination of _____ and _____	17) Bilirubin Glucuronic acid
18	Indoxyl sulphate is the product from the combination of _____ and _____	18) Indoxyl Sulphuric acid
19	_____ and _____ form hippuric acid	19) Benzoic acid Glycine

		Answers
20	Nicotinuric acid is formed from _____ and _____.	20) Nicotinic acid, Glycine
21	Bromobenzene and cysteine form _____.	21) P Bromophenyl mercapturic acid.
22.	N methyl pyridine is formed from _____ and _____.	22) Pyridine, Active methionine
23	Cyanide and sodium thiosulphate form _____ and _____	23) Thiocyanate, Sodium sulphate
24	The lymphocyte is the primary cell for _____ system.	24) Immune
25	The T lymphocytes are _____ derived.	25) Thymus.
26	The B lymphocytes are responsible for _____ immunity which is expressed by the production of specific circulating plasma proteins termed _____	26) Humoral, Antibodies
27	The basic unit of all immunoglobulin molecule consists of _____ chains linked by _____ bonds.	27) Four polypeptide, Disulfide
28	Immunoglobulins composed of more than one basic _____ unit are termed _____.	28) Monomeric; Polymers.
29	IgM are _____ and IgA are _____	29) Pentamers, Trimers.
30	The polypeptide chains are folded 3-dimensionally with _____ bonds to form areas called _____.	30) Disulfide, Domains.
31	The part of the antibody molecule which combines with antigens is formed by a few amino acids in the F region of _____ and _____ chains	31) H, L.
32	There are two major types of L chains in man, the _____ and _____ chains.	32) Kappa, Lamda
33	About 70 per cent of the human immunoglobulin molecules carry _____ light chains and 30 per cent carry _____ light chains.	33) Kappa, Lamda
34	In IgG, the H chain is termed a _____ chain, and in IgE, an _____ chain.	34) Gamma, Epsilon
35	The half of the light (L) chain toward the carboxy terminus is termed as the _____, while the amino terminal half is the _____ of the light chain	35) Constant region, Variable region
36	The portion of the immunoglobulin molecule which binds the _____ is formed by the _____ portions of both the H and L chains.	36) Specific antigen, Aminoterminal.
37	The domains of the protein chains form globular regions with _____ and _____ structure	37) Secondary; Tertiary
38	IgG comprises _____ per cent of the gamma globulins and contains 2-4 per cent _____	38) 80, Carbohydrates

		Answers
39	IgA has _____ per cent carbohydrate and does not cross the _____	39) 5 to 10 Placenta
40	IgM contains _____ amino acids and has a mass of _____ daltons.	40) 576 950 000
41	IgM is the major immunoglobulin expressed on the surface of _____ cells and its carbohydrate content is _____ per cent.	41) B 10 to 12
42	IgE is present in the serum in very _____ concentrations as a single basic unit with _____ E chains	42) Low Heavy
43	No antibody activity is associated with _____	43) IgD
44	Half of the patients with allergic diseases have increased serum _____ levels	44) IgE.
45	Immunoelectrophoresis consists of both _____ separation and immune precipitation of _____	45) Electrophoretic Proteins
46	The normal concentration of IgM _____/dl	46) 40 to 210 mg
47	The normal concentration of IgG _____/dl	47) 710 to 1530 mg
48	Substances which can give an immune response when introduced into an animal are called _____	48) Antigens
49	The combining sites on the surface of the lymphocytes are antibody like molecules called _____	49) Antigen receptors
50	The portions of antigenic molecules which are involved in actual binding with antibody combining sites are termed _____	50) Antigenic determinants.
51	The ability of the region of the antigen molecule to act as an antigenic determinant and to induce the formation of specific antibodies is called _____	51) Immunopotency

CHAPTER – 14
BLOOD, LYMPH AND CEREBROSPINAL FLUID

Write the correct answer number of the followings :

Answers

1 Under optimal conditions one gram of hemoglobin can carry oxygen in ml
(a) 1 0
(b) 1 14
(c) 1 24
(d) 1 34

1) d.

2 Cellular fraction of blood in volume per cent
(a) 40
(b) 45
(c) 50
(d) 55

2) b

3 Plama fraction of blood in volume per cent
(a) 40
(b) 45
(c) 50
(d) 55

3) d.

4 The diffusible constituent of plasma
(a) Urea
(b) Vitamins.
(c) Hormones.
(d) All of the above

4) d.

5 The catabolic products of diffusible consituents of plasma
(a) Uric acid.
(b) Creatinine
(c) Both of the above
(d) None of the above

5) c.

6 The normal concentration of fibrinogen of blood per 100 ml
(a) 0 2 – 0 6 grams
(b) 0 4 – 0 8 grams.
(c) 0 6 – 1 0 grams.
(d) 0 8 – 1 2 grams

6) a.

7 The normal level of free fatty acids in blood per 100 ml
(a) 110 – 400 mg
(b) 125 – 450 mg
(c) 140 – 480 mg
(d) 150 – 500 mg

8 The normal blood total lipids concentration in mg per 100 ml
(a) 500 – 750
(b) 550 – 800
(c) 570 – 820
(d) 600 – 850

9 The normal concentration of PBI of blood per 100 ml
(a) 2 – 6 µg
(b) 4 – 8 µg
(c) 6 – 10 µg
(d) 8 – 12 µg

10 The normal level of bilirubin in serum in mg per 100 ml
(a) 0.1 – 0.4
(b) 0.2 – 0.9
(c) 0.3 – 1.2
(d) 0.4 – 1.5

11 The normal concentration of serum lactate dehydrogenase per L
(a) 50 – 100 I.U
(b) 60 – 120 I.U
(c) 80 – 150 I.U
(d) 90 – 200 I.U

12 The normal level of NPN of blood in mg per cent
(a) 10 – 25
(b) 15 – 35
(c) 20 – 40
(d) 25 – 45

13 The normal concentration of pyruvic acid in blood in mg per cent
(a) 0.7 – 2.0
(b) 0.8 – 2.2
(c) 1.0 – 2.5
(d) 1.5 – 3.0

14 Human blood is thicker than water by the number of times
(a) 2

Answers

7) d

8) c

9) b

10) a

11) d

12) b

13) a

14) d

(b) 3.
(c) 4
(d) 5

15 The normal osmotic pressure of blood is equal to
(a) 0 915% NaCl
(b) 0 925% NaCl
(c) 0 945% NaCl
(d) 0 955% NaCl

16 The substance almost entirely confined in corpuscles is
(a) Sodium
(b) Calcium
(c) Potassium
(d) Magnesium.

17 The substances circulating in blood
(a) Enzymes
(b) Vitamins.
(c) Hormones.
(d) All.

18 Plasma contains solids in per cent
(a) 7 to 8
(b) 8 to 9
(c) 9 to 10
(d) 10 to 12.

19 The human plasma proteins are a mixture of
(a) Simple proteins.
(b) Glycoproteins.
(c) Lipoproteins.
(d) All of the above

20 The plasma proteins are separated by
(a) Salt precipitation.
(b) Electrophoresis.
(c) Immunoelectrophoresis
(d) All of the above

21 The numbers of amino acids arranged in a single peptide chain constituting albumin are
(a) 580

Answers

15) c.
16) c.
17) d.
18) b.
19) d.
20) d.
21) c

(b) 600
(c) 610
(d) 620

Answers

22. The normal concentration of serum albumin in gram per cent by precipitation method
(a) 4 5
(b) 5 0
(c) 5 2
(d) 5 4

22) a

23 The serum albumin concentration decreases in
(a) Severe protein deficiency
(b) Liver diseases.
(c) Nephritis.
(d) All of the above

23) d

24 The normal concentration of serum globulin in grams per 100 ml by precipitation method
(a) 2 0
(b) 2 5
(c) 2 9
(d) 3 2

24) b

25 β globulins contain
(a) Siderophilin
(b) Ceruloplasmin
(c) Both of the above
(d) None of the above

25) a

26 Serum β globulins normal concentration in gram per cent
(a) 0 82
(b) 0 85
(c) 0 91
(d) 0 95

26) c

27 The substances which contain more than 4% hexosamine are designated as
(a) Glycoproteins
(b) Mucoproteins
(c) Both of the above
(d) None of the above.

27) b

28. The normal concentration of serum α_1 globulins in gram per cent
    (a) 0.32
    (b) 0.35
    (c) 0.38
    (d) 0.42.

29 Metalloprotein concentration is reduced in
    (a) Pernicious anemia.
    (b) Chronic infections
    (c) Liver diseases
    (d) All of the above

30. Fibrinogen is precipitated by half saturation with
    (a) Ammonium sulphate
    (b) Magnesium sulphate
    (c) Barium sulphate
    (d) Calcium sulphate

31 Leukocytes contain
    (a) Proteins.
    (b) Nucleoproteins
    (c) Fats.
    (d) All of the above

32. Blood platelets contain large amounts of
    (a) Catecholamines.
    (b) Serotonin.
    (c) Histamine
    (d) All of the above

33. The minor blood groups are
    (a) M
    (b) N
    (c) Rh
    (d) All of the above

34 The commonest Rh antigen is
    (a) A.
    (b) B
    (c) D
    (d) AB

Answers

28) d.

29) d.

30) a.

31) d.

32) d.

33) d.

34) c

35 Hemolysis of blood is caused by
(a) Quinine
(b) Ether
(c) Both of the above
(d) None of the above

36 Thrombin consists of the numbers of polypeptide chains
(a) 1
(b) 2
(c) 3
(d) 4

37 The total volume of C S F in an adult human is about
(a) 130 ml
(b) 140 ml
(c) 150 ml
(d) 160 ml

38 *In the fasting adult, the sugar in C S.F in mg per 100 ml*
(a) 40 - 60
(b) 50 - 85
(c) 80 - 110
(d) 90 - 130

Answers
35) c
36) b
37) a
38) b

Fill up the blanks of the followings :

1 The sugar content of C S F is decreased in purulent _____

2. The reduction in chloride of C S F is most marked in _____

3. Increased level of calcium of C.S F is observed in all cases of _____ and _____

4 Isomerase activity of C S F is increased in malignant brain _____

5 The protein content of C S F is increased in _____

6 The term _____ denotes a fluid not only present in the lymphatic vessels but also the fluid which bathes the cells

7 The ratio between albumin and globulins in lymph is the same as in _____

8 The C S F is formed by the ultrafiltration of the _____ by the choroid plexuses of the _____

Answers
1 Meningitis.
2 Tuberculous meningitis
3 Meningitis Epidemic encephalitis
4 Tumors
5 Myxedema
6 Lymph
7 Plasma
8 Plasma Brain

		Answers
9	Plasmin normally exists in _____ in the inactive form _____	9 Plasma Plasminogen
10	Urokinase, the _____ enzyme of urine, is also a serine _____	10 Proteolytic Protease
11	Antithrombin 111 has a major _____ activity and has some _____ activity	11 Antithrombin Endogenous
12.	The common deficiency of factor _____ produces a disease known as hemophilia A	12 VIII
13	Von Willebrand s disease is caused by the defect in _____ and a deficiency of factor _____ clotting activity	13 Platelet adherence VIII
14	Factor VII is the GLA-containing _____ synthesized in the _____	14 Glycoprotein Liver
15	Thrombin converts factor XIII to _____ which is a _____	15 XIIIa Transglutaminase
16	Prothrombin is a single clain _____ with a molecular weight of _____	16 Glycoprotein 72000
17	The initial fibrin clot is _____ and held together only by fibrin _____	17 Weak Monomers.
18.	Fibrinogen is a soluble plasma _____ whose length is _____	18 Glycoprotein 46nm
19	The white thrombus is composed of _____ and _____ and is poor in _____	19 Platelets, Fibrin Erythrocytes
20	Stop of bleeding is said to be _____	20 Hemostasis
21	The hemolytic effect is due to the presence of the enzyme _____ which hydrolyzes _____	21 Phospholipase A Phospholipids
22	Persons of group O are called _____ donors	22 Universal
23	Blood platelets contain a _____ protein which is involved in the process of clot _____	23 Contractile Retraction
24	Blood platelets are _____ and contain no _____	24 Unnucleated DNA
25	Fibrinogen is highly elongated having an axial ratio of about _____	25 20 1
26	Some glycoproteins have specific binding function for _____ and _____	26 Thyroxine Cortisol
27	Metalloprotein concentration is increased in _____ deficiency or _____	27 Iron Pregnancy

Indicate "True" or "False" of the followings

		Answers
1	The viscosity of blood provides resistance to flow of blood in the blood vessels to maintain blood pressure at normal level	1 True

		Answers
2	Albumin exerts 60 per cent of the colloid osmotic pressure of plasma	2 False
3	γ- globulins are formed in the liver	3 False
4	α- and β- globulins are synthesized in the kidney	4 False
5	γ- globulins are immunoglobulins having antibody activity	5 True
6	α_1- globulins are of several complex proteins containing carbohydrates and lipids.	6 True
7	The normal concentration of α_2- globulins in serum is 0 42 gm/100 ml	7 False
8	About one third of the total plasma proteins is albumin	8 False
9	Plasma contains 15% to 16% solids composed largely of proteins.	9 False
10	The PH of blood of an individual is below 7 3 is considered in a condition of alkalosis	10 False
11	Blood is clotted within 2 or 3 minutes after shedding if it is left undisturbed	11 False
12.	The viscosity of blood is affected by the change in the numbers of red cells or white cells	12. True
13	Blood transports metabolites from one tissue to another	13 True
14	Plasma is about 2 83 litres in an adult weighing 70 kg	14 True
15	Enzymes and lipids are the diffusible constituent of plasma	15 False
16	The normal concentration of serum total iodine is 3—6 5 mg/100 ml.	16 False
17	Na^+, K^+, Ca^+, are the non diffusible constituents of plasma	17 False
18	The speeific gravity of plasma lies between 1 05 and 1 06	18 False
19	Plasma has a very much higher viscosity	19 False
20	The osmotic pressure of blood is increased on strenuous exercise	20 True
21	In normal individuals, the plasma proteins vary from 6 0% to 8 5%	21 True
22.	Albumin has a molecular weight of about 69,000 and is synthesized in the liver	22 True
23	Albumin plays an important role in the exchange of water between tissue fluid and blood.	23 True
24	The molecular weight of globulin ranges from 9000 to 130000	24 False
25	Bilirubin is associated with γ globulins	25 False
26	Thyroxine is transported in association with β-globulins	26 False
27	Many drugs and dyes are transported in the plasma in combination with globulins	27 False
28	Albumin is largely involved in the nutritive functions of the plasma proteins owing to its high concentration	28 True
29	Half of the calcium of plasma is bound to protein for transport	29 True
30	In kidney diseases large amount of water moves to the tissues	30 True

CHAPTER – 15
EXCRETION

Write the correct answer number of the followings

Answers

1 The weight of each kidney in grams is about
(a) 90 – 130
(b) 100 – 140
(c) 110 – 150
(d) 120 – 170

1) d.

2. The length of each kidney in cms is about
(a) 10 – 12
(b) 11 – 13
(c) 13 – 15
(d) 15 – 17

2) b

3 Urine formation involves the main steps
(a) The glomerular filtration
(b) The tubular reabsorption.
(c) The tubular secretion.
(d) All of the above

3) d.

4 The daily normal total amount of urine contains the number of grams of solids
(a) 35
(b) 40
(c) 60
(d) 80

4) c

5 The urinary excretion increases by the following substance having a diuretic action
(a) Urea
(b) Cellulose.
(c) Ammonium salts.
(d) Glycerol.

5) a.

6 The diuretic action of tea. coffee and cocoa is due to
(a) Theophilline
(b) Caffeine
(c) Bromophylline
(d) None of the above.

6) b

7 Increased urine volume are observed in
(a) Diabetes insipidus
(b) Diabetes
(c) Both of the above
(d) None of the above.

8 Decreased urine volume are found in
(a) Acute nephritis.
(b) Fevers.
(c) Diseases of the heart
(d) All of the above

9 The function of kidney is regulated by the hormone
(a) Aldosterone
(b) Parathormone
(c) Vasopressin
(d) All of the above

10 The chief pigment of urine
(a) Coproporphyrin
(b) Terochrome
(c) Urobilinogen
(d) Uroerythrin.

11 In liver disease the colour of urine may be
(a) Green
(b) Brown
(c) Deep yellow
(d) All of the above

12. The colour of urine is dark brown due to
(a) Methemoglobin
(b) Homogentisic acid
(c) Both of the above
(d) None of the above

13 A turbidity is developed in alkaline urine by precipitation of
(a) Calcium phosphate
(b) Magnesium sulphate
(c) Both of the above
(d) None of the above

14 Strongly acid urine is pink due to the precipitation of

Answers
7) c
8) d
9) d
10) b
11) d.
12) c
13) a

Answers

(a) Chloride salts.
(b) Ammonium salts.
(c) Uric acid salts.
(d) None of the above.

14) c.

15 The urine is acid in high protein intake because of the formation of excess

(a) Phosphates
(b) Sulpbates
(c) Both of the above
(d) None of the above.

15) c.

16. Urea excretion is increased in

(a) Fever
(b) Diabetes
(c) Excess adrenocortical activity
(d) All of the above

16) d.

17 Ammonia is formed by the kidney from

(a) Glutamate
(b) Glutamine
(c) Aspartate,
(d) Aneurine

17) b

18 There is high ammonia output in the urine in

(a) Uncontrolled diabetes mellitus.
(b) Cirrhosis of the liver
(c) Uremia.
(d) Nephritis.

18) a.

19 Creatine excretion is also found in

(a) Starvation
(b) Hyperthyroidism.
(c) Infections.
(d) All of the above

19) d.

20 Uric acid excretion is increased in

(a) Leukemia.
(b) Severe liver disease
(c) Various stages of gout
(d) All of the above

20) d.

		Answers
21	Deposits of uric acid and urates are coloured by absorbed urinary pigments, particularly the red	
	(a) Uroerythrin.	21) a
	(b) Uracil	
	(c) Urocatell	
	(d) Urostrepsin.	
22.	The number of mg of amino acid nitrogen excreted in the urine of adults daily is about	
	(a) 125 — 180	22) b
	(b) 150 — 200	
	(c) 160 — 240	
	(d) 180 — 280	
23	Premature infants excrete more amino acid nitrogen than that of full term infant by the number of times	
	(a) 6	23) c
	(b) 8	
	(c) 10	
	(d) 12	
24	Increased amounts of amino acids are excreted in	
	(a) Liver disease	24) c
	(b) Certain types of poisoning	
	(c) Both of the above	
	(d) None of the above	
25	The amino acids excreted in urine in cystinuria	
	(a) Arginine	25) d
	(b) Cystine	
	(c) Lysine	
	(d) All of the above	
26	Out of the total excreted sulphur the percentage of ethereal sulfate is about	
	(a) 8	26) b
	(b) 10	
	(c) 12	
	(d) 14	
27	The number of mg of indican excreted normally	
	(a) 5 — 20	27) a

	Answers
(b) 8 — 25	
(c) 10 — 30	27) a.
(d) 15 — 40	
28 Neutral sulfur is contained in	
(a) Cystine	28) d.
(b) Taurine	
(c) Thiocyanate	
(d) All of the above	
29 The greater part of the excreted phosphates is derived from inges ted food	
(a) Nucleoprotein.	29) d.
(b) Phosphoprotein	
(c) Phospholipids	
(d) All of the above	
30 Phosphate excretion is increased in	
(a) Osteomalacia	30) d.
(b) Wasting diseases of the nervous system	
(c) Renal tubular rickets.	
(d) All of the above	
31 Decrease in phosphate excretion is observed in	
(a) Hypoparathyroidism	31) c
(b) Infectious diseases	
(c) Both of the above	
(d) None of the above	
32. The normal amount of oxalate in urine in mg per day	
(a) 20	32) a.
(b) 25	
(c) 30	
(d) 35	
33 The excretion of oxalate is increased by ingestion of	
(a) Fruits.	33) c.
(b) Vegetables	
(c) Both of the above	
(d) None of the above	
34 [illegible] the activity of	
(a) A	34) b

		Answers
	(b) Adrenal cortex	
	(c) Thyroid.	
	(d) None of the above	
35	The number of mg of protein present in normal urine cannot be detected by the ordinary simple tests —	
	(a) 30 — 200	35) a
	(b) 50 — 250	
	(c) 80 — 350	
	(d) 100 — 400	
36	Proteinuria also results in poisoning of the renal tubules by heavy metals	
	(a) Mercury	36) d
	(b) Arsenic.	
	(c) Bismuth	
	(d) All of the above	

Fill up the blanks of the followings :

		Answers
1	Mucus is a _____ and is increased in _____ of the bladder	1 Mucin, Infection
2.	Bence jones proteins found in the urine are light chain fragments of _____ and most commonly occur in _____ are rarely in _____	2 Globulins Multiple myeloma, Leukemia
3	Normal individuals excrete not more than _____ of sugar per day	3 19 mg
4	In case of pregnant women and lactating mother, the _____ must be performed for urine glucose to eliminate the _____ present in urine	4 Osazone test, Lactose
5	Increased amount of ammonia is excreted in _____ accompanying _____	5 Acidosis Ketosis.
6	Bilirubinuria is accompanied by the excretion of _____	6 Bile salts
7	Bile salts may be excreted in urine without _____ in certain stages in _____ disease	7 Bile pigment, Liver
8	In excessive hemolysis, part of the bile pigment formed by breakdown of _____ is excreted in urine as _____	8 Hemoglobin, Urobilinogen
9	Urobilin is formed from colourless _____ when the urine is exposed to _____	9 Urobilinogen, air
10	Coproporphyrins are excreted more in certain _____ diseases	10 Liver
11	Diuretics, _____, enhance losses of water and salt via the urine through interference with normal _____ mechanisms	11 The drugs, Reabsorptive

	Answers
(b) 8 — 25	
(c) 10 — 30	27) a.
(d) 15 — 40	
28 Neutral sulfur is contained in	
(a) Cystine.	28) d.
(b) Taurine.	
(c) Thiocyanate	
(d) All of the above	
29 The greater part of the excreted phosphates is derived from inges ted food	
(a) Nucleoprotein.	29) d.
(b) Phosphoprotein	
(c) Phospholipids.	
(d) All of the above	
30 Phosphate excretion is increased in	
(a) Osteomalacia.	30) d.
(b) Wasting diseases of the nervous system.	
(c) Renal tubular rickets.	
(d) All of the above	
31 Decrease in phosphate excretion is observed in	
(a) Hypoparathyroidism.	31) c.
(b) Infectious diseases.	
(c) Both of the above	
(d) None of the above	
32. The normal amount of oxalate in urine in mg per day	
(a) 20	32) a.
(b) 25	
(c) 30	
(d) 35	
33. The excretion of oxalate is increased by ingestion of	
(a) Fruits.	33) c.
(b) Vegetables	
(c) Both of the above	
(d) None of the above.	
34 Sodium and potassium excretion are controlled by the activity of	
(a) Adrenal medulla.	34) b.

(b) Adrenal cortex
(c) Thyroid
(d) None of the above

35 The number of mg of protein present in normal urine cannot be detected by the ordinary simple tests —
(a) 30 — 200
(b) 50 — 250
(c) 80 — 350
(d) 100 — 400

36 Proteinuria also results in poisoning of the renal tubules by heavy metals
(a) Mercury
(b) Arsenic
(c) Bismuth
(d) All of the above

Answers

35) a

36) d

Fill up the blanks of the followings :

1 Mucus is a _____ and is increased in _____ of the bladder

2 Bence jones proteins found in the urine are light chain fragments of _____ and most commonly occur in _____ are rarely in _____

3 Normal individuals excrete not more than _____ of sugar per day

4 In case of pregnant women and lactating mother, the _____ must be performed for urine glucose to eliminate the _____ present in urine

5 Increased amount of ammonia is excreted in _____ accompanying _____

6 Bilirubinuria is accompanied by the excretion of _____

7 Bile salts may be excreted in urine without _____ in certain stages in _____ disease

8 In excessive hemolysis, part of the bile pigment formed by breakdown of _____ is excreted in urine as _____

9 Urobilin is formed from colourless _____ when the urine is exposed to _____

10 Coproporphyrins are excreted more in certain _____ diseases

11 Diuretics, _____, enhance losses of water and salt via the urine through interference with normal _____ mechanisms

Answers

1 Mucin Infection

2 Globulins Multiple myeloma Leukemia

3 19 mg

4 Osazone test Lactose

5 Acidosis Ketosis.

6 Bile salts

7 Bile pigment, Liver

8 Hemoglobin Urobilinogen

9 Urobilinogen air

10 Liver

11 The drugs Reabsorptive

		Answers
12.	Osmotic diuresis is responsible for the serious _____ which accompanies diabetic _____	12 Dehydration; Ketoacidosis.
13	Diamox blocks both _____ reabsorption in the _____ tubule.	13 HCO_3^-, Proximal.
14	Furosemide and mercurials inhibit _____ reabsorption in the _____ limb	14 Chloride; Ascending.
15	Clearance is measured to assess quantitatively the rate of _____ of a given substance by the _____	15 Excretion, Kidney
16	Diabetes insipidus is developed due to the non-production of _____ and the individual passes _____ litres of urine in 24 hours.	16 ADH, 5 to 20
17	In salt losing nephritis there is severe _____, _____ and _____	17 Dehydration, Hyponatremia Hypochloremia.
18	Renal tubular acidosis is accompanied by excessive mobilization and urinary excretion of _____ and _____	18 Calcium Potassium.
19	In hartnup syndrome the disturbances in tryptophan metabolism is suggested by the presence of inereased amounts of _____, _____ and _____ in urine	19 Tryptophan Indican Indole acetic acid
20	The clinical symptoms of hartnup syndrome are of _____ deficiency	20 Niacin.

Indicate "True" or "False" of the followings :

		Answers
1	In uremia, the concentration of urea and other NPN constituents in plasma are increased	1 True
2.	Acidosis develops due to the impairment of acid and phosphate excretion in acute uremia.	2. True
3	Not only the kidney performs excretory functions but it acts an endocrine organ.	3 True
4	Kidney also destroys several hormones which are liberated from other organs.	4 True
5	The juxtaglomerular cells of the renal cortex produce the proteolytic enzyme rennin.	5 True
6	Rennin acts on α_1 globulin which is normally present in plasma.	6 False
7	Rennin splits off a polypeptide fragment called angiotensin I which is a decapeptide containing 16 amino acids	7 False
8	Angiotensin decreases the force of the heart heat and relaxes the arterials.	8 False
9	Rennin angiotensin system is important in the maintenance of normal blood pressure	9 True

		Answers
10	Prostaglandins are the other hormones of the kidney and increase renal blood flow	10 True
11	Kininogen which is produced by the kidney has an antihyperten sive effect	11 True
12	Erythropoietin and erythrogonin have an effect on bone marrow to stimulate production of red cells.	12 True
13	Hypoxia stimulates production of erythropoietin	13 True
14	Amorphous calcium and magnesium phosphates may be deposited from acid urines	14 False
15	Calcium oxalate is found in alkine urine but may be found in acid urine	15 False
16.	Urates are usually found in alkaline urines	16 False
17	Meat diet increases the bulk of feces	17 False
18	One third of the dry matter of feces is represented by intestinal secretions	18 False
19	Greenish colour of feces is due to excessive consumption of green vegetables	19 True
20	Iron or Bismuth gives a green stool.	20 False
21	Feces are normally slightly acidic	21 False
22.	In diseases, fecal fat is increased when digestion or absorption of fat is impaired	22. True
23	An adult on a mixed diet usually excretes about 3 g of fecal nitrogen per day	23 False
24	Moist feces contain about 6—7% of salts	24 False
25	The PH of sweat is about 4 5, but if the skin is washed and dried sweat is slightly alkaline	25 True
26	The lactic acid content of sweat is more than normally found in blood	26 True
27	The tubular epithelium also removes a number of foreign substances	27 True
28	Mercaptans and H_2S may produce odour of feces	28 True

CHAPTER – 16
THE ENERGY REQUIREMENTS AND THE PRINCIPLES OF NUTRITION.

Write the correct answer number of the followings :

Answers

1. The R.Q. of mixed diets is about
   (a) 0.72.
   (b) 0.75.
   (c) 0.80.
   (d) 0.85

   1) d.

2. The BMR is influenced by the factor
   (a) Sex.
   (b) Climate.
   (c) Habit.
   (d) All of the above.

   2) d.

3. The BMR is subnormal in
   (a) Addison's disease.
   (b) Hyperthyroidism.
   (c) Hypothyroidism.
   (d) All of the above.

   3) a.

4. The BMR is below normal in
   (a) Starvation.
   (b) Lipoid nephrosis.
   (c) Both of the above.
   (d) None of the above.

   4) c.

5. The BMR is above normal in
   (a) Diabetes insipidus.
   (b) Leukemia.
   (c) Polycythemia.
   (d) All of the above.

   5) d.

6. When lard, glucose and protein are combined, the percentage of SDA is less than the sum of their individual SDA by
   (a) 20.
   (b) 22.
   (c) 24.
   (d) 25

   6) b.

7. When protein and lard are combined, the percentage of SDA is less than the sum of their individual SDA by
   (a) 45.
   (b) 50.
   (c) 54.
   (d) 58.

8. The SDA is decreased more by
   (a) Carbohydrate.
   (b) Fat.
   (c) Protein.
   (d) Mixture of all.

9. When different amino acids are fed, high SDA is found to be produced by
   (a) Glycine.
   (b) Alanine.
   (c) Phenylalanine.
   (d) All of the above.

10. The glands of internal secretion have no direct influence upon the SDA of
   (a) Protein.
   (b) Fat.
   (c) Carbohydrate.
   (d) All of the above.

11. Thyroidectomy in animals reduces the SDA of
   (a) Carbohydrate.
   (b) Fat.
   (c) Both of the above.
   (d) None of the above.

12. It is unfavourable to heavy muscular work by a diet rich in
   (a) Carbohydrate.
   (b) Protein.
   (c) Fat.
   (d) All of the above.

13. The extra heat is accelerated in the performance of work with
   (a) Carbohydrate.
   (b) Fat.
   (c) Both of the above.
   (d) None of the above.

Answers

7) c.

8) b.

9) d.

10) a.

11) c.

12) b.

13) c.

14 When new tissue is formed the usual SDA is not exerted by
(a) Carbohydrate
(b) Fat
(c) Vitamins
(d) Protein

15 A man of 70 kg. weight and 180 cm. height with a surface area of 1 8 square meter aged 30 and a B M.R. of 40 will have a basal metaboli sm of
(a) 1750 calories
(b) 1800 calories
(c) 1875 calories
(d) 1925 calories

16. Lumberman entails the expenditure of
(a) 8000 calories
(b) 9000 calories.
(c) 10000 calories
(d) 12000 calories.

17 In long bicycle races in 24 hours, there is the expenditure of
(a) 8000 calories
(b) 9000 calories
(e) 10000 calories
(d) 12000 calories.

18 Above the average for the six days the percentage of Sunday s con sumption is
(a) 24
(b) 28
(c) 32.
(d) 36

19 Women have that of men by
(a) 7% —
(b) 8% —
(c) 10% —
(d) 12% —

20 The a·
(a)

Answers
14) d.
15) b
16) a.
17) c
18) d.
19) a.
20) b

21 The daily average caloric requirement of the adult male
(a) 2000
(b) 2200
(c) 2500
(d) 3000

22. If the NPU (Net Protein Utilization) is low, the requirements for proteins are
(a) Low
(b) High
(c) Moderate
(d) None of the above

23 The relatively essential amino acid
(a) Glycine.
(b) Serine
(c) Alanine
(d) Histidine.

24 Vegetable proteins are almost associated with a large amount of
(a) Minerals
(b) Vitamins
(c) Carbohydrate
(d) Fat

25 Animal proteins are associated with fat but very little carbohydrate except
(a) Milk
(b) Peas.
(c) Beans
(d) Potatoes

26 The foodstuffs having the "protein sparing" effect
(a) Carbohydrate
(b) Fat
(c) Both of the above
(d) None of the above

27 Protein has a more catalytic function in the form of
(a) Hormones.
(b) Enzymes
(c) Antigens
(d) Antinogens

Answers

21) d

22) b

23) d

24) c

25) a

26) c

27) b

28 Proteins differ in "biologic value" depending on their contents of
(a) Non-essential amino acids
(b) Semi-essential amino acids.
(c) Essential amino acids.
(d) All of the above

29 Good quality proteins include
(a) Soyabeans.
(b) Peanuts
(c) Potatoes.
(d) All of the above

30 High quality proteins include
(a) Eggs
(b) Dairy products
(c) Kidney
(d) All of the above

31 Proteins are also important sources of
(a) Nitrogen.
(b) Sulphur
(c) Phosphorus.
(d) All of the above

32. The protective substances in a diet are
(a) First class proteins.
(b) Mineral elements.
(c) Vitamins.
(d) All of the above

33 The bulk of the food is reduced by
(a) Carbohydrate
(b) Fat.
(c) Protein.
(d) Minerals

34 The daily consumption of fat in grams is usually over
(a) 50
(b) 60
(c) 80
(d) 90

35 Animal fats have a better biologic value because they contain the vitamins

Answers

28) c.

29) d.

30) d.

31) d.

32) d.

33) b.

34) c.

Answers

(a) A
(b) D
(c) Both of the above
(d) B_{12}

35) c

36 The major symptoms of essential fatty acid deficiency are
(a) A scaly dermatitis.
(b) Hair loss.
(c) Poor wound healing
(d) All of the above

36) d

37 In societies where fat is the principal food for energy intake the population tends to develop
(a) Coronary heart disease
(b) Obesity
(c) Cancer of the bowel
(d) All of the above

37) d.

38 Carbohydrate is the cheapest source of food and for producing energy it can be readily
(a) Digested
(b) Absorbed
(c) Utilized
(d) All of the above

38) d

Fill up the blanks of the followings :

Answers

1 Blood cholesterol level can be reduced significantly by changing the diet containing less _____ fatty acids or _____

1 Saturated Cholesterol

2. Carbohydrate can furnish _____ of the total caloric intake in the scarcity of _____ and _____

2 60% — 80% Proteins Fats.

3. In gluconeogenesis, carbohydrate can be supplied from most _____ as well as from _____ moiety of fats.

3 Amino acids Glycerol.

4 Carbohydrate is bulky and liable to undergo _____ producing _____ if digestion is delayed

4 Fermentation Lactic acid.

5 A minimum of _____ of carbohydrate per 100 K cal of total diet is necessary to prevent the development of _____

5 5 grams Ketosis

6. Some cereals contain much _____ as to interfere with the absorption of _____

6 Phytin Calcium

7 Cellulose stimulates _____

7 Peristalsis.

		Answers
8.	Pentoses are absorbed and excreted _____ except _____ or _____	8 Unchanged Ribose Deoxyribose.
9	Sucrose is one of the major etiologic factors in _____ and it is not the cause of _____, _____ or _____	9 Dental caries; Diabetes; Heart disease Obesity
10	Large amounts of dietary fiber decrease bowel _____ time and alter the composition of _____ bacteria.	10 Transit, Intestinal.
11	Milk products supply the majority of the _____ and _____ in the diet.	11 Calcium Phosphorus
12.	The best sources of calcium are _____ and _____	12. Cheese Milk.
13.	Human milk contains _____ iron per _____	13 1 2 mg Later
14	Milk is nearest to a _____ and _____ food.	14 Perfect, Complete.
15	The chief proteins of milk are _____ and _____	15 Caseinogen Lact albumin.
16.	Milk kept on unsterilised becomes sour due to formation of _____ which is sufficient to precipitate the _____	16 Lactic acid, Caseinogen.
17	Vitamin B_{12} is synthesized by _____ and is present only in _____ and _____	17 Microorganisms; Meat, Dairy Products.
18.	The extra heat production over and above the caloric value of a given amount of food when used by the body is said to be _____	18 Specific dynamic action.
19	The total heat produced or the energy spent by the body under conditions to perform minimum possible work is known as _____	19 Basal metabolism.
20	The ratio of the volume of CO_2 eliminated to the volume of oxygen utilized on oxidation is called _____	20 Respiratory Quotients.
21	Zein is almost completely free from _____ and _____	21 Tryptophan, Lysine.
22.	Gelatin is also lacking _____ and casein lacks _____	22. Tryptophan Methionine
23	The incomplete proteins are _____ and _____	23 Zein Gelatin
24	The most suitable proteins for growth are _____, _____, _____	24) Lactalbumin Ovalbumin Ovovitellin.
25	Proteins of animal origin have the _____ digestibility with the wastage in digestion only _____ or _____	25) Highest 5% Less

		Answers
6	A balanced diet is one which contains all the food constituents in proper _____ to meet the energy and _____ requirements of the individual	26) Proportions, Nutritional
7	Milk fat contains all _____ fatty acids as well as _____ fatty acids.	27) Saturated, Unsaturated
8	The white portion of egg contains _____ and _____	28) Proteins, Salts
9	The egg-yolk contains _____ proteins, _____ cholesterol, and _____ mineral	29) 15%, 4%, 1%
30	The proteins of egg-yolk are _____ and _____	30) Vitellin Livertin
31	Meat contains about _____ protein and _____ vitamins	31) 22%; B group
32	During hanging nearly all the carbohydrates of meat are converted to _____ as well as _____	32) Lactic acid, Acid phosphates
33	Large fish is rich in _____ but deficient in _____	33) Phosphorus, Calcium
34	The crude cereals contain _____ protein, _____ carbohydrate	34) 11%, 70%
35	The main proteins of cereals are _____ and _____	35) Glutelins, Gliadins
36	The abundant mineral elements in cereals are _____ and _____	36) Calcium, Phosphate
37.	The dried pulses contain _____ protein, less than _____ fat.	37) 20%-25% 2%
38	Nuts contain _____ protein and fat but _____ carbohydrate	38) High, Low
39	The most important tuber used in this country is the _____ and _____	39) Potato, Sweat potato
40	The tubers are the valuable sources of _____ and _____	40) Iron, Vitamin C
41	The common roots are almost free from _____	41) Starch
42	Carrots the richest in _____, are the sources of _____	42) Sugars Carotene
43	Roots contain valuable salts but negligible _____ and _____	43) Protein, Fat
44	Arrowroot contains traces of _____ and _____	44) Protein Salts
45	Yellow pumpkin is a fair source of _____	45) Carotene
46	The energy value of fruits is due to _____ and _____	46) Sugars Starch
47	Many fruits contain _____ and _____	47) Pentoses, Pectons
48	Banana contains _____ as well as _____	48) Starch, Sugar
49	Tea has a _____ and _____ effect	49) Stimulant, Diuretic
50	Strong tea disturbs _____ digestion due to _____	50) Gastric, Tannic acid

	Answers
51 Coffee contains _____ and _____.	51) Caffeine Tannic acid.
52. Chocolate consists of ground _____ mixed with _____	52) Cocoa nibs; Sugar

Indicate "True" or "False"	Answers
1 Tea has negligible caloric value.	1 True
2. Cereals provide proteins, certain minerals and vitamins in addition to energy in the diets of the low-income groups.	2. True
3. Pure carbohydrates and fats provide energy only	3 True
4 The protective foods are rich in proteins, vitamins and minerals.	4 True
5 Foods rich in vitamins, minerals and proteins of high biologic value e.g. milk, eggs, meat, fish.	5 True
6. Tartaric acid, citric acid and malic acid are present in cereals.	6 False
7 Green leafy vegetables are lack in calcium and vitamin C.	7 False
8. Nuts contain high fat and carbohydrate but low proteins.	8. False
9 The main protein of pulses is a globulin called legumin.	9 True
10 . The fats of cereals contain considerable amounts of olein to make them liquid at ordinary temperature	10 True
11 All cereals contain the enzyme phytase which hydrolyzes phytic acid.	11 False.
12. Carotene and riboflavin of cereals are lost by heating.	12. False.
13. Small fish eaten with bones are good source of calcium.	13. True.
14 The dried fish may contain about 20% carbohydate.	14 False
15 The flavour of meat is due to the organic substances extracted from it by boiling water	15 True
16 The minerals in egg-yolk are sodium, potassium and carbonate.	16 False
17 The egg-yolk is very rich in vitamin C.	17 False.
18 The other proteins of egg-white are conalbumin, ovoglobulin and ovomucoid.	18. True
19 The proteins of milk neutralize gastric HCl to allow clotting	19 True.
20 Milk is rich in vitamin B_1, C, and D	20 False
21 The iron content of milk is very high which is quite sufficient.	21 False
22. Proteins of meat, glutenin (wheat), and glycinin (soyabean) support growth when given in a higher concentration.	22. True.
23 The vegetable proteins legumin (pea) and hordein (barley) are only suitable for maintenance	23 True.
24 Zein and gelatin are neither suitable for growth nor maintenance.	24 True.

		Answers
25	The proportions of protein, fat and carbohydrate should approximately be 1 1 2 3 8	25 False
26	Vitamin B_{12} is fairly available in cereals and vegetables	26 False
27	Human and cow s milk contain copper only about 0 6 mg per liter	27 True
28	The more concentration of iron is from bread meat and potatoes	28 True
29	A diet lacking in fat causes a feeling of hunger shortly after a meal.	29 True
30	The biologic value of protein depends on the content of essential amino acids	30 True
31	Hard water is a source of calcium	31 True
32.	In prolonged starvation the concentration of triiodothyronine falls which is responsible for the reduced metabolism	32 True
33	Starvation patients often suffer from infections — malaria cholera pneumonia and gastroenteritis	33 True
34	In nervous cretinism there is mental deficiency deaf mutism spasticity and ataxia	34 True
35	In myxoedematous cretinism there is dwarfism	35 True
36	Where goitre is endemic daily intake of dietary iodine are likely to be less than 100 µg	36 False
37	Xerophthalmia means dry eye is a condition caused by vitamin A deficiency	37 True
38	Xerophthalmia arises when the diet contains whole milk and butter	38 False
39	Osteomalacia is sometimes found in patients with cirrhosis of the liver due to failure to form 25(OH) D	39 True
40	In osteoporosis there is muscle weakness	40 False
41	In osteomalacia the plasma alkaline phosphatase is normal	41 False
42	All people can be protected from rickets by a supplement of 10 µg of vitamin D daily	42 True
43	In wet beriberi anorexia and dyspepsia are not present.	43 False
44	In the treatment of infantile beriberi the mother should receive 10 mg thiamin twice daily in severe cases	44 True
45	Warnicke s disease and Korsakoff's psychosis are manifestations of thiamin deficiency	45 True
46	In pellagra the tongue characteristically has a raw beef appearance	46 True
47	Pellagra is often accompanied by signs of protein energy malnutrition	47 True
48.	Tropical ulcers are often caused by minor injuries in people living in poor hygienic condition infested by dysentery and malaria	48 True

		Answers
49	Cheilosis is characterized by a zone of red, denuded epithelium at the line of closure of the lips.	49 True
50	Angular stomatitis occurs in association with iron deficiency, anemia and other diseases.	50 True
51	In corneal vascularisation small greyish-white opacities may also be seen on the surface of the cornea.	51 True
52.	Nutritional glossitis is a feature of pellagra, sprue and the various types of nutritional anemias.	52. True
53	In chronic atrophic glossitis the tongue is small with an atrophic mucous membrane	53. True
54	In Von Gierke s disease growth is retarded and marked enlargement of the liver	54 True
55	Emotional stress may bring an angina.	55 True

CHAPTER – 17
VITAMINS

Write the correct answer number of the followings :

1 Retinal is reduced to retinol by retinene reductase in presence of the coenzyme
   (a) NAD^+
   (b) $NADP^+$
   (c) $NADH + H^+$
   (d) $NADPH + H^+$

2. Retinol exists as an ester with higher fatty acids in the
   (a) Liver
   (b) Kidney
   (c) Lung
   (d) All of the above

3. Retinol is transported to the blood as retinol attached to
   (a) α_1 Globulin
   (b) α_2 Globulin
   (c) β Globulin
   (d) γ-Globulin

4 Carotenes are transported with the
   (a) Proteins
   (b) Lipids.
   (c) Lipoproteins.
   (d) Minerals.

5 The dietary vitamin A which is in the form of Vitamin A esters are hydrolyzed in the lumen of the intestine by the enzyme
   (a) Amylase
   (b) Lipase
   (c) Peptidase
   (d) Nuclease

6 In the blood the vitamin esters are attached to
   (a) α_1 Lipoproteins
   (b) α_2 Lipoproteins
   (c) β– Lipoproteins.
   (d) γ– Lipoproteins

Answers

1) c

2) d

3) a

4) c

5) b

6) c

7 The percentage of vitamin A in the form of esters is stored in the liver
(a) 80
(b) 85
(c) 90
(d) 95

8 The preformed vitamin A is supplied by foods such as
(a) Butter
(b) Eggs
(c) Fish liver oil
(d) All of the above

9 One I U is equal to the number of micrograms of β–carotene
(a) 0 3
(b) 0 4
(c) 0 6
(d) 0 8

10 The non protein part of rhodopsin is
(a) Retinal
(b) Retinol
(c) Carotene
(d) Repsin

11 Lumirhodopsin is stable only at a temperature below
(a) −35°C
(b) −40°c.
(c) −45°c
(d) −50°c.

12 Metarhodopsin is hydrolyzed to retinal and opsin at a temperature below
(a) −8°c
(b) −10°c.
(c) −15°c
(d) −20°c.

13 The normal concentration of vitamin A in blood in I U /dl.
(a) 20 - 55
(b) 24 - 60
(c) 30 - 65
(d) 35 - 70

Answers

7) d.

8) d.

9) c

10) a

11) d.

12) c.

13) b

14 Continued intake of excessive amounts of vitamin A especially in children produces
(a) Irritability
(b) Anorexia
(c) Headache
(d) All of the above

15 Vitamin D_2 is also said to be
(a) Activated ergosterol
(b) Ergocalciferol
(c) Viosterol
(d) All of the above

16 The poor sources of vitamin D
(a) Eggs
(b) Butter
(c) Milk
(d) Liver

17 The normal concentration of vitamin D in blood in I U /l
(a) 600 – 2500
(b) 700 – 3100
(c) 800 – 4100
(d) 850 – 4700

18 The activity of tocopherols is destroyed by
(a) Oxidation
(b) Reduction
(c) Conjugation
(d) All of the above

19 Some tocopherols are
(a) Terpenoid in structure
(b) Dionol in structure
(c) Isoprenoid in structure
(d) Farnesyl in structure

20 The methyl groups in the aromatic nucleus of α–tocopherol are
(a) 2
(b) 3
(c) 4
(d) 5

Answers
14) d
15) d
16) c
17) b
18) a
19) a
20) b

21 Vitamin E is stored in
(a) Mitochondria.
(b) Microsomes.
(c) Both of the above
(d) None of the above.

22. Vitamin E protects the polyunsaturated fatty acids from oxidation by molecular oxygen in the formation of
(a) Superoxide
(b) Peroxide.
(c) Trioxide
(d) All of the above

23 The tocopherols prevent the oxidation of
(a) Vitamin A
(b) Vitamin D
(c) Vitamin K
(d) Vitamin C

24 Vitamin E protects enzymes from destruction in
(a) Muscles.
(b) Nerves
(c) Gonads
(d) All of the above

25 Vitamin K_2 was originally isolated from
(a) Soyabean
(b) Oysters.
(c) Putrid fish meal
(d) Alfalfa.

26 The poor sources of vitamin K_1 are
(a) Milk
(b) Meat
(c) Fish
(d) All of the above

27 Vitamin K regulates the synthesis of blood clotting factors
(a) VII
(b) IX
(c) X
(d) All of the above

Answers

21) c.

22) b

23) a.

24) d.

25) c.

26) d

27) d.

28 Ascorbic acid can reduce
(a) 2 6 — dichlorophenolindophenol
(b) 2 4 — dinitrobenzene
(c) 2 6 — dioxypyridine
(d) 2 6 — dibromobenzene

29 Sterilised milk is devoid of
(a) Vitamin A
(b) Vitamin D
(c) Vitamin C
(d) Vitamin B1

30 Vitamin C is required in the metabolism of
(a) Phenylalanine
(b) Tryptophan
(c) Both of the above
(d) None of the above

31 The symptoms of scurvy are
(a) Poor healing of wounds
(b) Loosening of the teeth
(c) Anemia
(d) All of the above

32 Thiamine is also said to be
(a) Antiberiberi substance
(b) Antineuritic vitamin
(c) Aneurine
(d) All of the above

33 Lipoic acid is also termed as
(a) Thioctic acid
(b) Protogen
(c) Acetate replacement factor
(d) All of the above

34 Folic acid is also termed as
(a) SLR factor
(b) Pteroyl glutamic acid
(c) Liver lactobacillus casei factor
(d) All of the above

35 Thiamine is oxidized to thiochrome in alkaline solution by

Answers
28) a
29) c
30) c
31) d
32) d
33) d.
34) d

Answers

(a) Potassium permanganate. 35) b
(b) Potassium ferricyanide
(c) Potassium dichromate
(d) Potassium chlorate

36 The requirement of vitamin B_1 is increased when metabolism is elevated as in
(a) Fever 36) d.
(b) Hyperthyroidism
(c) Increased muscular activity
(d) All of the above

37 Riboflavin in alkaline solution when exposed to ultra violet light is converted into lumiflavin which in ultra violet light has a
(a) Greenish yellow fluorescence 37) a.
(b) Bluish yellow fluorescence
(c) Reddish yellow fluorescence
(d) Light yellow fluorescence

38 The normal concentration of riboflavin in plasma in ug/100 mL
(a) 1 5 to 3 5 38) b
(b) 2 5 to 4 0
(c) 3 5 to 5 5
(d) 4 5 to 7 5

39 Riboflavin is involved in the regulatory functions of some hormones connected with the metabolism of
(a) Carbohydrate 39) a.
(b) Fat
(c) Protein
(d) Minerals

40 FMN is a constituent of the
(a) Warburg yellow enzyme 40) d.
(b) Cytochrome C reductase
(c) L amino acid dehydrogenase
(d) All of the above

41 The majority of the excess nicotinic acid is excreted in the urine in the form of
(a) N methyl nicotinamide 41) d.
(b) 6-pyridone of N-methylnicotinamide
(c) N methyl nicotinic acid
(d) All of the above

42 Niacin is present in the maize in the form of
(a) Niatin
(b) Niacytin
(c) Nicotin
(d) Nicyn

43 The normal concentration of niacin in blood in mg /100 ml
(a) 0 3 to 0 5
(b) 0 4 to 0 6
(c) 0 5 to 0 8
(d) 0 6 to 0·9

44 Nicotinic acid is essential for the normal functioning of
(a) Skin
(b) Intestinal tract
(c) Nervous system
(d) All of the above

45 Pyridoxine is a mixture of
(a) Pyridoxine
(b) Pyridoxal
(c) Pyridoxamine
(d) All of the above

46 Pyridoxine produces a coloured compound with
(a) 2 6 dichloroquinone chlorimide
(b) 2 6 dichloroquinone
(c) 2 4 nitroquinone
(d) All of the above

47 Pyridoxal phosphate is involved in the desulphuration of
(a) Cysteine
(b) Homocysteine
(c) Both of the above
(d) None of the above

48 In some subjects pyridoxine deficiency causes
(a) Lymphopenia
(b) Peripheral neuropathy
(c) Both of the above
(d) None of the above

Answers

42) b

43) c

44) d.

45) d

46) a

47) c

48) c

49 In the deficiency states of vitamin B_6 there are inborn errors of metabolism including
(a) Cystathioninuria
(b) Familial xanthurenic aciduria.
(c) Some pyridoxal responsive anemias
(d) All of the above

50 Pantothenic acid exists in the tissues as
(a) β–mercaptoethylamine
(b) Coenzyme A
(c) Pantoic acid.
(d) β–alanine

51 Pantothenic acid deficiency causes
(a) Nausea
(b) Irritability
(c) Anemia
(d) All of the above

52. Folacin is made up of
(a) Pteridine nucleus
(b) P aminobenzoic acid.
(c) Glutamic acid
(d) All of the above

53 Folic acid coenzymes take part in the synthesis of
(a) Purines
(b) Thymine
(c) Both of the above
(d) None of the above

Answers

49) d.

50) b

51) d

52) d.

53) c.

Fill up the blanks of the followings

1 The folic acid inhibitors are _____ and _____.

2 Aminopterine has been used in the treatment of _____ particularly in children

3 Folic acid deficiency causes increased output of _____ in the urine after histidine loading

4 Folic acid is converted to tetrahydrofolate catalyzed by _____ which use _____

5 Folic acid as a coenzyme is involved in the transfer and utilization of the single _____ moiety

Answers

1) Aminopterine Amethopterine

2) Leukemia.

3) Farmiminoglutamic acid (FIGLU)

4) Folic acid reductase Reduced NADP

5) Carbon

		Answers
6	The single (formyl) carbon present in the tetrahydrofolic acids is utilized as a source of carbons _____ and _____ in the purine nucleus.	6) 2 8
7	Folic acid is transported to the plasma as _____ bound to _____	7) Methyltetrahydrofolate Protein
8	The normal level of folic acid in serum is _____ per l of serum in healthy subjects.	8) 3 to 25 µg
9	The central structure of vitamin B_{12} is referred to as a _____ ring system	9) Corrin
10	Vitamin B_{12} contains cobalt _____ per cent	10) 4 to 5
11	Vitamin B_{12} activity is slowly destroyed by the reducing action of _____	11) Ascorbic acid
12	The absorption of vitamin B_{12} depends on the presence of _____ and a constituent of normal gastric juice called the intrinsic factor (IF) a _____ secreted by the parietal cells of the gastric mucosa	12) Hydrochloric acid Mucopolysaccharide
13	Cobalamin is bound to intrinsic factor in the proportion of _____ mol of cobalamin to _____ mol of IF	13) 2 1
14	Intrinsic factor is found in the _____ and _____ of the stomach	14) Cardia Fundus
15	Vitamin B_{12} also binds to the proteins of gastric juice _____ and _____	15) Bile Saliva
16	The intrinsic factor possesses two receptor sites one for _____ and other for ileal intestinal _____	16) Vitamin B_1 Microvilli
17	Intrinsic factor protects _____ against bacterial attack	17) Vitamin B_2 peptide
18	Transcorrin I is a _____ binder of cobalamin and transcorrin II is a _____ binder of cobalamin	18) Strong Weaker
19	The total amount of cobalamin in the bodies of adults is _____ of which about _____ is in the liver	19) 2 5 mg 1 5 mg
20	The normal concentration of vitamin B_{12} in serum is _____ per ml	20) 160 to 1000 pg
21	Vitamin B_{12} is present only in the foods of _____ origin	21) Animal
22	Vitamin B_{12} along with _____ is required for the development of red blood cells beyond _____ stage	22) Folic acid Megaloblast
23	Vitamin B_{12} as the coenzyme is involved with _____ in the synthesis of labile _____ groups	23) Tetrahydrofolate Methyl
24	Vitamin B_{12} is also a coenzyme for the _____ which converts methyl malonyl-CoA into _____	24) Mutase Succinyl CoA
25	The carboxyl group of biotin combines with the terminal nitrogen of _____ residue of enzyme protein forming _____	25) Lysine Biocytin

		Answers
26	The conversion of pyruvate to oxaloacetate is also a _____ dependent carboxylation reaction catalyzed by _____	26) Biotin, Pyruvate carboxylase.
27	Biotin is involved in the fixation of _____ for the formation of carbon _____ in purine synthesis.	27) CO_2, 6
28	The deficiency of biotin may result from the adequate intake of raw _____ which contains the protein _____	28) Egg white, Avidin.
29	Inositol exists in the form of phospholipids, _____ and _____ in plant tissues	29) Phytic acid, Phytin
30	Large amounts of inositol containing phosphatides are present in _____ and _____	30) Mitochondria Microsomes
31	Along with _____ inositol exerts a _____ effect.	31) Choline, Lipotropic
32.	Inositol is oxidized to _____ in the liver by _____	32) Glucuronic acid, Oxygenase
33	Choline takes part in _____ reactions in the formation of _____ from homocystine	33) Transmethylation, Methionine.
34	Choline acts as a _____ donor only after oxidation to _____	34) Methyl Betaine.
35	Choline is first oxidized to _____ and then to _____	35) Betainealdehyde, Betaine
36	Osteomalacia can be prevented by the administration of _____ and _____	36) Vitamin D, Calcium.
37	In pernicious anemia the tongue is _____ and _____	37) Sored Inflammated.
38	The antagonists of vitamin B_6 are _____ and _____	38) Isonicotinic acid hydrazide Hydralazine.

Indicate "True" or "False" of the followings :	Answers
1 Renal ricket is caused by defective transport of phosphate by the renal tubules.	1) True
2 The symptom of osteomalacia is the progressive calcification of bones and bones become hard	2) False
3 In infantile scurvy purpura occurs in the sternum.	3) False
4 Palpitation and breathlessness appear in the wet beriberi	4) True
5 Pellagra is not caused by chronic infection.	5) False
6 In pernicious anemia the RBC count is very high.	6) False
7 The best sources of vitamin K are cauliflower and soyabean.	7) False
8 Vitamin K is involved in oxidative phosphorylation in animal tissues.	8) True

		Answers
9	The daily requirement of vitamin C by adult is 80 mg	9) False
10	Vitamin C is involved in the hydroxylation of steroids in the adrenal cortex	10) True
11	Vitamin C is required for the formation of growth hormone	11) False
12.	The deficiency of vitamin C causes susceptibility to infection	12) True
13	The daily requirement of thiamine by pregnant women is 0 5 to 0 8 mg	13) False
14	In dry beriberi the muscles become wasted and weak	14) True
15	No toxicity results in the intake of excessive amounts of thiamine	15) True
16	Vitamin B_2 is destroyed when kept in cold.	16) False
17	The best sources of riboflavin are cereals and germinating wheat	17) False
18	Niacin is chemically pyridine 3-carboxylic acid	18) True
19	Nicotinamide when heated in a strong alkaline or acid solution, it is converted into nicotinic acid	19) True
20	The best sources of niacin are fruits and vegetables.	20) False
21	The daily requirement of niacin by children is 5 to 8 mg	21) False
22.	The reduced NADP is also required in enzyme reaction in the formation of tetrahydrofolate	22) True
23	In large doses nicotinic acid causes burning sensation	23) True
24	Pyridoxamine contains a primary amine side chain in no 4 of pyridine nucleus	24) True
25	Pyridoxal phosphate acts as a cotransaminase in the transamination reactions	25) True
26	Liver phosphorylase also contains pyridoxal phosphate as coenzyme	26) False
27	Pyridoxal phosphate is concerned with immune response	27) True
28	The fair source of pantothenic acid is jelly	28) False
29	The daily requirement of pantothenic acid by infants is 5 to 10 mg	29) False
30	Pantothenic acid is a constituent of coenzyme A	30) True
31	Coenzyme A is involved in the metabolism of propionate and of branch chain fatty acids	31) True
32	Riboflavin diminishes the photo-oxidation of folic acid.	32) False
33	Dihydrofolate is reduced to tetrahydrofolate by folic acid reductase	33) True
34	2 to 5 mg of folic acid is excreted in the urine daily	34) False
35	Folic acid is incorporated into the erythrocytes during erythropoiesis.	35) True
36	Pregnant women require 800 mg of folic acid daily	36) False

37	Rhodopsin is a conjugated protein with a molecular weight of about 40000	37) True.
38	Retinene reductase is very similar to alcohol dehydrogenase of liver	38) True
39	Hypervitaminosis A causes drowsiness, severe headache, sluggishness and peeling of the skin about the mouth	39) True
40	Vitamin D is stored largely in heart and pancreas	40) False
41	Eggs and butters are the poor sources of vitamin D	41) False
42.	Adults daily require 800 I.U of vitamin D	42) False
43	The major function of vitamin D is to stimulate transcription of the mRNA for a calcium transport protein.	43) True
44	The effect of vitamin D is not much related to the activity of parathormone and calcitonin.	44) False
45	In fully growing bones in adults, there is a type of defective mineralization of osteoid tissue termed "Osteomalacia"	45) True
46	The symptoms of hypervitaminosis D are thirst, constipation and polyuria.	46) True
47	Vitamin E prevents the hepatic necrosis produced by the lack of sulphur containing amino acids in dietary proteins.	47) True
48	The tocopherols play a part in the cellular oxidation	48) False
49	The normal concentration of vitamin E in blood is 20 mg/L	49) False
50	The tocopherols are largely methyl derivatives of the parent compound tocol.	50) True
51	Vitamin E refers to a group of compounds known as tocopherols.	51) True
52.	Vitamin E is involved in heme synthesis.	52) True
53	Vitamin C is stable below pH 6 8 at room temperature and readily oxidized in an alkaline medium.	53) True
54	Human body can not synthesize vitamin C.	54) True

PRACTICAL PART

Section I – QUALITATIVE

Section II – QUANTITATIVE

PRACTICAL BIOCHEMISTRY

SECTION I

QUALITATIVE

CHAPTER I

CARBOHYDRATES

1. MOLISCH'S TEST:

Principle: Alcoholic alpha naphthol forms furfural and furfural derivatives, such as hydroxymethylfurfural, by the concentrated sulphuric acid acting on the sugar. This compound forms a reddish-violet coloured ring at the junction of the two liquids.

Molisch's reagent: A 5 per cent solution of alpha naphthol in alcohol

Proecdure: Add 2 drops of molisch's reagent to 2 ml. of sugar solution in a test tube. Mix thoroughly. Add 2 ml. of Conc. H_2SO_4 by the side of the test tube slanting the tube. Then errect the test tube slowly. The formation of reddish-violet ring at the junction of two liquids indicates the presence of carbohydrate.

Discussion: Concentrated solutions of organic compounds may give a red instead of a violet colour due to the charring action of the sulphuric acid. In case of doubt the experiment should be repeated on a more dilute solution of the substance to be tested.

2. IODINE TEST:

Principle: The composition of the blue- or red- or wine red-coloured substance is not well defined. This may be an adsorption complex of starch or dextrins or glycogen with iodine rather than a definite compound.

Iodine reagent: 0·5 ml. of iodine diluted to 5 ml. with distilled water.

Procedure: Add 1 or 2 drops of dilute iodine solution to 2-3 ml. of dilute starch or dextrin or glycogen solution. A blue, red and brown colour develop in case of starch, dextrin, and glycogen respectively. In case of starch, the blue colour disappears on heating and reappears on cooling. But the red colour and the brown colour in cases of dextrin and glycogen respectively do not reappear on cooling as in the case of starch.

3. REDUCTION TESTS:

Carbobydrates with free aldehyde or ketone groups have the ability to reduce solutions of various metallic ions. These properties are mentioned below:

A. FEHLING'S TEST:

Principle: Carbohydrates with free aldehyde or ketone groups reduce copper sulphate to cuprous oxide forming a yellow- or brownish-red-coloured precipitate.

Fehling's reagent: Prepare freshly by mixing equal volumes of two stock solutions A and B.

Solution A: 6 93 gms. of $CuSO_4$, $5H_2O$ per 100 ml. of water.

Solution B: 20 gms. of KOH and 34 6 gms of sodium potassium tartarate (Rochelle salt) per 100 ml. solution.

Procedure: Add a few drops of sugar solution at a time to 5 ml of fehling's solution and heat the mixture after each addition. The production of yellow or brownish-red cuprons oxide precipitate indicates the presence of reducing sugars.

B. BENEDICT'S TEST:

Principle: Carbohydrates with free aldehyde or ketone groups reduce copper sulphate of benedict's solution to cuprous oxide on boiling forming a red, yellow or green coloured precipitate depending on the concentration of the sugar.

Benedict's qualitative solution: Dissolve with heat 173 gms. of sodium citrate and 100 gms of anhydrous sodium carbonate in 600 ml of water in a beaker Into this with constant stirring, run slowly a solution of copper sulphate ($CuSO_4$, $5H_2O$) containing 17 3 gms dissolved in about 100 ml. of water Cool and transfer to a litre flask and make to the mark with water

Procedure. Add 8 drops of sugar solution to 5 ml of the benedict's qualitative reagent in a test tube. Boil vigorously for 2 minutes or place in a water bath for 3 minutes Allow to cool spontaneously (do not cool it by immersion in cold water) A red, yellow or green precipitate develops depending on the concentration of sugar present.

Colour	Approximate amount of reducing sugar
No change of blue colour	Absence of Reducing sugar
Blue changes to green ppt.	0 1—0 5 gm% of reducing sugar
Blue changes to yellow ppt.	0 5—1·0 gm% ,, ,, ,,
Blue changes to orange-red ppt.	1 —2·0 gm% ,, ,, ,,
Blue changes to Brick red ppt	over 2 0 gm% ,, ,, ,,

C. BISMUTH REDUCTION TEST (NYLANDER)

Principle: Carbohydrates with free aldehyde or ketone groups reduce to cause the black precipitation of metallic bismuth

Procedure: Add 8 drops of Nylander's reagent to 5 ml. of sugar solution in a test tube and heat for 5 minutes in a boiling water bath A black coloured precipitate develops on standing for a few minutes.

D COLE'S TEST

Principle: Carbohydrates with free aldehyde or ketone groups reduce copper sulphate to cuprous oxide on boiling forming a yellowish precipitate.

Procedure: Add a large quantity of anhydrous sodium carbonate to 5 ml. of 0 1% sugar solution and add 3 drops of glycerol and 3 drops of 5% $CuSO_4$ solution. A yellowish precipitate develops on boiling

E REDUCTION OF METHYLENE BLUE

Principle: Carbohydrates containing free aldehyde or ketone groups can reduce methylene blue to leuco-methylene blue with the discharge of the blue colour

Procedure: Add one drop of 1% methylene blue, a few drops of 5% NaOH to 2 ml of water and boil taking care that the blue colour does not disappear Now add a few drops of 0 2% sugar solution and boil again. The colour is now discharged Shake and note the reoxidation of leuco-methylene blue by oxygen of air to the coloured dye again.

Discussion of reduction tests

(i) Ammonium salts interfere with fehling's test. If present in excess, the solution should be made alkaline with Na_2CO_3 and boiled in order to decompose the ammonium salts Prolonged contact with hot strong alkali may destruct the sugar present The solution under examination by fehling's test must be made neutralized or alkaline before the commencement of the test.

If the sugar is preserved by Chloroform, a positive reaction may be obtained in the absence of sugar even. This statement is based on the fact that bot alkali produces reducing subtances from the sugar present. Uric acid and creatinine interfere this test

(ii) Benedict's test responds to even a very small quantities of sugar (0 1%) yielding a very good precipitate

Chloroform does not interfere with this test and even uric acid or creatinine do not interfere to such an extent as in the case of fehling's test.

This test may produce a white precipitate if considerable amounts of phosphates are present

On the above grounds benedict's test is better than fehling's test

(iii) Cole's test is suitable for detecting a small quantity of sugar (0 1 mg)

(iv) Bismuth reduction test responds to albumin, if present Chloroform also interferes with this test Uric acid and creatinine do not interfere This test can detect sugar to the extent of 0 08 per cent

4 BARFOED'S TEST

Principle: The monosaccharides with free aldehyde or ketone groups can cause the reduction of copper sulphate to cuprous oxide with the development of a red precipitate

Barfoed's reagent: Dissolve 24 grams of copper acetate in 450 ml boiling water If a precipitate form, do not filter Immediately add 25 ml 8 5 per cent lactic acid to the hot solution Shake, cool and dilute to 500 ml and filter off the impurities

Procedure: Add 8 drops of sugar solution to 5 ml of barfoed's solution Heat to boiling for 30 seconds A red precipitate appears indicating the presence of monosaccharides

Discussion: This test is a reduction test for monosaccharides and the reduction is brought about in an acid solution If the solution with the sugar is boiled for a few minutes, the disaccharides also are hydrolysed giving a positive test This test is specific for monosaccharides

5 BIAL'S TEST FOR PENTOSES

Principle: Hydrochloric acid acting on pentoses yields substances which link with orcinol to form green compounds

Bial's reagent: Dissolve 300 mg of orcinol to 100 ml of Conc HCl and add 5 drops of 10 per cent ferric chloride solution

Procedure: Mix 5 ml of bial's reagent with 0 5 ml of the sugar solution Heat to boiling The development of green colour indicates the presence of pentoses

Discussion: This test is sensitive to 0 1 per cent of Pentoses Glucuronates can give a similar colour if the boiling is prolonged due to the conversion of glucuronates to pentoses

6 TAUBER'S BENZIDINE TEST FOR PENTOSES

Procedure: To 1 ml of Benzidine solution add 2 drops of sugar solution Boil and cool quickly A violet colour indicates the presence of Pentoses

7. SELIWANOFF'S TEST

Principle· Fructose is acted on by hydrochloric acid to form a derivative of furfuraldehyde which gives a red coloured compound when linked with resorcinol

Seliwanoff's reagent: Dissolve 50 mg of resorcinol in 33 ml of concentrated hydrochloric acid and dilute to 100 ml with water

Procedure: Add a few drops of sugar solution (0 5 ml) to 5 ml of seliwanoff's reagent in a test tube Heat to boiling for 30 seconds Formation of red colour indicates the presence of fructose The test may be positive for sucrose also if it is hydrolyzed during the course of the test

Discussion: A similar colour may also develop in case of glucose or maltose if the boiling is prolonged due to the transformation of glucose into fructose by the catalytic action of the hydrochloric acid

This test is sensitive to 0 1 per cent of fructose if glucose is absent But if glucose is present, it is less sensitive to fructose Large amounts of glucose can give a similar colour

8 HYDROLYSIS TEST FOR SUCROSE

Principle: Sucrose on hydrolysis by HCl is converted into glucose and fructose. The presence of these two monosaccharides are detected by tests mentioned above.

Procedure: Add two drops of dil HCl and one drop of thymol blue to 5 ml. of sucrose solution. The development of pink colour indicates the solution acidic. Divide it into two equal parts Boil one portion for about one minute and then cool it under the tap Neutralize both portions by adding 2% Na_2CO_3 drop by drop Formation of blue colour indicates the neutralization. The sucrose in the boiled portion has been hydrolyzed to form glucose and fructose which can be detected by Benedict's test and Selwanoff's test. But the unboiled sucrose does not reduce benedict's solution.

9 METHYLAMINE TEST FOR LACTOSE

Procedure: Add one ml. of 0·2 per cent solution of methylamine hydrochloride in water, followed by 0 2 ml. of 10 per cent sodium hydroxide, to about 5 ml. of sugar solution. Mix by inversion, cover the tube with a glass bulb and beat at 56°C for 30 minutes Remove from the bath and stand at room temperature. The solution will show a red colour before the end of the heating if much lactose is present Colour increases on standing

Discussion: The only sugars which give a red colour are the disaccharides, lactose and maltose.

Glucose, fructose, galactose, xylose and sucrose in large amounts give a yellow colour which is easily distinguished

10 OSAZONE TEST

Principle: A solution of reducing sugar when heated with phenylhydrazine, characteristic yellow crystalline compounds called osazone are formed. Simple sugars like glucose, fructose and mannose produce the same osazone because of the similarities in their molecular structures.

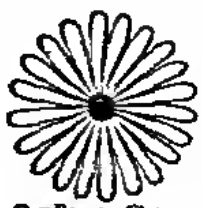
Procedure: Add 10 drops of glacial acetic acid to 5 ml. of sugar solution in a test tube Then add a knife point of phenylhydrazine hydrochloride and double the amount of sodium acetate crystals Mix and warm a little to see that the solids are dissolved Filter the solution in another test tube and keep the filtrate in a boiling water bath for 20 minutes Allow the tube to cool slowly in the water bath without cooling it hurriedly under the tap to have better crystals and examine the crystals under the microscope.

Discussion: Formation of osazone crystals of different sugars depends on the time schemed below

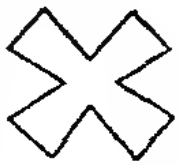
Osazones	Minimum time of formation of crystals	Appearance of Crystals
Glucosazone	5 minutes	Broomstick like
Fructosazone	2 minutes	Broomstick like
Galactosazone	7 minutes	Rhombic like
Lactosazone	10-12 minutes	Powderpuff like
Maltosazone	10-15 minutes	Sunflower like.

Broomstick like

Powderpuff like



Sunflower like



Rhombic like

Sucrose does not produce osazone crystals But it produces osazone crystals when the solution is kept in the boiling water bath for 30 minutes because of the hydrolysis of sucrose to glucose and fructose

11 PRECIPITATION REACTION FOR POLYSACCHARIDES

Principle: Generally, the bigger molecules are more easily precipitated by salts The starch is precipitated when its solution is half saturated with ammonium sulphate Glycogen is completely precipitated only when its solution is fully saturated with ammonium sulphate, whereas dextrin is not completely precipitated even on full saturation

Procedure: Add 5 ml of saturated solution of ammonium sulphate to 5 ml of starch solution to make the solution half saturated with ammonium sulphate Mix well by inverting the test tube several times and allow it to stand for 5 minutes Flocculent precipitate of starch will be formed Filter Perform iodine test with the residue as well as with the filtrate Blue colouration with the residue indicates the presence of starch and no blue colouration with the filtrate indicates the complete precipitation of starch

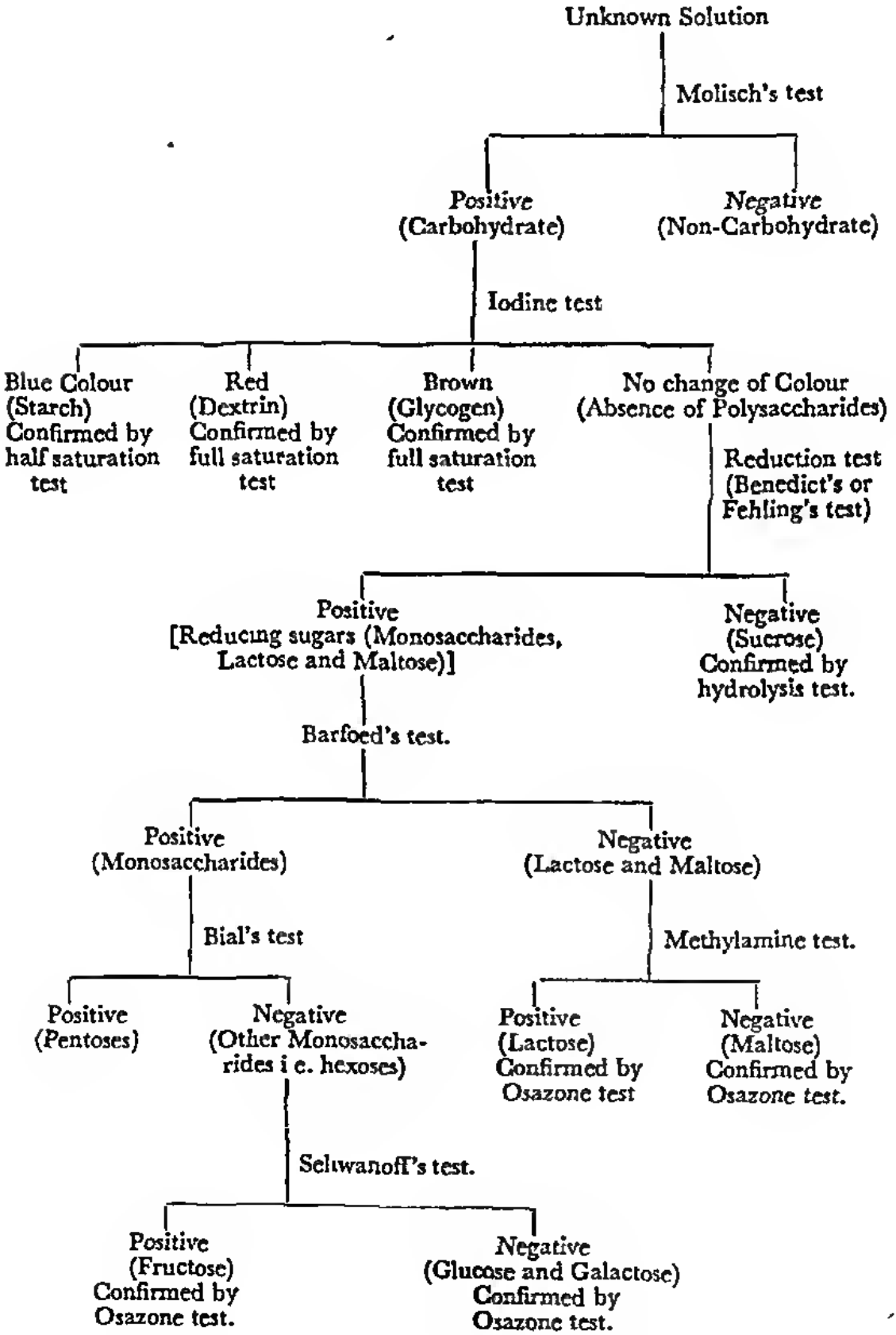
Now take 5 ml of glycogen or dextrin solution in a test tube and saturate it fully with solid ammonium sulphate Wait for 5 minutes and filter Perform iodine test with the filtrate In case of dextrin solution, the filtrate showing red colour indicates that dextrin is *not completely precipitated even on* full saturation But in case of glycogen solution, the filtrate will develop no brown colouration indicating the complete precipitation of glycogen by full saturation

REVIEW OF THE TESTS OF CARBOHYDRATES

A chart showing different tests is given below for the facility of students to put + and − signs for positive and negative reactions respectively on the basis of their practical observations

Carbohydrates	Molisch's test	Iodine test	Benedict's test	Fehling's test	Barfoed's test	Bial's test	Tauber's test	Selwanoff's test	Hydrolysis test	Methylamine test	Osazone test	Precipitation by half saturation	Precipitation by full saturation
Glucose													
Fructose													
Galactose													
Pentose													
Maltose													
Lactose													
Sucrose													
Starch													
Glycogen													
Dextrin													

DETECTION OF CARBOHYDRATE OF AN UNKNOWN SOLUTION



Questions to answer

1 Discuss the principle of Molisch s test State why some of the substances other than Carbohydrates give positive Molisch's test

2 Name the tests for reduction reaction and the sugars for reduction reaction

3 Which test is suitable for detecting reducing sugars and why?

4 Why does lodine solution develop blue colour with starch solution?

5 How will you distinguish fructose from sucrose and other monosaccharides?

6 How will you perform hydrolysis test for sucrose?

7 What is the basis for osazone formation? Name the characteristic osazone crystals for different sugars

8 Why do glucose, fructose and mannose form similar osazone crystals?

9 Why sucrose does not form osazone crystals and why does it form crystals on long boiling?

10 Why do reducing sugars form red precipitate by Benedict s qualitative solution and why sometimes form yellow or green precipitate?

11 What is the composition of Benedict s qualitative solution?

12 Why does Seliwanoff's test produce red colour?

13 How will you detect pentoses from other monosaccharides and how will you perform the test?

14 Represent systematically to detect carbohydrate of an unknown solution

CHAPTER 2

FAT

1 SOLUBILITY

The glycerides of lower fatty acids are slightly soluble in water All glycerides are soluble in ether, cloroform and benzene They are slightly soluble in cold methyl alcohol, ethyl alcohol and acetone but highly soluble in hot methyl and ethyl alcohol, acetone.

Procedure: Arrange five dry test tubes in a rack. Add 2 ml of water, ether, chloroform, benzene, and ethyl alcobol to each test tube followed by one drop of mustard oil Shake and observe. In water, oil is broken into small droplets and float at the surface indicating that oil is insoluble in water But in other solvents, oil disappears

2 EMULSIFICATION

Principle· Oil or liquid fat becomes finely divided and is dispersed in water when shaken with water to form emulsification. Emulsification is permanent and complete in the presence of emulsifying agents The important emulsifying agents are bile salts, proteins, soaps, mono- and diglycerides Emulsification is important in the processes of fat digestion in the intestine Emulsifying-agents lower surface tension of the liquid

Procedure: Take 2 clean and dry test tubes In one tube, put 2 ml. water and in the other 2 ml dilute bile salt solution. Now to each add 2 drops of mustard oil and shake vigorously for about one minute. Allow the tubes to stand for 2 minutes and note that in water, oil is broken in small pieces and floated on the surface, whereas in bile salt solution, the oil can be seen in minute droplets suspended in the liquid (e g , permanent emulsification)

3 SAPONIFICATION

Principle: Oil or liquid fat when boiled with an alkali is hydrolyzed and the liberated fatty acids form salt with alkali (soap) This process is said to be saponification. The saponification number is the number of milligrams of KOH required to saponify one gram of fat.

Procedure. Take 4 ml. of 2% Sodium Carbonate Solution in a test tube and add 2 drops of mustard oil Shake vigorously and boil. A clean soapy solution is formed Cool and divide it into three parts for further study on the properties of soap

(*i*) In one test tube, add a few drops of Conc. HCl and observe that the fatty acid separates out and floats up This is due to the hydrolysis of soap by the acid

(*ii*) In another test tube, dissolve sufficient amount of finely powdered NaCl. White precipitates of soap separate out and float on the surface This process is called 'salting out' of soap

(*iii*) In the third test tube, add a few drops of $CaCl_2$ solution. A precipitate of insoluble calcium soap is obtained

4 GREASE-SPOT TEST

Procedure: Put a drop of oil over a piece of ordinary writing paper (not filter paper) The translucent spot indicates the presence of fat.

5 UNSATURATION TEST

Principle: All neutral fats contain glycerides of some unsaturated fatty acids These unsaturated fatty acids become saturated by taking up iodine. If the fat contains more unsaturated fatty acids, it will take up more iodine

Procedure: Add 10 drops of *Hubl's iodine reagent* (alcoholic solution of iodine containing some mercuric chloride) to 10 ml chloroform The chloroform shows pink colour due to the presence of free iodine Divide this solution equally into 4 test tubes

(i) To one test tube, add mustard oil drop by drop shaking the tube vigorously for about 30 seconds after addition of each drop until the pink colour is discharged and count the number of drops The pink colour is discharged owing to the taking up of iodine by the unsaturated fatty acids of the oil

(ii) Repeat the above experiment with the remaining three test tubes taking fats (coconut oil, dalda and ground nut oil) Now compare their unsaturation *It should be remembered that more the number of drops required to discharge the pink colour, the less is the unsaturation*

Iodine value: It is the number of grams of iodine taken up by 100 grams of fat

CHOLESTEROL

1. SALKOWSKI'S TEST (H_2SO_4 TEST)

Procedure: Dissolve a little cholesterol in 2 ml chloroform in a dry test tube Add an equal volume of Conc H_2SO_4 Shake gently, The upper layer of chloroform turns red and the sulphuric acid layer shows a yellow colour with a green fluorescence

2 LIEBERMANN-BURCHARD REACTION (ACETIC ANHYDRIDE H_2SO_4 TEST)

Principle: Addition of H_2SO_4 to cholesterol in the presence of acetic anhydride gives a green (e g , red-absorbing) chromphore

Procedure: Dissolve a few crystals of cholesterol in 2 ml of chloroform in a dry test tube Now add 10 drops of acetic anhydride and 1 to 3 drops of Conc. H_2SO_4 Mix well A red rose colour is formed which quickly changes through blue to green

3 FORMALDEHYDE-H_2SO_4 TEST

Procedure: Add 2 ml of formaldehyde—H_2SO_4 solution (1 part of 40% formaldehyde to 50 parts of the acid) to 2 ml of chloroform solution in a dry test tube The cherry colour is developed in the chloroform Pour off the chloroform into another test tube and add 2 to 3 drops of acetic anhydride The blue colour develops

This test is said to be more delicate than Salkowski's test

Questions to answer

1 Why is bile salt important for emulsification?
2 What is the importance of emulsification in the digestion of fat?
3 What is saponification value and what is the significance of high saponification value?
4 Why does the pink colour of chloroform disappear on addition of oil?
5 What is iodine value of fat and what is its significance?
6 Which test is important in the detection of cholesterol and how to perform the test?
7 Name the test for detecting fat and discuss its procedure

CHAPTER 3

PROTEINS

A. COLOUR REACTIONS OF PROTEINS

Proteins respond to some colour reactions due to the presence of one or more radicals or groups of the complex protein molecule. All proteins do not contain the same amino acids and hence they do not respond to all colour reactions giving positive findings The material under examination should, therefore, be subjected to several test before concluding its nature.

1 BIURET TEST

Principle: This test is positive for all compounds containing more than one peptide linkage (-CO NH) e g , Proteins and their hydrolytic products (metaproteins, proteoses, peptones, polypeptides except dipeptides and amino acids) This test is also positive for substances which contain two carbamyl ($-CONH_2$) groups joined directly or through a single atom of nitrogen or carbon and similar substances which contain $-CSNH_2$, $-C(NH)NH_2$ or $-CH_2NH_2$ groups also respond to this biuret test. Hence nonproteins e g , oxamide $\begin{bmatrix} CONH_2 \\ | \\ CONH_2 \end{bmatrix}$ and biuret $\begin{bmatrix} CONH_2 \\ | \\ NH \\ | \\ CONH_2 \end{bmatrix}$ give positive with this test.

Procedure: To 2 ml of Protein solution add 1 ml of 40% NaOH solution and 1 or 2 drops of 1% $CuSO_4$ solution A violet colour indicates the presence of Peptide linkage of the molecule

Note: Care must be taken that not more than 2 drops of dilute copper Sulphate (1%) be added, otherwise blue colour will develop instead of violet colour

2 NINHYDRIN TEST (THE TRIKETOHYDRINDENE HYDRATE TEST)

Principle: This test is positive for all amino acids containing free amino and carboxylic groups Hence, it is positive for proteins, peptones, peptides. It is also positive with other primary amines including ammonia. The triketohydrindene hydrate forms a complex with the amino or carboxylic group of the amino acids or other primary amino developing a blue colour on heating

Procedure: To 1 ml of Protein solution add 2 drops of freshly prepared 0 2% of ninhydrin solution and heat. A blue colour develops indicating the presence of proteins, peptides or amino acids

3 MILLON'S TEST (Test for tyrosine)

Principle: The neutral protein solution reacts with acidified mercuric chloride to form a yellow precipitate of Mercury—Protein complex. This complex forms a nitrite complex with sodium and nitrite solution on warming, developing a red colour This test is given by phenols or phenolic substance such as Salicylic acid

Procedure: Add 1 ml of Protein solution acidified with H_2SO_4 to 1 mL of acid mercuric sulphate solution (10% $HgSO_4$ in 10% H_2SO_4) Boil gently for about one minute A yellow precipitate forms Cool under the tap and add a drop of 1% of $NaNO_2$ (Sodium nitrite) solution On gentle warming it turns red. This indicates the presence of hydroxy phenyl group Tyrosine is the only amino acid which responds positively to this test.

Note: Excess of chloride interferes by forming unionised $HgCl_2$ If it be so, more $HgSO_4$ must be added.

4 XANTHOPROTLIC REACTION (For tyrosine, phenylalanine and tryptophan)

Principle: Proteoses and peptones do not form precipitate with HNO_3, but their solutions become yellow and then orange when made alkaline

The white precipitate of protein after the addition of HNO_3 is due to the formation of metaproteins insoluble in HNO_3 The nitro-compounds from the protein molecule containing *benzene ring* develop a yellow colour These nitro compounds in alkaline medium ionise freely and produce deep yellow or orange colour

Procedure: To 2 ml of protein solution add carefully 1 ml of Conc HNO_3 A white precipitate forms Boil and the colour changes to yellow Cool the test tube and add 2 ml of 20% NaOH (or ammonia) to make it alkaline The colour changes to orange indicating the presence of aromatic amino acids

Note: Phenylalanine does not respond to this test as it is ordinarily performed This test is not a satisfactory one for use in urinary examination because of the colour of the end reaction

5 HOPKINS—COLE—ADAM—KIEWICZ REACTION (Test for tryptophan)

Principle: Tryptophan forms a condensation product with the aldehyde in presence of Conc H_2SO_4 developing a purple ring

Procedure: To about 1 ml of the protein solution add one drop of very dilute (1 500) solution of commercial formalin (40% formalin) and one drop of mercuric sulphate solution (10% $HgSO_4$ in 10% H_2SO_4) Mix and incline the tube to add carefully at least 1 ml Conc H_2SO_4 by the side of the tube A purple ring at the junction of the two liquids indicates the presence of *tryptophan*

6 GLYOXALIC ACID REACTION FOR TRYPTOPHAN (HOPKINS COLE)

Principle: Tryptophan forms condensation product with glyoxalic acid in presence of pure Conc H_2SO_4 developing a purple ring

Procedure: About 2 ml of Protein solution is mixed thoroughly with 2 ml of glyoxalic acid reagent Add this mixture to another test tube containing 4 ml of pure Conc H_2SO_4 in such a way that the two liquids do not mix Rotate gently A purple ring develops at the junction of the two liquids

Note: This test does not respond in presence of strong oxidizing agents like nitrates and chlorates Sulphuric acid must be highly pure, otherwise, the impurities may act as oxidizing agents

7 SAKAGUCHI REACTION (Test for Arginine)

Principle: Arginine in presence of alcoholic α naphthol forms a complex with sodium hypochlorite developing a bright red colour

Procedure: To about 3 ml of protein solution add 1 ml of 5% NaOH, 2 drops of pure alcoholic alpha naphthol and one drop of 10% sodium hypochlorite Mix well The development of bright red colour indicates the presence of *arginine*

Note: This is an extremely sensitive test and may be used as a general test for protein, because all known proteins contain sufficient arginine

8 SULPHUR REACTION (For Cysteine, Cystine and methionine)

Principle: Sulphur of the sulphur-containing amino acids reacts with the sodium hydroxide forming sodium sulphide A black or brown precipitate of lead sulphide is formed as a result of the reaction between sodium sulphide and lead acetate This lead sulphide is insoluble in dilute HCl

Procedure: Boil 1 mL. of Protein solution (concentrated solution of egg white) with 1 ml of 40% NaOH for 1 minute Add a drop of lead acetate solution A black or brown precipitate is formed which is insoluble in dilute HCl

9 MOLISCH S TEST

Some proteins (glycoproteins) contain carbohydrate Hence this test is done for detecting the carbohydrate present in glycoprotein The method of this test is already discussed in carbohydrate chapter

B COAGULATION REACTION

Principle: Coagulation of Protein is caused by the denaturation of Protein structure by heat or acids

1 HEAT COAGULATION

Procedure: Take protein solution of about two third of the test tube and heat the upper portion of the solution. An opalescent appears which becomes deep on the addition of a few drops of 2% acetic acid This indicates the presence of *albumin*

2 COAGULATION WITH CONC HNO_3

Procedure: Take 2ml. of Conc. HNO_3 in a test tube and add the protein solution by the side of the test tube A white ring appears at the junction of the two liquids

C. PRECIPITATION REACTION

Principle: (i) Proteins are precipitated by salts of the heavy metals (e.g , $HgCl_2$, $AgNO_3$ $CuSO_4$, etc.)

(ii) These may he precipitated by certain acids some of which are called alkaloidal reagents (Picric acid, phosphotungstic acid, tannic acid and metaphosphoric acid etc.)

(iii) These may also be precipitated by concentrated solutions of ammonium sulphate, sodium sulphate and sodium chloride. These salts precipitate proteins by 'salting out' methods

(iv) The proteins are precipitated by dehydrating agents such as alcohols and acetone. These agents convert them into suspensoids which flocculate upon the addition of a few drops of salt solution Alcohol also causes denaturation of proteins Alcohols bring protein solution into isoelectric point at which it is precipitated

1. Precipitation by salts of heavy metals.

(*a*) **Procedure:** To 2 ml of dilute protein solution (solution of egg white) add a few drops of dilute solution (1%) of Zinc Sulphate drop by drop A white precipitate is formed indicating the presence of Protein

(*b*) **Procedure:** To 2 ml of dilute protein solution (solution of egg white) add a few drops of 0 5 per cent ferric chloride solution drop by drop An opalescent appears which disappears on addition of excess of ferric chloride solution.

2 Precipitation by alkaloidal reagent.

(*a*) **Procedure** Add 1 ml of Esbach's reagent (Solution of Picric acid and citric acid) to 1 ml. of protein solution A yellow precipitate is formed indicating the presence of protein

(*b*) **Procedure** To 2 ml of protein solution add a drop or two of 2% solution of salicylsulphonic acid (a solution of salicylic acid in sulphuric acid) The formation of white precipitate indicates the presence of protein.

3 Precipitation by Neutral salts.

(*a*) Full Saturation:

Procedure: Take 5 ml of Protein solution in a test tube and add solid ammonium sulphate until it becomes saturated A gelatinous precipitate forms which indicates the presence of *Albumin*

(*b*) Half Saturation:

Procednre: Prepare a *saturated solution of ammonium sulphate in* a test tube Add 3 ml of saturated solution to 3 ml of protein solution Shake vigorously A gelatinous precipitate formed indicates the presence of *globulin*

4 Precipitation by alcohol

(*a*) **Procedure:** To 1 ml of protein solution (concentrated egg white solution) add 2 to 3 ml of alcohol A white opalescent formed indicates the presence of *protein* Filter after 10 minutes Trv to dissolve the residue by *water* The ppt does not dissolve

(*b*) **Procedure:** Take 1 ml of protein solution in a test tube and 2 to 3 ml of alcohol in another test tube Keep both the tubes in the freezing mixture for 10 minutes and then mix the contents of the two tubes A white opalescent appears Filter at once Try to dissolve the residue on the filter paper and observe that the precipitate redissolves

CASEIN

Precipitation at isoelectric point: Add a drop of bromocresol green indicator to 3 ml of casein solution A blue colour develops Now add 2% acetic acid drop by drop until the colour changes to green at pH 4 7 A flocculent precipitate of casein is formed Boil, cool and then add dil NaOH to it The precipitate dissolves again

TEST FOR PHOSPHORUS IN CASEIN

Neumann's test: Take dry casein in a dry test tube Add 2 drops of Conc HNO_3 and 10 drops of Conc H_2SO_4 to it Heat the contents strongly over a flame in a fume cupboard with constant stirring to avoid spurting The organic material chars and nitrous fumes are evolved Now allow the cupboard to cool in the rack and add *about 2 ml of distilled water* and *2 ml of concentrated ammonia* solution Mix. Now add 2 ml of ammonium molybdate solution and warm The development of lemon yellow precipitate of ammonium phosphomolybdate indicates the presence of *phosphorus*

Questions to answer

1 Why biuret test is done? Why violet colour is produced in that test?
2 What is the name of the test for detecting amino acid and how to perform the test?
3 If amino acid is detected, what other tests should be done for that?
4 If protein is detected what other tests should be done for it?
5 Why does protein coagulate? By which test. you can have coagulation of Protein?
6 Discuss the principle for precipitation of Protein
7 Name the reagents for precipitating protein from a solution
8 Which test should be performed for detecting albumin and globulin and how to perform?
9 What is the principle of sulphur test for sulphur containing amino acids?
10 Name and perform the test for detecting aromatic amino acids.
11 Name and perform the test for the detection of Phosphorus in casein

CHAPTER 4

CHEMISTRY OF MILK

Specific Gravity: (*i*) The specific gravity of milk is determined by lactometer (hydrometer)

(*ii*) The sp gr of whole milk is usually around 1 30

(*iii*) The sp gr increases with the removal of fat from milk and decreases with the addition of water to it

Reaction:

(*i*) A fresh milk is practically neutral in reaction

(*ii*) It becomes acidic on keeping milk at room temperature owing to the production of lactic acid by bacterial fermentation

COMPOSITION OF MILK

1 It is a complete food as it contains almost all the elements for growth and maintenance But it is not quite suitable for adults as it is poor in iron and contains high proteins and fat

2 The composition of milk varies with the species of the animals The composition of human and cow's milk are mentioned below

3 It's dietary value is also progressed by the presence of inorganic salts and vitamins A, C, D, and B complex

Constituents in gram/100 ml	Human milk	Cow's milk
Protein	1 4	3 5
Fat	3 5	4 0
Carbohydrate	7 0	4 8
Mineral	0 2	0 7

4 The principal protein is casein (a phosphoprotein) It also contains lactalbumin and lactglobulin

5 The fat contents present in fine droplets in emulsion form a neutral fat, cholesterol and lecithin

6 The minerals are calcium, potassium, phosphates in large amounts, sodium , magnesium and chloride in fair amounts, but iron in poor amounts

EXPERIMENTS ON CHIEF CONSTITUENTS OF MILK

1 10 ml. of milk is diluted by 10 ml of distilled water Then add 2% acetic acid drop by drop till a heavy precipitate of casein is formed Allow it to stand for 5 minutes and filter Residue and filtrate

2 A small portion of the residue is allowed to dissolve in 4 ml of 2% Na_2CO_3, and with this solution perform (*a*) Biuret test, (*b*) Ninhydrin test and (*c*) colour test for amino acids to justify that the residue contains *protein*

3 Take a portion of the residue and perform Neumann's test for combined *phosphorus*

4 A portion of the dried residue is shaken up vigorously with ether in a test tube for about 1 to 2 minutes and allow it to stand for a while Decant the supernatant ether layer in a dry basin and allow it to evaporate spontaneously at room temperature Rub a piece of ordinary writing paper on the portion left in the basin A translucent grease spot on the paper indicates the presense of *fat*

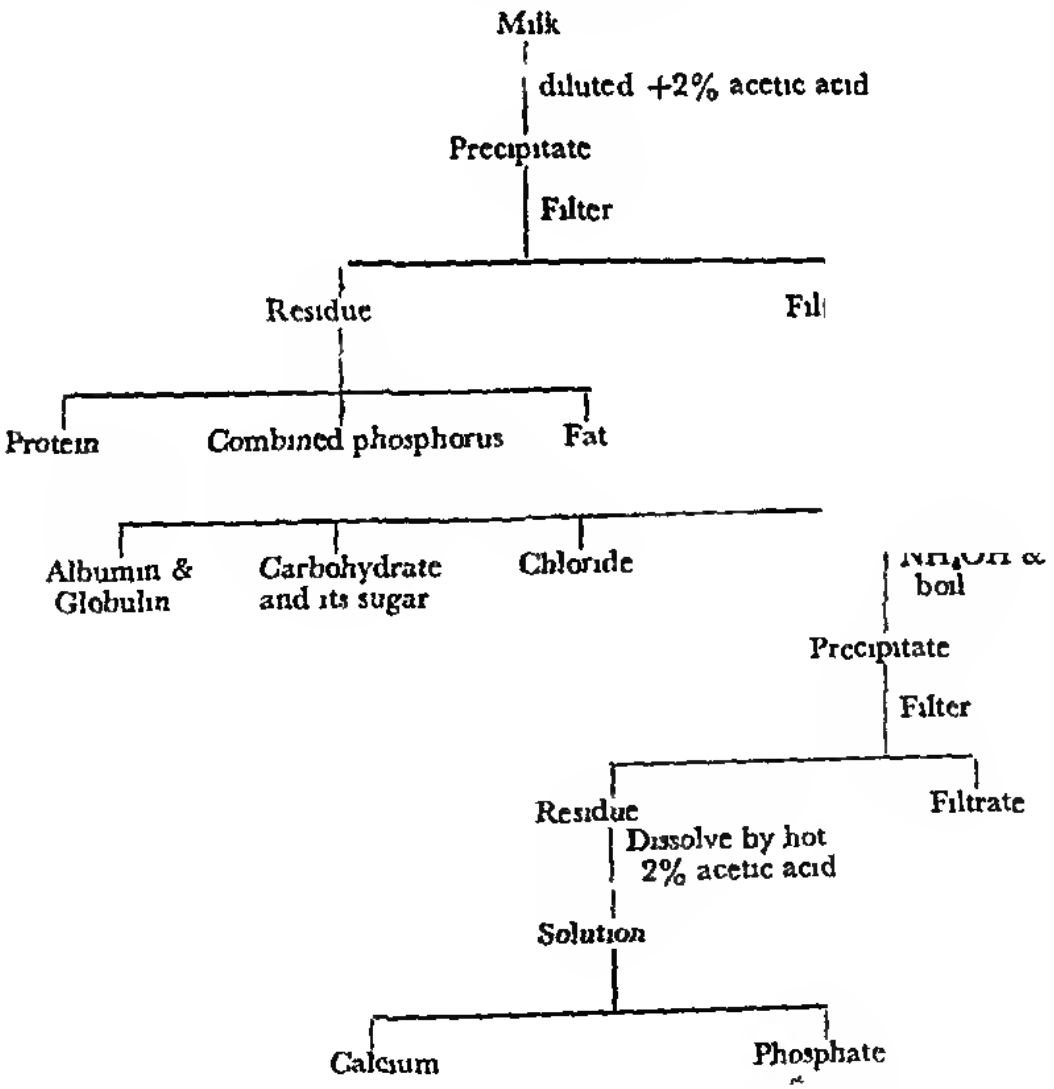
5 To 10 ml of the filtrate add a drop of chlorphenol red indicator Then add 2% Na_2CO_3 solution or 2% acetic acid to bring the pH 5 4 showing the change of red to yellow colour Boil the solution A coagulum appears owing to the coagulation of *albumin and globulin* Filter and keep the filtrate for the following experiments

6 With the portion of this filtrate perform molisch's test to know the presence of *carbohydrate* and other tests for the detection of the nature of *sugar present*

7 Perform silver nitrate test for detecting *chloride* with a portion of the filtrate

8 To the remaining portion of the filtrate add a few drops of NH_4OH to make it alkaline and boil A slight gelatinous precipitate of calcium and magnesium phosphates are produced Filter and dissolve the residue on the filter paper by pouring over it 5 ml of hot 2% acetic acid (*a*) To a portion of this solution add a clear solution of Potassium oxalate A turbidity of *Calcium oxalate* appears. (*b*) To the remaining solution add 2 ml of ammonium molybdate solution Mix and heat Lemon yellow precipitate of phosphomolybdate indicates the presence of *phosphate*

Schematic representation of the experiment for finding ont the constituents of milk



CHAPTER 5

CHEMISTRY OF WHEAT FLOUR

COMPOSITION

1 Wheat flour contains about 75% starch, 8 to 10% protein and about 1% fat

2 The remaining constituents are water, cellulose, inorganic salts and some vitamins

3 Starch is insoluble in cold water On boiling, cellulose layer (the covering of starch granules) breaks and starch granules go into solution

4 *The proteins* are gliadins *(soluble in 70—80% ethanol)* and glutelin (soluble in alkali) On treatment with water these proteins form a sticky mass called *gluten*, the viscidity of which is due to gliadin Rice and maize are poor in Gliadin for which dough is not formed when mixed with water

5 White flour is poorer in protein, fat, cellulose and vitamins

DETECTION OF THE CONSTITUENTS OF WHEAT FLOUR

1 Mix some wheat flour with a little water to make a stiff dough and allow it to stand for 10 minutes

2 Wrap a small amount of dough in a piece of cloth and knead it well in a small amount of water kept in a basin. Continue kneading until the washings are no longer turbid.

3 The yellowish sticky mass, known as gluten, remains in the muslin which is kept for performing experiment (A) and the washings for experiment (B)

Experiment (A)

Dissolve a portion of the sticky mass in 5 ml of 5% NaOH by just heating over a small flame. *After standing for a few minutes, decant the supernatant* Cool and perform the following tests

(*i*) Biuret test for protein

(*ii*) Ninhydrin test for amino acids

(*iii*) Millon's test for tyrosine

(*iv*) Xanthoproteic reaction for tyrosine, phenylalanine and tryptophan.

(*v*) Hopkins Cole Adam Kiewicz reaction for tryptophan

Experiment (B)

1 Examine a drop of the washing under a microscope for starch granules. Sketch the diagram

2 To a small portion of the washing add a drop of dilute iodine solution The development of violet colour indicates the presence of *amylopection* of starch granules

3 Boil a portion of the washing and cool it thoroughly under the tap Add a drop of dilute iodine solution to it A deep blue colour formed indicates the presence of *amylose* of starch granules

Grease Spot test for fat

The fat of flour is extracted by shaking a pinch of it with 3 to 4 ml of ether in a test tube for 2 to 3 minutes Allow the test tube to stand for a while and decant the supernatant into a clean and dry porcelain basin. Keep the basin exposed to evaporate the ether Then rub a piece of ordinary writing paper on the area where fat layer is left. A translucent grease spot is visible if fat is present

CHAPTER 6

ENZYMES

1 Enzymes are proteins having catalytic properties

2 They are formed by living cells—both animals and plants

3 Digestive enzymes are formed by digestive glands, such as salivary, gastric, pancreatic etc -

4 The enzymes need an optimum temperature (37°C) for most of the animal enzymes and an optimum pH for their highest activity

5 Their activity is lost by boiling and they become inactive at low temperature

6 They are specific for a particular substrate for a chemical reaction to bring about

DIGESTIVE ENZYMES

STUDY OF SALIVARY AMYLASE

1 Salivary amylase or ptyalin is the digestive enzyme of saliva which is secreted by the salivary glands

2 This enzyme digests boiled starch into maltose Dextrins are also formed as an intermediate product during hydrolysis of starch

3 It works best at an optimum pH 6 8 and optimum temperature 37°C

4 It is activated by chloride ions

5 During hydrolysis of starch, amylodextrin, erythrodextrin, achrodextrin and maltose formed are detected by the colour such as blue, purple, red and no colour respectively by the addition of dilute iodine solution

Collection of diluted Saliva

1 Clean your mouth with water first

2 Take about 20 ml of 0 2% NaCl solution in your mouth and move it about in the mouth with the help of tongue for a minute or two and collect the fluid in a clean hard glass test tube

3 Shake the contents of the tube vigorously

4 Filter to remove any epithelial cells etc present

Experiment

Take 3 test tubes and mark them 1, 2 and 3 with a marking pencil

Test tube No 1 —5 ml diluted saliva + boil and cool + 5 ml of 1% starch solution

Test tube No 2 —5 ml diluted saliva + 5 drops of dil HCl + 5 ml of 1% starch solution

Test tube No 3 —5 ml of diluted saliva + 5 ml of 1% starch solution

Place all the three test tubes in a beaker full of water at about 37°C

Take a drop of fluid from test tube No 3 at an interval of one minute and test it with a drop of dilute iodine till you get a faint iodine colour The successive colours blue, purple, red and no colour are seen Remove this test tube from the bath

Now take a drop of fluid from each of test tube No 1 and 2 at an interval of one minute and test with iodine Only blue colour will be seen This indicates that salivary amylase has not at all digested in these two test tubes This explains,

the fact that in test tube No 1 the activity of the enzyme is lost by boiling and in test tube No 2 the optimum pH of the solution is not maintained.

Perform Benedict's test in test tube No. 3 This will give positive because of the formation of maltose from starch hydrolysis by the enzyme. The enzyme has worked best owing to the optimum pH and temperature maintained.

STUDY OF PEPSIN IN GASTRIC JUICE

1 Pepsin is secreted in the form of Pepsinogen by the peptic glands of the gastric mucosa.

2. Pepsinogen is activated to pepsin by HCl.

3 It is inactivated in alkaline medium and is destroyed by boiling

4 To accomplish the experiment, carmine-fibrin is used as a substrate for pepsin. Carmine-fibrin is a blood fibrin stained with carmine.

Experiment

Arrange 3 numbered test tubes in a rack and put the following contents into them

Test tube No 1 —1 ml. pepsin — boil and cool + 1 ml of 0 4% HCl — 2 small flakes of carmine-fibrin.

Test tube No. 2.—1 ml. pepsin + 1 ml. water + 2 small flakes of carmine-fibrin.

Test tube No. 3 —1 ml. pepsin solution + 1 ml. of 0 4% HCl + 2 small flakes of carmine-fibrin.

Keep the three tubes in a water bath at about 37°C and observe the test tube after 5, 10 and 15 minutes. It will be seen that the digestion has taken place in test tube No. 3 but not in No. 1 and 2 The digestion has been indicated by the red colour developed as a result of the liberation of carmine from the digested part of carmine-fibrin.

CHAPTER 7

VITAMINS

Detection of Vitamin A in cod liver oil.

(i) Dissolve 1 drop of Cod liver oil in 0 5 ml of chloroform

(ii) Add 2 ml of Carr Price reagent (saturated antimony trichloride in chloroform) to it and mix

(iii) A transient blue colour is developed indicating the presence of vitamin A and its precursor (carotene)

This test is very sensitive

Precaution: Carr price reagent is highly poisonous

Detection of riboflavin in milk.

(i) 2 ml of 40% trichloracetic acid is added to 8 ml of milk to precipitate protein, mix well and filter

(ii) Take 5 ml of the filtrate in a boiling tube, add to it 1 ml of glacial acetic acid and 2 ml of Pyridine Shake well by the use of stopper

(iii) 2 drops of saturated solution of potassium permanganate is added to oxidize interfering substances and after 1 minute, add 2 drops of 10% (V/V) H_2O_2 to decolorise excess of permanganate

(iv) Add 5 grams of anhydrous Sodium sulphate and shake gently

(v) Add 10 ml of n butanol and shake vigorously for 2 minutes by the use of stopper Centrifuge

(vi) Pour the clear supernatant into a clean dry test tube and examine it by ultra violet light The development of greenish-yellow flourescence indicates the presence of riboflavin The intensity of flourescence is proportional to the concentration of riboflavin

Detection of Nicotinic acid

Principle: When nicotinic acid cyanogen at 60°C and pH 6 5 is incubated, Pyridine ring is opened to produce an aldehyde which couples with P amino-acetophenone in acid solution to give a yellow product

Reagent:

Cyanogen bromide: This is prepared by adding chilled 10% KCN to chilled bromine water until the colour is just discharged It is to be prepared in a fume cupboard

Precaution: Cyanogen bromide solution is highly poisonous

Procedure:

(i) Put 5 ml of nicotinic acid solution (140 µg per ml) in a test tube and add to it 2 ml of cyanogen bromide solution from a burette Mix well and incubate the tube at about 60°C in a water bath for 4 minutes

(ii) Then cool the tube under the tap for 5 minutes avoiding strong light

(iii) Add 1 ml of 10% P aminoacetophenone in ethanol and mix well

(iv) Examine after 5 minutes An unstable yellow colour develops

Discussion: All these above quabtative methods can be used with known standards to determine the quantities of these vitamins

CHAPTER 8

URINE

PHYSICAL CHARACTERISTICS

Frequency of Urination

(i) It is generally dependent upon the amount of fluid in the bladder

(ii) The frequency is increased in the inflammation of the urinary tract, in the affections of the spinal Cord and in weakening of the sphincter

Preservation of the Urine sample

(i) Toluene is a very satisfactory preservative for urine

(ii) Formaldehyde (2 drops per 50 ml of urine) or a bit of Camphor or thymol are also satisfactory preservatives

Volume:

(i) Normally, the volume of urine excreted by an adult individual ranges from 1200 to 1800 ml per day

(ii) The volume is influenced by the diet and by the temperature which causes the loss of water through perspiration. Hard physical exercise diminishes output of urine

(iii) Polyuria causes the increased output of urine, but oliguria causes diminished excretion

(iv) Certain pathological conditions such as diabetes mellitus, diabetes insipidus, contracted kidney, and amyloid degeneration of the kidney etc. cause increased volume of urine

(v) A decreased volume is also observed in acute nephritis, fevers, diarrhea, vomiting, and diseases of the heart and lungs

Colour

(i) Normal human urine is yellow in colour This colour is due to the pigment urochrome which is a compound of urobilin and urobilinogen.

(ii) The urine may be deeper yellow when it gets concentrated as in the case of low-output of urine

(iii) The urine colour may be very dark brown or black or may assume the colour of the drugs or their degradation products on the administration of various drugs or antiseptics

(iv) The Urine colour may be greenish yellow due to the excretion of large amounts of bile pigments in the urine and may be reddish due to the presence of blood

(v) In chyluria, the urine appears milky and in alkaptonuria, the urine becomes dark on standing

Specific Gravity:

(i) The specific gravity of urine of normal individuals varies between 1 006 and 1 025

(ii) Following copious water—or beer drinking the specific gravity may go down to 1 002 but in case of excessive perspiration, it may go upto 1 040

(iii) The specific gravity depends on the amouot of solutes present in urine When large amounts of glucose or albumin is passed in urine, the specific gravity rises

(iv) The determination of specific gravity is done by *Urinometer* This instrument is calibrated at a specific temperature

Appearance:

(i) Normal urine is perfectly clear and transparent when freshly voided

(ii) On standing for a variable time, the appearance becomes turbid due to the formation of the Crystals of phosphates or urates or oxalates and also for the formation of nucleoproteins or mucoid and epithelial cells

(iii) The phosphates are deposited in alkaline urine (or sometimes in slightly acid urine) Calcium oxalate is deposited in all urines Uric acid and Urates are deposited when the urine cools and redissolved on warming Phosphates dissolve in dilute acetic acid

(iv) The turbidity also appears in the presence of W B C , R B C or bacteria A coloured deposit is also observed in the presence of R B C

Odour:

(i) Fresh normal urine has an aromatic odour

(ii) On standing for a long time, it undergoes bacterial decomposition producing a very unpleasant ammoniacal odour

(iii) Under normal conditions, the urine possesses a peculiar odour due to the ingestion of certain drugs or vegetables

CHEMICAL CHARACTERISTICS

A. Reaction:

(i) Freshly voided normal human urine has a pH 6 (acidic to litmus)

(ii) On standing for a long time, it may be alkaline due to the formation of ammonia from bacterial decomposition

(iii) Ingestion of acid fruits (Oranges, lemons etc) causes the formation of alkaline urine because the ash of such fruits is alkaline Bread, Cereals, meats etc produce an acid urine

B. Urine Contains normal or abnormal constituents which are detected by chemical tests The normal constituents can be divided into nitrogenous and non-nitrogenous constituents Urine also contains some pigments derived from blood pigments Several enzymes are also present in normal urine e g amylase and trypsin In certain abnormal conditions, some of the normal constituents of urine are excreted in abnormal amounts

The Chief nitrogenous constituents of normal urine·

1. (a) Specific Urease test for Urea·

Principle: Soyabean powder contains the enzyme *Urease* This *Urease* under optimum pH and temperature decomposes Urea into ammonia and Carbon-dioxide which together form ammonium carbonate (the alkaline substance) which changes the slightly acid reaction (Yellow colour) to alkaline reaction (Pink colour)

Procedure: Take 2 ml urine in one test tube and 2 ml water in another Add a drop of phenol red indicator to each Then add 2% Na_2CO_3 drop by drop till the pink colour develops (just alkaline) Now add 2% acetic acid to each drop by drop till the pink colour just disappears (just acidic)

Add a pinch of Soyabean powder to each and rotate the tubes between the palms or warm both the tubes to about 60°C

The pink colour appears in the tube containing urine but not in the other tube containing water

Precaution: Over heating should be avoided Otherwise the enzyme will be destroyed 60°C temperature is maintained by touch only which is likely to be

pleasant This 60°C temperature is the optimum temperature for urease for its maximum activity

Discussion: This test is said to be the specific test for urea because the enzyme urease shows its specificity for the substrate urea

The optimum pH (just acidic) and temperature (60°C) must be maintained for the highest activity of the enzyme urease Urea is formed in the liver from ammonia and carbon dioxide Ammonia is the product in the deamination of amino acids Therefore, urea excretion in urine is dependeot on the amount of Protein ingested

(b) Biuret test

Principle: Urea when heated decomposes with the liberation of ammonia and the formation of biuret The biuret is dissolved in water and develops a violet colour forming a complex with the alkaline Copper sulphate solution

Procedure Place a small amount of urea Crystals in a dry test tube and heat it in a low flame Urea melts with the liberation of ammonia On further beating it solidifies (In case of urine the urine is heated to solidify) Cool the tube Add 3 ml of water and shake

Add to it 1 ml of dil NaOH and 1 or 2 drops of 1% $CUSO_4$ solution The pink colour develops indicating the presence of urea

Precaution: More drops of $CUSO_4$ should not be added, otherwise $CUSO_4$ will form $CU(OH)_2$ with NaOH forming a blue colour This is sometimes mistaken for a positive biuret test

2.(a) Murexide test (for uric acid):

Principle: Uric acid is oxidized to dialuric acid and alloxan which condense to form alloxantin This reacts with ammonium hydroxide to form the purplish red compound, *ammonium purpurate or murexide*

Procedure: Take 5 ml of the urine and saturate it fully with solid ammonium chloride Add a few drops of stroog ammonium hydroxide A gelatinous precipitate of ammonium urate is formed Allow the excess of ammonium chloride to settle and filter off the supernatant

Take the residue on the filter paper in a porcelin dish Add 2 drops of concentrated HNO_3 and heat to dryness A reddish yellow residue remains Cool aod add a few drops of very dilute ammoonium hydroxide The development of Purplish red colour is due to the formation of murexide

(b) Benedict's test for uric acid and urates

Principle: Uric acid is soluble in alkali The blue colour is developed due to the reductioo of phospbotungstic acid by uric acid

Procedure: To 2 ml of urine add a few drops of Benedict's uric acid reagent and a pinch of anhydrous soldium Carbonate Mix A deep blue colour indicates the presence of uric acid

Discussion: Uric acid is formed from adenine and guanine of nucleic acids Ordinarily it occurs in urine as urates Alkalis dissolve uric acid in the form of urates It is insoluble in water In gout, the kidney loses the power of elimmating Uric acid properly and it is collected in the blood in high concentration This is accompanied by the deposition of uric acid in the joint Uric acid is greatly excreted in urine in leukemia

3. Jaffe's test (for Creatinine):

Principle: Creatinine, the anhydride of Creatine, forms Creatinine Picrate in alkaline medium developing a deep reddish oraoge colour

Procedure: To 4 ml of saturated solution of Picric acid add 2 ml of 10% NaOH Divide it equally into two test tubes

Add 3 ml of urine to one test tube and 3 ml of water to the other

The test tube containing urine produces a deep reddish orange colour due to the formation of creatinine picrate But this does not happen in other test tube containing water

Discussion: Creatine occurs in small amounts in the urine of normal adults It is found in larger amounts in the urine of children and pregnant women till it is taken to be the normal constituent

It is increased in fasting and after high water ingestion Increased amounts are found in malnutrition, disintegration of muscular tissue and in Carcinoma of the liver

A decrease of creatinine excretion is found in anemia, Paralysis, leukemia, etc

Under normal conditions about 1 to 1 8 grams of Creatinine are excreted by an adult man per day

4. Test for ammonium salts

Principle: The Pink colour in the urine developed by the phenolphthalein shows the urine in the alkaline medium The ammonia evolved due to the decomposition of ammonium salts makes the phenolphthalein layer over the glass tube pink

Procedure: To 2 ml of urine add a drop of phenolphthalein and 2% Na_2Co_3 drop by drop till pink colour is produced

Boil the faintly alkaline fluid and keep a glass tube previously dipped in phenolphthalein over the mouth of the test tube This turns pink

Precaution: Do not touch the mouth of the test tube by the glass tube previously dipped in phenolphthalein

Discussion: The average excretion of ammonia is about 0 7 grams per day

The kidneys manufacture ammonia in proportion to the amount of acid radicals excreted in urine In alkaline urine, ammonium salts are absent

Imperfect protein metabolism increases the output of ammonia in urine

The Chief Non Nitrogenous constituents of normal urine

1. Chlorides:

Principle: A white precipitate of AgCl is formed when acidified urine reacts with $AgNO_3$ solution

Procedure: Acidify 2 ml of urine with 2 drops of Conc HNO_3 and add to it 2 ml of $AgNO_3$ A white precipitate of silver chloride indicates the presence of chlorides

2. Sulphates:

Principle: Urine being acidified with HCl forms a white precipitate of barium sulphate by the reaction with barium chloride solution

Procedure: Add a few drops of Conc HCl and 1 ml of barium chloride solution to about 3 ml of urine A white precipitate of barium sulphate indicates the presence of sulphates

Note: The presence of HCl prevents precipitate of phosphates

3. Phosphates:

Principle: Phosphates of calcium and magnesium are precipitated by ammonium hydroxide on boiling and these phosphates are dissolved in hot dilute acetic acid This forms yellow precipitate of ammonium phosphomolybdate reacting with ammonium molybdate

Procedure Add a little ammonium hydroxide to a test tube containing urine three fourth of the test tube and then boil A white flaky precipitate of phosphates of calcium and magnesium is formed

Filter this precipitate and wash the residue on the filter paper with water Dissolve the residue in about 3 ml of hot dilute acetic acid by pouring the acid on the filter paper Collect the solution in a clean test tube and divide it into two parts Retain one part for detecting Calcium

To the other part add a drop of Cone HNO_3 and a few drops of ammonium molylbdate solution Boil A lemon yellow precipitate indicates the presence of *phosphates*

4. Calcium·

Principle· Calcium forms a white precipitate of calcium oxalate on addition of Potassium oxalate to the solution for calcium detection

Procedure· To one part of the just above experiment add about 1 ml of Potassium oxalate solution A white precipitate of Calcium oxalate is formed immediately

5. Ethereal Sulphates·

Principle: Ethereal sulphates on treatment with baryta mixture forms soluble salts with barium On hydrolysis with strong acid and with excess baryta it forms a white precipitate of barium sulphate

Procedure To 10 ml of urine add 10 ml of haryta mixture, [Saturated solution of $Ba(NO_3)_2$ and $[Ba(OH)_2]$ Phosphates and inorganic sulphates are precipitated Filter repeatedly to have a clear filtrate

To the filtrate add 5 ml of Cone Hcl and boil in a dish for about 5 minutes Allow to stand for a few minutes, A faint white precipitate indicates the presence of *ethereal sulphates*

Discussion of Non nitrogenous constituents of urine·

(i) Sodium and potassium in the form of Na_2O and K_2O are found to the extent of 5 grams and 3 grams respectively in 24 hours urine

(ii) 0 1 to 0 2 gram of calcium is excreted in urine per day Its excretion increases in hyperparathyroidism The excretion of magnesium is also low which approaches to 0 0o to 0 2 gram per day

(iii) Very small quantity of iron is found in urine

(iv) *About 10 12 grams of chloride (in the form of NaCl) is excreted daily* The amount of chloride excreted depends upon the intake of food and the quantity of its loss through sweat

(v) Phosphates are present in urine as salts of sodium, Potassium, ammonium, Calcium and magnesium These are crystallized out in alkaline urine

(vi) Sulphates found in urine are derived from the oxidation of sulphur containing substances It is available in urine in three forms—(a) neutral sulphur (b) Sulphates of sodium and potassium (c) ethereal sulphates

(vii) Ethereal sulphates in urine are the Conjugated phenols, phenol sulphuric acid and indoxysulphuric acid etc The total output of it various forms 0 04 to 0 1 gram per 24 hours These ethereal sulphates result from phenols produced during putrefaction of protein material in the intestine Phenol passes to the liver where part of it is coojugated to form phenol potassium sulphate and appears in urine in this form This indoxysulpburic acid (indican) under normal condition is excreted from 10 to 20 mg per day Variation also results in depending upon the protein diet Pathologically, the indicans are excreted highly in increased putrefaction and stagnation of intestinal contents Bacterial decom-

position of body protein as in gangrene and putrid pus formation etc result in the increased excretion of indican

(viii) Normal human urine also contains fluorides, nitrates, silicates and hydrogen peroxide Nitrates are available from water and food vegetable diet provide more quantities of nitrates than meat diet

(ix) The pigments urochrome, urobilin and uroerythrin are also found in urine Urochrome is the principal pigment of the normal urine and the Pale yellow colour of the normal urine depend on the colour of it Urobilin is the oxidative product of urobilinogen In fresh urine urobilinogen is present Urobilinogen is excreted in less amount in normal urine But its concentration in urine increases in the diseases of liver and bile passages, in fever, in malaria and in R B C destruction

The total reducing substances equivalent to about 0 05 to 0 15 per cent of glucose are present in normal human urine Fermentable sugars are about 0 01 per cent Most of the sugar of normal urine is not glucose but includes Pentose, lactose and altered carbohydrates formed through the action of bacteria in the intestinal tract

Glucose is excreted ordinarily in traces only Lactose is found in the urine of pregnant women and lactating mother in large quantities Detectable amounts of Pentose sugar and fructose are found in pentosuria and fructosuria respectively Otherwise reducing sugars are not normally found in sufficient amounts to respond to the ordinary tests

ABNORMAL CONSTITUENTS OF URINE

Substances which are not present in easily detectable amounts in Urine of normal healthy individuals but are present in urine under certain diseased conditions are said to be *"Abnormal"* or *"Pathological"* constituents of urine

Before detecting the abnormal constituents of urine, note the physical characteristics (Colour, Volume, odour, appearance, specific gravity and sediment) and chemical reaction with litmus paper

The abnormal constituents for routine purposes are reducing sugars, acetone bodies, proteins, blood pigments, bile salts, bile pigments and Pus

1. Reducing sugars·

To detect reducing sugars in urine, Benedict's test and Fehling's test are done To be sure of the presence of glucose, lactose, pentose, fructose and glucose *etc , osazone test is to be performed*

Benedict's test is preferred than Fehling's test on the fact that the strong alkalinity of Fehling's solution destroys traces of sugar, whereas Benedict's solution with its weak alkalinity responds to a little amount of sugar present in urine Hence it is more sensitive than Fehling's test

Presence of streptomycin and certain preservatives such as chloroform and formaldehyde give Benedict's test positive

Benedict's test:

Principle: Copper sulphate of Benedict's qualitative solution is reduced by reducing substances on boiling to form the coloured precipitate of cuprous oxide The light green, green, yellow and brick red precipitates of cuprous oxide depend on the concentration of reducing substances present in urine

Benedict's qualitative reagent: Copper sulphate + sodium carbonate + sodium citrate

Procedure: To about 5 ml of Benedict's qualitative reagent add 0 5 ml (8 drops) of urine and boil for 2 minutes holding the test tube firmly with a test

tube holder A light green, green, yellow and brick red precipitate indicates the presence of reducing substances in urine

The various coloured precipitate depends on the concentration of reducing sugars in urine which gives a rough estimate of the concentration given below

Light green precipitate	0 1 to 0 5% of reducing sugars.
Green precipitate	0 5 to 1 0% ,, ,, ,,
Yellow precipitate	1 to 2 % ,, , ,,
Brick red precipitate	above 2 % ,, ,, ,,

Note The variation in coloured precipitate relating to the concentration of reducing sugar is found in urine only, whereas this variation does not occur in pure sugar solution in water This difference may possibly be due to the presence of creatinine and other constituents of urine

Precaution (i) Boil the urine for atleast 2 *minutes*

(ii) During boiling, the contents of the test tube get a tendency to spurt out. Hence it is wise to keep the test tube shaking after holding it in the inclined position near the flame which does not cause overboiling it

Discussion

(i) Normal urine also contains a trace of glucose and glucuronates, but their amount is too small to cause reduction in Benedict s test

(ii) Benedict s test is not necessarily indicative to glucose in urine only but it too indicates the presence of other reducing sugars such as lactose (in case of pregnant women and lactating mothers) fructose (in fructosuria), galactose (in galactosuria), Pentose (in Pentosuria), homogentisic acid (in alkaptonuria), glucuronates and mucin If mucin is suspected, Benedict s test is repeated after removing mucin with Kaolin The different reducing sugars (glucose, lactose, galactose, fructose) may be identified by establishing the performance of Seliwanoff's test osazone test and fermentation test etc

(iii) In diabetes mellitus and in renal glycosuria glucose is found in urine This gives a benedict s test positive

2 Acetone bodies

(a) Rothera's test

Principles Acetoacetic acid forms a complex with sodium nitroprusside in alkaline solution developing a permanganate colour

Procedure Saturate 5 ml of urine with solid ammonium sulphate by shaking it vigorously Then add 2 drops of freshly prepared 5% solution of sodium nitroprusside and 1 ml of ammonium hydroxide Allow it to stand in the rack for a while A permanganate colour develops just above the layer of the undissolved ammonium sulphate crystals indicating the presence of acetone bodies

(b) Gerhardt test for acetoacetic acid

Acetoacetic acid gives a red colour with ferric chloride

Procedure Add 5% ferric chloride solution drop by drop to about 5 ml urine till no more precipitate of ferric phosphate is formed Filter

To the filtrate add some more ferric chloride The development of red colour indicates the p esence of acetoacetic acid

To the filtrate add some more ferric chloride The developmnet of red colour indicates the presence of acetoacetic acid

Precaution A large number of substances such as aspirin, antipyrin, salicylates etc may develop similar red colour If the urine is boiled, acetoacetic acid is converted into acetone, but the other substances remain unchanged Now if the urine gives negative test it indicates the presence of acetoacetic acid

Discussion:

(i) In diabetes mellitus and in prolonged starvation, fat is catabolised to produce acetoacetic acid, acetone and β-hydroxybutyric acid which are together termed as acetone or ketone bodies These acetone bodies are accumulated in blood and excreted in urine. This condition is called Ketosis. The excretion of Ketone bodies in urine is called Ketonuria.

(ii) Ketosis also occurs in the acetonemic vomiting of childhood, and frequently in pregnancy, fevers, ether and chloroform anesthesia, malnutrition, prolonged feeding of a carbohydrate poor diet high in meat and fat.

(iii) Total Ketone bodies are found in normal urine to the extent of about 20 mg in 24 hours. Pathologically, values from 0 02 to 6 grams or more per day have been observed.

(iv) β-hydroxybutyric acid as such does not give Rothera's or Gerhardt's test positive unless converted to acetoacetic acid and then to acetone by oxidation

3. Albumin.

(a) Sulphosalicylic acid test:

Principle: Albumin, the protein is denatured by sulphosalicylic acid causing a coagulation.

Procedure: Add a few drops of sulphosalicylic acid to 2 ml of clear urine (filter if not clear). A turbidity indicates the presence of albumin

This test is used for routine owork

(b) Heat Coagulation test:

Principle: The urine containing albumin is coagulated after being heated

Procedure: Fill 3/4th of the test tube by clear urine (If the urine is turbid; filter it before performing the test) Heat the upper one-third of the test tube by a small flame. A turbidity is found on the heated portion of the urine

Add a drop of 33% acetic acid to the urine Phosphate is dissolved but not the protein albumin

(c) Heller's Nitric acid ring test:

Principle: Nitric acid causes the precipitation of Protein

Procedure: To 3 ml of nitric acid in a narrow tube add 3 ml of urine by means of a pipette in such a way that the two liquids do not mix

The presence of a white ring at the junction of two fluids, indicates the presence of *albumin.*

Discussion:

(i) A trace of protein which is less than 250 mg in 24 hours urine found in normal urine. This amount is so slight that it escapes detection by any of the simple tests.

(ii) Some proteins (albumin and globulin) are found in the urine under pathological conditions known as *albuminuria.*

(iii) In kidney disturbance and [illegible] h blood pressure, albumin is found in urine significantly.

(iv) [illegible] exercise or cold baths

(v) [illegible] line in cases of multiple

4 Blood pigments (Benzidine test).

Principle Hemoglobin of blood decomposes hydrogen peroxide catalytically and liberates oxygen This oxygen oxidizes benzidine to a blue or Green Compounds. This colour changes to brown within a few minutes on exposure to air

Procedure: Take a pinch of solid benzidine in a test tube and 2 ml. of glacial acetic acid till the benzidine dissolves Add 2 ml of hydrogen peroxide (H_2O_2) to it Divide it into two portions

To one portion add urine drop by drop with shaking The appearance of deep blue colour indicates the presence of blood pigments This quickly changes to brown within a few minutes

To the other part add water drop by drop No blue colour appears. This part is treated as Control

Discussion:

(i) Blood occurs in urine under hematuria and hemoglobinuria. Hematuria consists of hemoglobin and unruptured corpuscles Hematuria is brought about by blood passing into the urine because of some lesion of the kidney or of the urinary tract Hemoglobinuria results from hemolysis This occurs in malaria, typhoid, hemolytic jaundice, yellow fever and in transfusion with incompatible blood

(ii) The presence of high concentration of ascorbic acid in urine is oxidized more readily than benzidine by oxygen liberated from hydrogen peroxide The benzidine reaction then becomes negative although sufficient blood is present in urine

(iii) The benzidine test is a very sensitive test

5 Hay's test for bile Salts:

Principle Bile salts reduce the surface tension present in the urine for which sulphur powder sinks.

Procedure: Fill half of a test tube with urine and another test tube with water Sprinkle gently some sulphur powder on the surface of two liquids.

The sulphur powder spontaneously sinks in the test tube containing urine which indicates the presence of bile salts But in the other test tube containing water, no sulphur powder sinks

Discussion:

Bile duct obstruction by inflammation or gall stones in gall bladder leads to the obstruction of bile into the general circulation The subcutaneous tissues are deeply stained resulting in the yellow complexion which is the characteristic of jaundice In such cases, bile salts and bilepigments are detected in urine

6 Bilepigments

A. Fouchet's test

Principle: Bilirubin is precipitated by barium chloride. This bilirubin is oxidized to green biliverdin by fouchet s reagent (Ferric Chloride in trichloroacetic acid)

Procedure Acidify 10 ml of urine with a few drops of dilute acetic acid and add 5 ml of 10% $BaCl_2$ solution If there is not much precipitate, add 2 drops of saturated solution of magnesium sulphate. Mix and allow to stand for a few minutes

Filter and unfold the filter paper Add one drop of fouchet's reagent to the precipitate The development of green colouration indicates the presence of bile pigments

B. Gmelin's test

Procedure: To 5 ml of concentrated nitric acid in a test tube add an equal volume of urine carefully, as if the two fluids do not mix The various coloured rings (green, blue, violet, red and reddish yellow) will be formed at the point of contact of the two liquids

C. Test for urobilinogen:

Add 0 5 ml of Ehrlich's reagent [Prepared by dissolving 2 grams of P-dimethyl amino benzaldehyde in 100 ml of 20% HCl] to 5 ml of urine

Discussion·

(i) A urine containing bile may be yellowish green to brown in colour and when shaken foams readily

(ii) Bilirubin formed from hemoglobin is conjugated with glucuronic acid in the liver and then excreted

Indican

Principle: Indoxyl is oxidized to form indigo blue developing a blue colour which is soluble in chloroform

Procedure: Take 5 ml of urine and 5 ml of Conc HCl in a test tube Add one drop of Potassium chlorate and 3 ml of chloroform, to it Mix thoroughly Allow the chloroform to settle down The development of blue colour indicates the presence of indican If no blue colour is developed, add another drop of Potassium chlorate and mix If still no blue colour develops, it indicates the absence of indican

Discussion:

(i) Tryptophan undergoes bacterial decomposition in the intestine forming indoxyl

(ii) Indican is the potassium salt of the indoxyl sulphuric acid and hence it is an ethereal sulphate

(iii) Sometimes in case of large abscess, indican may be formed due to putre faction

PUS

Microscopic indentification of Pus· The urine is centrifuged and the sediment is put on the slide under microscope In acid urine, the pus corpuscles appear as round, colourless cells, refractive, granular and nucleated Sometimes they exhibit ameboid movements if the slide containing the sediment be warmed slightly In alkaline urine, the pus corpuscles occur as swollen, often degenerated transparent and non granular

Guaiac test· The urine is acidified with acetic acid and filtered To the sediment on the filter paper add a few drops of tincture guaiac followed by a few drops of H_2O_2 Blue colour indicates the presence of Pus or blood or both

Biol 5 ml of urine in another test tube for 30 seconds and repeat the test as above The development of blue colour indicates the presence of blood Pus does not respond to this test after boiling the urine

Disenssion:

(i) Pus is present in urine under various inflammatory condition Such a condition is termed *Pyuria* Albumin always accompanies the pus

(ii) Pus is also present in the urine in catarrh of the urinary bladder and in inflammation of the urethra or pelvis of kidney

(iii) Rupturing of an abscess in some part of the urinogenital tract causes a high concentration of pus in the urine

Allow to stand for 3-5 minutes for the development of colour

No red colour	Urobilinogen absent
Faint pink	Urobilinogen present in small amounts
Distinctly red colour	Increased amount of urobilinogen present

QUESTIONS TO ANSWER

1 What is the volume, colour, specific gravity, odour and appearance of normal urine? How to preserve urine?

2 What are the chief nitrogenous and non nitrogenous constituents of normal urine?

3 Name the specific test for detecting Urea in urine What is the principle of the test and how to perform it?

4 Name the test for detecting Uric acid in urine Discuss the principle and performance of the test

5 Why red colour is developed in Jaffe's test for creatinine? Discuss creatine and creatinine

6 What do you mean by phosphaturia? How to differentiate phosphate and albumin by tests?

7 Where is ammonia manufactured in the body? How to detect ammonium salt in the urine?

8 What do you mean by ethereal sulphates? What is the procedure for detecting it?

9 Give the discussion of the non nitrogenous constituents of urine

10 Name the abnormal constituent of urine What do you mean by 'abnormal constituents of urine?

11 Enumerate the tests for detecting abnormal constituents of urine Discuss the principle of those tests.

12 Give the discussions of the abnormal constituents of urine

CHAPTER 9

IDENTIFICATION OF AN UNKNOWN SUBSTANCE OF PHYSIOLOGICAL IMPORTANCE

The unknown physiological substance may be given in the solid state or in solution to detect If the substance is solid (powder), it is to be dissolved either of the following ways

Cold water
Hot water
Alcohol (95%)
2 % Na_2CO_3 or 5% $NaOH$
2% acetic acid or N/10 HCl

Substances which are soluble in dilute alkali are uric acid, Protein (casein) globulin etc

Substances which are soluble in dilute acids are Calcium oxalate and alkali metaproteins

After dissolving the substance, proceed like the substance in the solution form in the following manner

(i) **Reaction to litmus paper**: Protein in acidic medium or free HCl will be acidic in reaction If alkaline, it may be uric acid, casein alkalimetaprotein If neutral, it may be carbohydrates, urea, acetone etc

(ii) **Appearance**: The solution of protein, polysaccharide or fat emulsion is opalescent

(iii) **Colour**:	Reddish brown	Blood
	yellow or Greenish yellow	Bile
	Light yellow	Dextrin or urine or Peptone
(iv) **Smell**:	Peculiar smell	urine or bile
	Aromatic odour	Acetone bodies
	Characteristic odour	Peptone etc

First perform test for protein, then carbohydrate, fat and the nitrogenous and non-nitrogenous constituents of urine

Tests for protein

Perform biuret test If it is positive, Perform ninhydrin test, coagulation test, Precipitation test and tests for different amino acids

Tests for Carbohydrate

If biuret test is negative, perform Molisch's test If molisch's test if positive Perform other tests for individual carbohydrates given in carbohydrate chapter

Tests for fat

If Molisch's test is negative, perform Grease spot test. If this test is positive, perform other tests for fat mentioned in fat chapter

Tests for nitrogenous and non nitrogenous constituents of urine

If Grease-spot test is negative, perform tests for nitrogenous and non-nitrogenous constituents or urine mentioned in urine chapter

N B While reporting in the answer scripts, if suppose protein is detected, one must mention all other tests although negative for a correct and complete answer for having the maximum credit

SECTION II

QUANTITATIVE

CHAPTER 10

INSTRUCTIONS FOR QUANTITATIVE ESTIMATION

Qualitative tests are done only to ascertain the presence of a substance But quantitative analysis shows the exact amount of a substance in a particular sample

The solution which contains an exact amount of a substance in a definite volume is termed as the *standard solution* For preparing standard solution the chemical balance and the clean pipette must be used Standard solution is required for finding out the amount of a substance in an unknown sample Standard solution is always required in estimation by photoelectric colorimeter

For the use of water, distilled water must always be used All chemicals meant for use must be of great purity Analar or C P quality chemicals must be used

The titations are generally performed in cold, sometimes performed when the solutions are hot or even boiling Burettes with jets and pinch cocks should be used During titration the end point should be carefully taken by the addition of a single drop of solution from burette More than a drop at a time should not be added to have the correct end point The first reading of titration is taken to be approximate but not accurate. Hence, a second and a third reading must be taken The difference between two consecutive readings must not be more than 0 1 ml Out of three readings if two readings are almost similar than the third one, the third one should not be taken in this case All possible errors must be avoided While using pipettes last drop at the tip of these pipettes should be blown out if measurement upto the tip is wanted

The results of some of the substances are best expressed in milli equivalent (m Eq) units

$$mEq/L = \frac{mg \text{ Per } 100 \text{ ml} \times 10}{\text{Eq Wt of the substance}}$$

Recently, all values in clinical chemistry are expressed in SI units (Syste'me International d' Unite's) which was adopted by the International Federation for clinical chemistry in 1967

PHOTOELECTRIC COLORIMETER

Photoelectric colorimeter is an instrument, which measures the intensity of colour of a solution by registering the amount of light absorbed by the given solution Hence this instrument can be better termed *absorptiometer*

This instrument consists of the following essential parts

(*i*) A source of light

(*ii*) **The monochromatic light:** The monochromatic light (a narrow wave band of light from the source) passes through the test solution This arrangement is being done by placing selected filter in the path of light

(*iii*) **A photosensitive element:** In most instrument barrier layer cells are used The cells are provided with a layer of selenium It is activated when light falls on the selenium and it emits electrons according to the proportion of light falling on it The galvanometer is connected to measure the amount of electron flow and it indicates the amount of light falling on the cells

(*iv*) **Cuvettes or tubes:** These are neutral glasses and are used to contain test solutions All cuvettes, or tubes for a particular instrument should have the same absorptive capacity

(*v*) **A sensitive Galvanometer:** A certain percentage of light is absorbed when monochromatic light passes through a coloured solution The extent of this absorption obeys two laws—Lambert and Beer According to these laws the optical

density or Extinction of a coloured solution is proportional to the concentration of the coloured substance

The optical density (O D) is defined as the logarithm of the ratio of the incident to transmitted light (the light emerging out of the solution)

$$\text{Therefore O D} = \frac{\text{Log incident light}}{\text{transmitted light}}$$

$$= \frac{\text{Log } 100}{T}$$ [Where incident light is taken as 100% and T is the percent of light transmitted (transmittance)]

$$= \text{Log } 100 - \text{Log } T$$

$$= 2 - \text{Log } T$$

When the transmittance of a solution is 50

Then, O D $= 2 - \text{Log } 50$

$= 2 \quad 1\,699$

$= 0\,3$

Since, O D is directly proportional to the concentration

$$\frac{\text{Concentration of unknown}}{\text{Concentration of standard}} = \frac{\text{O D of unknown}}{\text{O D of standard}}$$

$$\text{Concentration of unknown} = \frac{\text{O D Unknown}}{\text{O D Standard}} \times \text{Coucentration of standard}$$

Most instrument show calibration both in optical density (Extinction) and percent transmission scale If the readings are taken in percent transmission, it is to be converted into optical density according to the formula O T $= 2 - \text{Log T}$

Selection of filters In almost all cases, the correct wave length is the wave length of light which is most strongly absorbed by the coloured solution. The colour of the filter is complementary to the colour of the solution i e , for blue solution red filter, for red solution green filter and for yellow or brown solution a blue filter A filter is designated in terms of the wavelength of peak transmittance, thus a filter called ' No 540 or No 54 has its peak transmittance at a wave length of 540 mμ

Importance of Blanks The perparation of blank is essential in colori metric analysis because of the fact that some colour comes from the reagents etc , in addition to the colour produced by the substance for estimation Therefore, a blank is prepared using all reagents except the substance to be estimated The optical density (O D) value of the blank is subtracted from the O D values of the unknown and the standard Hence, the remaining values are the true O D values of the unknown and the standard

Many types of photoelectric colorimeters with different designs are made available in the market. However, these photoelectric colorimeters are basically of two types—single cell type and double cell type

(i) Single cell type In this type, the light transmitted through a solution placed between the *light source* and the cell is measured directly by the current output of the cell in relation to that obtained with blank and standard solutions

(ii) Double cell type This type is concerned with the use of two photo cells in the same light source and balanced against each other by means of a null point instrument like a galvanometer The solution to be estimated is placed between one cell and the light source. The extent to which the two cells are thrown out of balance as regard to each other is a measure of the light transmitted by the solution.

Operation of the instrument:

It is better to use the instruction manual of the instrument The chief steps are as follows

(i) The appropriate filter is advised to be inserted in the proper place

(ii) One cuvette or tube with distilled water is to be put at the place provided for it in the machine and the instrument is to be switched on It is to be left for warm up for about 4 to 5 minutes The galvanometer is then to be switched on The needle of the galvanometer is to be adjusted and brought it to position 100% transmission (zero optical density)

(iii) Another cuvettes or tubes are to be filled with the blank, standard and test solutions and are put these in place of water The optical densities of these are to be read

(iv) Then the instrument is to be switched off and disconnected from the mains after taking all readings

(v) The cuvettes or the tubes are to be thoroughly cleaned with running water first and then rinsed with distilled water both inside and outside of the cuvettes or tubes The optical parts of the cubettes or tubes should never be touched with fingers to avoid finger marks on these Very clean and tranparent cuvettes or tubes are to be required

Calculation:

Concentration of the substance of the unknown Per 100 ml

$$= \frac{\text{O D of unknown} - \text{O D of blank}}{\text{O D of standard} \quad \text{O D of blank}} \times \text{Concentration of the substance per 100 ml of the standard solution}$$

COLLECTION AND PRESERVATION OF URINE SAMPLES

Single specimens of urine are used for most qualitative tests and ward examinations But for quantitative analysis of urine, a 24 hour sample of urine helps in accurate diagnosis and interpretation For this purpose it is most convenient to collect the specimen from one morning to the next The individual is instructed to empty the bladder immediately preceding the initiation of collection to avoid bladder catheterization Samples collected for prompt chemical examination only need not be sterile or preserved

Urine on keeping undergoes changes by bacterial action Bacteria chiefly converts urea into ammonium carbonate Such urine is unsuitable for the determination of urea, ammonia, p^H, total nitrogen Microorganisms both bacteria and yeast may act on glucose If the urine becomes alkaline, phosphates may be precipitated On standing Uric acid and urates are deposited because they are less soluble in cool urine Other changes are the oxidation of urobilinogen to urobilin and the rapid oxidation of ascorbic acid

It is advised to collect the urine in a clean, well washed container Still little change takes place in a twenty four hour specimen by the time it is received in the laboratory If urine is to be kept unchanged, preservatives are required to be added The preservatives chloroform, toluene, light petroleum, thymol and formalin may be used but they have disadvantages too Toluene and light petroleum form a thin layer on the surface and this tends to contaminate pipettes This can be overcome by running off the lower layer of urine They do not stop the growth of bacteria already introduced during the collection of urine *Chloroform* is satisfactory in this respect But it may interfere with the detection of glucose as it reduces Fehling's solution Chloroform can be boiled off as it mixes with any deposit 5 ml of 10% *thymol* is found satisfactory as preservatives for many substances as Sodium, Potassium, chloride, bicarbonate, Calcium, phosphorus, urea, ammonia, amino

acids, creatinine, creatine, proteins, reducing substances Acetone bodies and amylase etc , 3 to 4 drops of *formalin* to 100 ml of urine can be used. If it is more added, it may give positive results for the test for glucose. 10 percent *acetic acid* can be added to preserve urine for determination of ascorbic acid. In addition to the use of preservatives, the urine must be kept cool.

Before carrying out estimations, any deposit must be well mixed with the urine , before sampling, the total volume of the 24-hour urine must be measured before quantitative analysis This volume is taken as a basis for calculating output per unit of time

Precautions to avoid hemolysis of blood

Hemolysis makes the serum unsuitable for some determinations Therefore, it is better to obtain serum free from hemolysis The following precautions should be taken to avoid hemolysis

(*i*) The syringe and needle must be dry

(*ii*) When taking blood, a minimum amount of constriction is applied to the arm The blood should flow slowly and steadily into the syringe The blood should be expelled slowly into the tube after removing the needle.

(*iii*) Tubes into which blood is kept should be clean and dry

(*iv*) Any anticoagulant should be mixed with the blood by gentle rotation. Excessive amount of anticoagulant should not be used as excessive amount can cause lysis of the blood

(*v*) In case of serum preparation, the blood is allowed to clot and then centrifuged or the serum is drained off when the blood is firmly clotted

CHAPTER 11

URINE

ESTIMATION OF CREATININE IN URINE

(Folin s method)

Principle: Creatinine develops an orange red colour when treated with picric acid in the presence of strong alkali This colour is due to the formation of creatinine picrate The colour so produced depends on the amount of creatinine present

Reagents:

1 **Creatinine standard solution:** Dissolve 0 1 gram creatinine (0 1611 gram of creatinine Zinc Chloride) in 100 ml of 0 1 N HCl Keep in a cool place in a well stoppered bottle 1 ml of the solution contains 1 mg of creatinine

2 Saturated solution of Picric acid (about 1%)

3 Sodium hydroxide solution (10%)

4 Hydrochloric acid solution (0 1N)

Procedure:

In three 50 ml volumetric flask, the following contents are taken

	Blank (B)	Unknown (U)	Standard (S)
Urine	—	0 5 ml	—
Distilled water	0 5 ml	—	—
Standard solution of creatinine	—	—	0 5 ml
10% NaOH	1 ml	1 ml	1 ml
Saturated Picric acid solution	10 ml	10 ml	10 ml

Make up the volume by distilled water

Mix and allow them to stand for 10 minutes for the development of colour Bring up the volume in each flask to the mark. Mix well and make photoelectric colorimetric reading using green filter at 520 $m\mu$

Calculation

Let U, S, and B the optical densities of the unknown, standard and Blank respectively

$$\frac{\text{Creatinine in unknown}}{\text{Creatinine in standard (0 5mg)}} = \frac{U-B}{S-B}$$

$$\text{or, creatinine in 0 5 ml Urine} = \frac{U-B}{S-B} \times 0\,5 \text{ mg}$$

0 5 ml urine was taken for estimation and 0 5 ml

Standard creatinine taken which contains 0 5 mg creatinine

$$\text{Creatinine in 100 ml Urine} = \frac{U-B}{S-B} \times 0\,5 \times \frac{100}{0\,5}$$

$$= \frac{U-B}{S-B} \times 100 \text{ mg}$$

Discussion:

1 Creatinine in urine appears from creatine of the skeletal muscle.

2 Normal adults excrete creatinine from 1 to 1 8 grams daily Under certain conditions it may be higher But the value is constant from day to day for a given normal individual, being influenced by the diet which contains significant amounts of creatinine e g , heavy meat diet

3 Nitrogen metabolism in the body does not influence creatinine excretion.

4 Obese individuals excrete more creatinine than in persons with less body fat

5 Creatinine coefficient is defined as the number of milligram of creatinine excreted daily per kg body weight The normal range is 19 to 30

6 Exercise can cause the increase in creatinine excretion due to the release from muscle stores

7 Increased creatinine excretion occurs in wasting diseases, prolonged starvation, fever, etc due to the break-down of muscle

8 Creatinine excretion is also increased in the diseases of the muscle such as progressive muscular dystrophy (myopathy), amyotonia congenita etc

ESTIMATION OF INORGANIC PHOSPHATE IN URINE

(Fiske and Subbarow)

Principle: The inorganic phsophate of urine reacts with molybdic acid to form phosphomolybdic acid, which is reduced to a blue coloured compound (molybdenum blue, which is a mixture of lower oxides of molybdenum) by 1,2,4-amino naphthol sulphuric acid. The intensely of blue colour depends on the amount of inorganic phosphate in urine

Reagents:

1) Sulphuric acid (10N)

2) **Molybdic acid solution:** Dissolve 2 5 grams of ammonium molybdate in about 20 ml of water in a 100 ml volumetric flask. Add 30 ml. of 10 N Sulphuric acid to it Make up the volume upto 100 ml with distilled water

3) **1,2,4—aminonaphthol sulphonic acid (ANSA):** Take 195 ml of 15 per cent sodium bisulphite solutionin a glass stoppered cylinder Add 0 5 gram of 1,2,4- amino naphthol sulphonic acid and 5 ml of 20 percent sodium sulphite Stopper and sbake until the powder is dissolved If solution is not complete, add more sodium sulphite (1 ml at a time with shaking) Transfer the solution to a brown glass bottle and store in the cold This solution can be used for four weeks

4) **Standard inorganic phosphate solution (Stock)** Dissolve 439 grams of Potassium orthophosphate in 100 ml water (100 mg%)

Working standard: Prepare as if 5 ml contains 0 4 mg of phosphate

Procedure:

In three 100 ml volumetric flask, the following contents are taken

	Blank (B)	Unknown (U)	Standard (S)
Urine	—	1 ml +70 ml distilled water	—
Blank	70 ml water	—	—
Standard solution	—	—	5 ml + 65 ml water
Molybdic acid solution	10 ml	10 ml	10 ml
1,2,4-Amino naphthol sulphonic acid	4 ml	4 ml	4 ml

Make up the volume by distilled water Mix well by by inversion Allow to stand for 5 minutes Take readings in the photoelectric colorimeter using red filter at 660 mμ

Calculation:

Let U be the O D of the unknown, S the O D of the standard and B the O D of the blank

$$\frac{\text{Phosphate in unknown}}{\text{Phosphae in standard (0 4 mg)}} = \frac{U-B}{S-B}$$

$$\text{Inorganic phosphate in 100 ml Urine} = \frac{U-B}{S-B} \times 0\,4 \text{ mg}$$

Discussion:

1 The inorganic phosphate (expressed as P) of urine is mainly of acid sodium phosphate (NaH_2PO_4) and basic sodium phosphate (Na_2HPO_4) besides other salts of phosphoric acid

2 The phosphate content of normal urine varies with diet and lies between 1 to 3 grams in 24 hours The greater part of the phosphate excretion arises from ingested food

3 The phosphate excretion is increased in fevers

4 Its excretion is decreased in low phosphorus intake, in vitamin D deficiencies resulting in rickets and osteomalacia and in hypoparathyroidism

5 In hyperparathyroidism its excretion is increased

6 Phosphaturias always represent decreased acidity and not increased phosphate content of the urine

ESTIMATION OF UREA IN URINE

(Doremus)

Principle: Urea in the urine is decomposed when treated with an alkaline solution of hypobromite The nitrogen thus formed is a measure of the quantity of urea present

$$CO(NH_2)_2 + 3\,NaBrO + 2\,NaOH = N_2 + Na_2CO_3 + 3\,NaBr + 3\,H_2O$$

One molecule of urea (60 g) produces one molecule of nitrogen (28g)

Reagent:

Sodium hypobromite solution (freshly prepared) 2 5 ml of bromine is added to 25 ml of 40% of NaOH Use fume cupboard for handling bromine

Procedure

1 Close the stop cock of the Daremus Ureometer Fill the closed limb with a freshly prepared alkaline hypobromite solution bearing no air bubbles at the top Remove the air bubbles by tilting the Ureometer

2 Add urine into the open side tube upto the zero mark by the help of a glass tubing

3 Open the stop cock a little at a time and allow 1 ml of urine to run slowly into the hypobromite solution taking about 10 to 15 minutes time In case, the urine is allowed to flow too rapidly, the CO_2 produced is not completely absorbed by NaOH present in the hypobromite solution which will lead the result too high

4 When the evolution of gases has stopped and the hypobromite solution becomes clear, the reading on the closed limb should be taken

5 Add another 1 ml of the urine slowly into the open side tube and take the reading again as before The mean of these two readings gives the result

Calculation:

The *Doremus* apparatus is graduated to give direct readings in grams of urea contained in the volume of urine reacted. The gas collected in the apparatus is nitrogen which is liberated by the amount of urea marked on the apparatus.

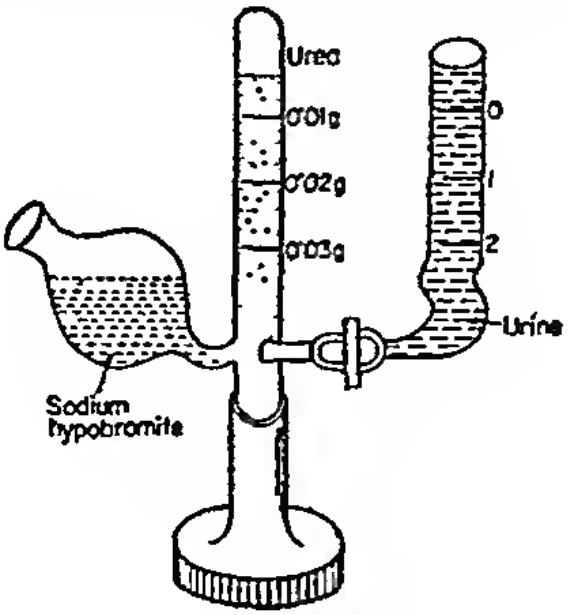


Fig 11.1. Doremus Ureometer.

Discussion:

1. Urea is the end product of Protein metabolism and hence its excretion depends on the amount of Protein ingested. The average daily excretion of urea by normal adults is usually about 30 grams. On a low protein diet, it may be as low as 3 grams per day.

2. In increased tissue catabolism, as in fevers, the excretion of urea is increased

3. Urea excretion may be dimmished in pronounced kidney and liver disorders due to decreased formation and decreased power of elimination.

4 In marked acidosis, its excretion may be decreased due to the utilization of *ammonia* to trap *hydrogen ion*

ESTIMATION OF PROTEIN (ALBUMIN) IN URINE.

(Aufrecht's method)

Principles: The protein of urine is completely precipitated with the help of an alkaloid reagent. The precipitate is centrifuged down in a tube (Aufrecht's tube) which is graduated to indicate the amount of protein present in 100 ml of the urine

Reagents:

Esbach's reagent: Prepare 1% solution of citric acid and saturate it with puric acid. It is a saturated solution of Picric acid containing 1% citric acid.

Procedure:

1. Filter the urine using a dry filter paper until the filtrate is clear.
2. Fill up the Aufrecht's tube to the mark 'U' with the clear filtered urine.
3 Add Esbach's reagent to the mark 'R'.

4 Stopper and invert the tube a few times to mix the contents thoroughly, but do not shake A turbidity appears due to the precipitation of protein

5 Stand for 5 minutes and then centrifuge for 10 minutes At the end of it take the readings of the horizontal level of the precipitated protein

6 Centrifuge again for 5 minutes and read

7 If the two readings are not the same, recentrifuge till the last two readings of the protein is constant

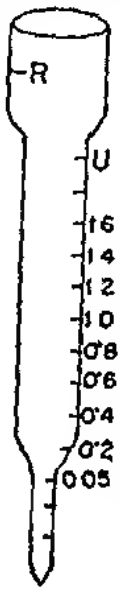


Fig 11 2
Aufrecht s tube

Discussion:

1 Detectable amount of Protein is not found in normal urine

2 The presence of protein in urine (Proteinuria) is abnormal The types of protein in pathological urine are plasma albumin, globulin and fibrinogen, hemoglobin and methemo globin, and sometimes Bence Jones proteins and Proteoses, or proteins from Pus and mucus discharged from urinary tract infection

3 In Kidney diseases (Nephritis and nephrosis), large amount of protein is excreted In many other conditions where Kidney is secondarily involved, there is moderate to heavy loss of protein in urine In such conditions, the loss of protein is essential to assess by measuring the quantity of it in urine

ESTIMATION OF REDUCING SUGAR IN URINE

(Benedict's method)

Principles: $CUSO_4$ of the quantitative Benedict's reagent is reduced to Cu_2O by the reducing sugar present in urine when boiled The cuprous oxide then reacts with Potassium thiocyanate (KCNS) to form white precipitate of cuprous thiocyanate Potassium ferrocyanide of quantitative Benedict's reagent helps to prevent the precipitation of cuprous oxide for which the red coloured cuprous oxide does not appear as precipitate The alkali Na_2Co_3 has the advantage over NaoH that it does not cause destruction of small amounts of sugar

The same principle does apply on the estimation of fructose, lactose and maltose too

Reagents:

1 Sodium Carbonate (anhydrous or Crystalline salt)

2 **Benedict's quantitative reagent:** Take weight of 200 g of sodium citrate, 75 g of anhydrous sodium carbonate (or 200 g of crystalline salt), and 125 g of Potassium Thiocyanate Dissolve them one by one in sufficient hot distilled water upto a total volume of about 800 ml Filter and cool to room temperature Now accurately weight 18 g of crystalline copper sulphate ($CuSO_4$, $5H_2O$) and dissolve it separately in about 100 ml of distilled water Pour the Copper sulphate solution into the other solution slowly and with constant stirring Add 5 ml of 5% Potassium ferrocyanide solution and make up the volume to a litre with distilled water If the solution is not clear, filter

Procedure·

Qualitative Benedict's test should be performed for the sugar of the urine to assess the concentration of sugar in urine The following dilution of urine

should be made for the different coloured precipitate by qualitative Benedict's test

Green precipitate	3 times dilution
Yellow ,	6 , ,,
Brick red	10

Measure accurately 10 ml of quantitative Benedict s reagent into a porcelain Basin Add 2 grams of anhydrous sodium Carbonate (or 5 grams of crystalline salt) and a pinch of Pumice stone

Fill a 10 ml microburette with the urine (or its dilution if necessary) Now heat the porcelain Basin on a low flame. When the solution of the procelain basin begins to boil add urine from the burette rapidly first but continuously until white precipitates begin to appear At this stage add urine drop by drop at an interval of about 10 seconds until the last trace of blue colour is discharged

Allow the titration mixture to cool If the mixture still shows bluish tinge boil the contents again and add a few drops of urine until the trace of blue colour disappears Take the readings in the burette Repeat it again twice. All the readings should very nearer

Precaution

1 The boiling should never be interrupted

2 If the mixture in the Porcelain basin becomes too concentrated during boiling some quantity of hot distilled water should be added to replace the loss by evaporation

3 Reading in the burette must be taken carefully

Calculation

10 mL quantitative Benedict s reagent is reduced by 0 02 g glucose, 0 0212 g fructose 0 027 g lactose 0 03 g maltose

10 ml quantitative Benedict s reagent ≡ Y ml of urine

Y ml of urine contain 0 02 gm of glucose

100 ml of urine contain $\frac{0\ 02 \times 100}{Y}$ gm

$= \frac{2}{Y}$ gm

Discussion

1 The normal urine contains a very small amount of glucose which can not be detected by ordinary routine test

2 The presence of sufficient amount of reducing sugar in urine under pathological condition is said to be glycosuria (Glucosuria) Besides glucose, urine may contain lactose (in pregnant and lactating mothers), Galactose, Pentose.

3 Persistent glucosuria is the common indication of diabetes mellitus The volume of urine in this disease excreted per day is usually large

4 The estimation of sugar in urine aids us to assess the severity of the disorder as well as to follow its course under treatment

ESTIMATION OF CHLORIDE IN URINE

(Whitehorn method)

Principle A measured volume of urine is treated with a known volume of a standard silver nitrate solution containing concentrated nitric acid As a result, the chloride of the urine is precipitated as silver chloride The unreacted silver nitrate is estimated by titrating it against a standard Potassium thiocyanate solution

using ferric alum as indicator Ferric alum yields red colour with potassium thiocyanate which disappears in presence of silver nitrate

Silver nitrate combines with potassium thiocyanate forming silver thiocyanate Silver chloride can not react with KCNS due to the presence of excess of nitric acid The amount of silver nitrate used up for the precipitation of chloride as silver chloride is a measure of the amount of chloride in urine

Reagents:

1 **Acid silver nitrate reagent:** 29 061 grams of pure silver nitrate is dissolved in water and the volume made upto 1 litre with distilled water The solution is further mixed with 1 litre of Pure nitric acid 1 ml of this silver nitrate solution is equivalent to 0 005 grams NaCl

2. **Standard Potassium thiocyanate solution:** A 2% solution of KCNS is titrated against the acid silver nitrate solution with ferric alum as indicator and its concentration is adjusted so that 1 ml of the thiocyanate solution corresponds to 2 ml of the acid silver nitrate solution

3 Ferric alum (finely powdered)

Procedure:

1 Take exactly 5 ml urine in a porcelain basin and add 10 ml of acid silver nitrate reagent to it Mix well slowly with the help of a glass stirrer and allow it to stand for 5 minutes for the precipitate of silver chloride to aggregate

2 Add about 0 3 gm of finely powdered ferric alum (or 6 ml of 5% solution of ferric alum) Stir slowly again and let the precipitate settle down

3 Titrate the supernatant liquid against KCNS solution from a microburette

4 Stir carefully during titration without disturbing the precipitate and continue titration until a red colour is obtained which remains for at least 30 seconds after careful stirring

5 Take exact reading in the microburette and repeat the experiment thrice avoiding error All the three readings must be very nearer for a better result to be obtained

Precaution

1 During titration the mixture in the porcelain basin should not be stirred vigorously Vigorous stirring tends to break up the silver chloride aggregate which also take up some KCNS and make the colour disappear leading to unusual high titration value

$$AgCl + KCNS = AgCNS + Kcl$$

2 If the permanent red colour of the whole mixture appears by the addition of one or two drops of KCNS solution from the microburette, it indicates that 10 ml of silver nitrate solution taken is just barely sufficient for the precipitation of all the urinary chlorides

Therefore, a further 10 ml of acid silver nitrate reagent and 0 3 gram of ferric alum should be added to the mixture in the porcelain basin Mixed, allowed to stand for 5 minutes and the titration carried out further In calculating the result, particular attention must be given for the increased volume of silver nitrate used

Calculation

Volume of acid silver nitrate reagent originally used = 10 ml

Volume of KCNS solution used for titration of remaining $AgNO_3$ = A ml

Volume of $AgNO_3$ reagent remaining unused = 2A ml = B ml

[Because 1 ml of KCNS = 2 ml of acid $AgNO_3$ reagent]

Volume of $AgNO_3$ used up by chlorides present in 5 ml. of urine $= (10-B)$ ml

$$\text{Chloride in 100 ml urine} = \frac{(10-B) \times 0.005 \times 100}{5}$$

$$= (10-B) \times 0.1 \text{ gms NaCl}$$

[1 ml of acid $AgNO_3$ reagent $\equiv$ 0.005 gm. of NaCl]

Daily excretion of chloride $= (10-B) \times 0.1 \times 1500$ gms NaCl.

The average daily excretion is assumed to be 1500 ml

Discussion:

1 Chloride is the chief anion of urine. The amount of chloride excreted per day by normal adult on a normal diet varies from 10 to 15 gms. (expressed as NaCl)

2 In fasting, the chloride excretion falls rapidly and on high water ingestion, it is increased.

3 In Pneumonia and certain other acute infectious diseases, the excretion of chloride is significantly diminished

4 The decreased excretion of chloride has been found in nephritis associated with edema.

5 Chloride excretion increases in adrenal cortical hormone deficiency as in Addison's disease.

ESTIMATION OF TOTAL TITRATABLE ACIDITY IN URINE

(Folin method)

Principle: The titratable acidity of urine is expressed in terms of the ml. of standard alkali necessary to bring the urine from its original pH to Phenol phthalein end point pH 8.5 or 9 by the use of the phenolphthalein indicator

In normal urine, the "acid" titrated consists entirely of $H_2PO_4^-$. The reaction is as follows:

$$H_2PO_4^- + OH^- = HPO_4^{--} + H_2O$$

The urine is titrated with standard 0.1 N NaOH using Phenolphthalein as an indicator. Potassium oxalate is added to precipitate calcium as calcium phosphate on neutralization

Reagents:

(1) 0.1 N NaOH solution.

(2) 1% Phenolphthalein solution.

(3) Potassium oxalate.

Procedure:

1 Take 25 ml of urine in a conical flask.

2 Add to it 5 gms of finely Powdered Potassium oxalate and 0.5 ml of phenolphthalein solution.

3 Shake vigorously and titrate immediately with 0.1 N NaOH solution from a burette till a permanent pink colour appears.

4 Take burette reading and take such readings thrice. Take the mean of the three readings for calculation

Calculation:

$$\frac{25}{a} = \frac{b}{x}$$

$$x = \frac{a \times b}{25}$$

where, a = ml of 0·1 N NaOH solution used

b = Volume of urine excreted in 24 hours

x = Total acidity of the 24-hours urine specimen

The result is expressed as ml of 0·1 N NaOH

Discussion:

1 The total acid excreted per day under normal condition by normal adults varies from 200 to 500 ml of 0·1 N NaOH

2 The urine is acidic on meat diet and on vegetable diet it is less acidic or alkaline

3 During active gastric digestion the urine may temporarily be acidic

4 In renal diseases, the urine becomes acidic due to the incapability of neutralizing acids

5 In fasting, the titratable acidity may rise to 800 in a few days Values upto 1500 may be found in severe diabetic acidosis

6 The acidity of the urine may increase by the administration of mineral acids, while it is diminished on the administration of sodium bicarbonate

7 The acidity of the urine is decreased, if the urine is allowed to stand for sometimes, due to the formation of ammonia from urea by bacterial decomposition

ESTIMATION OF TOTAL NITROGEN IN URINE

(Kjeldahl method)

Principle: The various nitrogenous constituents of urine are converted into ammonium sulphate by digestion (boiling) with concentrated sulphuric acid Traces of copper sulphate is added to catalyse the oxidation and potassium sulphate is used to raise the boiling point of sulphuric acid The ammonium sulphate thus formed is heated with a strong solution of sodium hydroxide and the liberated ammonia is distilled into a measured volume of standard acid At last, this partly neutralized acid solution is titrated with standard alkali solution The amount of acid neutralized by ammonia is a measure of the nitrogen content of urine

Regents:

1 Sodium hydroxide (40%)
2 Methyl red (0·2%)
3 Saturated solution of copper sulphate
4 Potassium sulphate
5 NaOH (0·1N)
6 H_2SO_4 (0·1 N)

Procedure:

1. Digestion:

(a) Put 1 ml. urine in a 300 ml. Kjeldahl flask and also 2 ml. conc. H_2SO_4, 2 drops of saturated solution of copper sulphate, 1 gm. of potassium sulphate.

(b) Heat the flask in a fume cupboard over a small flame of microburner. The fume should just touch the bottom of the flask. Use a fume absorber (fig. 11.3). Continue the gentle boiling until the solution turns bluish green.

(c) Disconnect the flask and swirl the contents gently to dislodge and black particles which may be sticking to the walls of the flask. Connect again and heat as before for another 5 minutes.

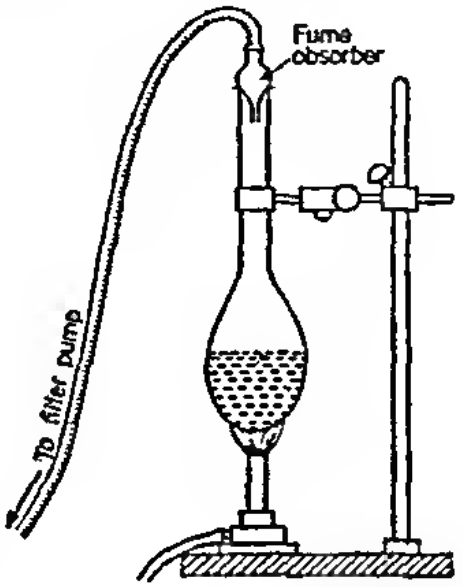


Fig. 11.3. The arrangement for the digestion of nitrogenous compound.

2. Distillation:

(a) Allow the flask to cool till it is just warm. Add 20 ml. water and a few pieces of broken porcelain and shake immediately to prevent separation of potassium hydrogen sulpbate.

(b) Take 20 ml. of standard 0.1N. H_2SO_4, 10 ml. water and 3 drops of methyl red in a 250 ml. Conical flask. Assemble the apparatus according to the diagram (fig. 11.4). Take care that the delivery tube of the condenser just dips below the surface of the acid. Start running water through the condenser.

(c) Take down the Kjeldahl flask and gently run 10 ml. of 40% sodium hydroxide down its side so that it sinks to the bottom of the flask.

(d) Clamp the flask into position and connect it to the condenser through the still-head.

(e) Fix the burner in such a position that the top of the flame just touches the bottom of the flask. Start heating the flask till boiling commences. Continue boiling for another 10 minutes. At the end of the distillation lower the conical flask till the tip of the condenser is out of solution. Then remove the burner.

(f) Disconnect the condenser from the flask. Wash down the inside of the condenser and outside of the delivery tube with a jet of distilled water receiving the washing into the conical flasks.

(g) Titrate the contents of the conical flask against the standard 0 1N NaOH solution provided Also titrate 20 ml 0 1N H_2SO_4 with 0 1N NaoH and the two solutions should closely match

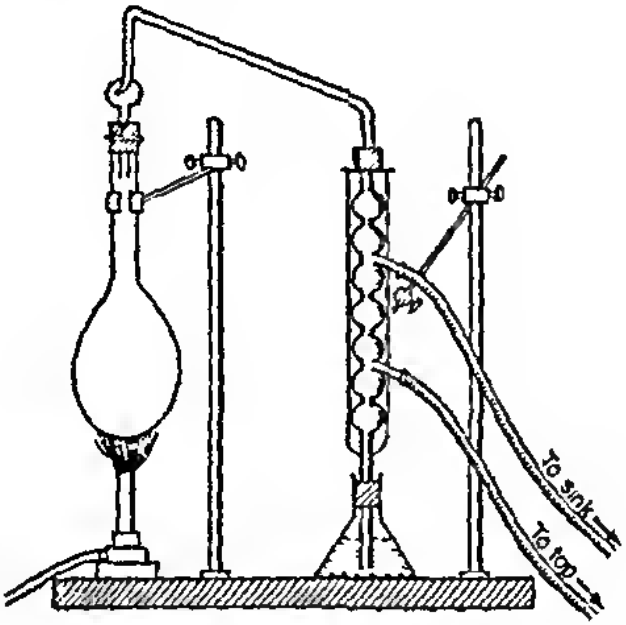


Fig 11 4 The nitrogen distillation set up

Calculation:

Since 1 litre of N/1 acid	=	1 litre of N/1 Nitrogen
∴ 1 ml of N/10 acid	=	0 0014 g nitrogen
The acid used for neutralization of the ammonia in the above experiment	=	The total acid originally taken in the conical flask—unneutralized acid that remains in the conical flask at the end of distillation
	=	(20 ml acid—the volume of NaOH used for the back titration)
	=	Y ml N/10 acid
Hence the total nitrogen in 1 ml Urine	=	(Y×0 0014 g]
The daily excretion of total nitrogen in urine	=	(Y×0 0014×1500) g

Discussion:

1 The daily excretion of total nitrogen in urine by a normal adult on a mixed diet varies from 12 to 18 gms On a low protein diet, the urinary nitrogen may fall to 5 gms per day and on a high protein diet, it may rise to 22 to 25 gms

2 The chief nitrogenous constituents of normal urine are urea, uric acid, creatinine, ammonium salts, amino acids etc

3 In a normal adult, the total nitrogen of the urine and of the faeces are often almost exactly equal to the total nitrogen of the diet Such a condition is said to be "Nitrogen equilibrium" The feces usually contain very little nitrogen

4 Persons when excrete more nitrogen than what he ingests is said to be "negative nitrogen balance" But if intake of nitrogen exceeds nitrogen excretion, it is said to be ' Positive nitrogen balance"

ESTIMATION OF AMYLASE (DIASTASE) IN URINE
(Wohlgemuth's method)

Principle: Different amounts of urine are incubated with a fixed amount of 0.1% starch solution for 30 minutes. The minimum amount of urine necessary for converting 2 ml. of starch solution into erythrodextrin at 37°C in 30 minutes is an index of the quantity of enzyme in the urine.

Reagents:

1. **Stock Starch solution (2% in 10% NaCl):** Make 2 gms. of starch into a paste with a few ml. of distilled water and pour into it about 60 ml. of boiling water. Add 10 gms. of sodium chloride and dissolve. Cool, transfer 100 ml. Volumetric flask and make up to the mark. This keeps for a few weeks.

2. **0.1 per cent starch solution for use:** Dilute the stock solution 20 times with distilled water. Prepare each day.

3. **N/50 iodine solution approximately.** Prepare by diluting a small amount of N/10 iodine 1 in 5.

Procedure:

1. All pipettes for this experiment should be lightly plugged on the top with a little cotton wool to prevent contamination with saliva.

2. *Dilute the urine 1 in 10.*

3. Take 10 clear and dry test tubes and number them from 1 to 10. Arrange the tubes in serial order in a rack.

4. Measure different amounts of undiluted or diluted urine according to the table given below into ten test tubes by 1 ml. graduated pipette.

5. *Add required amounts of distilled water as indicated in the table to make* the total volume in each test tube to 1 ml. See that the first five test tubes contain diluted urine and the other five undiluted urine.

6. Place all test tubes in a water bath at 37°C and after 2 minutes add 2 ml. of 0.1% soluble starch solution to each and shake gently to mix. *Note the time* and leave the test tubes in the bath for exactly 30 minutes.

7. Remove them from the bath and put them in a tumbler of ice cold water for 2 minutes to stop the reaction. Arrange the test tubes again in the rack.

8. Add 2 drops of dilute iodine solution to each of the test tubes, mix and note the colour. If the colour fades away on shaking, add a drop or two of the iodine solution to the test tubes. Too much iodine should be avoided.

9. The test tubes which show blue or purple colour still contains starch or amylodextrin. But the tube which does not show blue or purple colour but show reddish colour contains erythrodextrin. The amount of Urine in this tube is to be recorded.

Test tube numbers	1	2	3	4	5	6	7	8	9	10
Ml. of diluted urine (1 10)	0.5	0.6	0.7	0.8	0.9					
Ml. of undiluted urine						0.1	0.2	0.3	0.4	0.5
Ml. of distilled water	0.5	0.4	0.3	0.2	0.1	0.9	0.8	0.7	0.6	0.5
Calculated diastase index	40	33.3	23.6	25	22.2	20	10	6.7	5	4

Calculation:

Noting down the minimum amount of urine necessary for the digestion of 2 ml of 0 1 per cent starch solution, calculate what volume of the starch will be digested to erythrodextrin by 1 ml of urine under the specified condition of temperature and time

$$\text{Thus } d\frac{37^\circ}{30} = \frac{2}{\text{Smallest volume of urine noted to digest the starch to erythrodextrin}} \text{ units}$$

As for example, if tube No 7 shows red colour, the smallest volume of urine required is 0 2 ml undiluted urine Therefore, diastatic index is

$$d\frac{37^\circ}{30} = \frac{2}{0\ 2} = 10 \text{ units}$$

Discussion:

1 The enzymes pepsin, trypsin, amylase and lipase have been identified in urine

2 Normally, very small amylase activity is present in urine But its concentration increases highly in acute pancreatitis and Parotitis

3 Amylase is normally secreted by salivary glands and Pancreas It digests starch into maltose through amylodextrin , erythrodextrin and achrodextrin

4 The activity of amylase in urine is usually expressed as "diastatic index" which is defined as the number of millilitre of 0 1% starch digested into erythrodextrin by 1 ml of urine in 30 minutes at 37°C

5 The diastatic index of a normal urine ranges between 5 to 30 units Concentrated urine may show higher diastatic index

ESTIMATION OF VITAMIN 'C' (ASCORBIC ACID) IN URINE

(Harris and Ray, 1935)

Principle: L ascorbic acid is a strong reducing agent and it reduces the dye 2 6 dichlorophenolindophenol into the colourless leucobase This dye is red in acid solution and becomes colourless when fully titrated with vitamin C Ascorbic acid in urine is determined by titrating it directly into a known amount of dye to the colourless end point

Reagents:

(1) Glacial acetic acid

(2) **2 : 6 dichlorophenolindophenol solution:** Weight out accurately 40 mg of the dye and dissolve it in water Make the volume to 100 ml with water 0 5 ml of this solution is equivalent to 0 1 mg of ascorbic acid It should be stored at 4°C Its keeping qualities are poor Hence, it is to be prepared at frequent intervals (at least weekly)

Procedure:

Take 0 5 ml of 2 6 dichlorophenolindophenol solution in a test tube and add 1 ml of glacial acetic acid to it

Collect your urine and immediately fill a 10 ml microburette with it Titrate the dye with the urine shaking well the test tube during the titration till the red colour is just discharged Take the burette reading Repeat it thrice Take the mean of the three readings for calculation

Precaution:

Ascorbic acid is a labile substance and is quickly oxidized to dehydroascorbic acid by atmospheric oxygen. Therefore, it is wise to estimate ascorbic acid of urine sample within a few minutes after passing it.

Sometimes acetic acid is added immediately in the proportion of 1 part to 9 parts of urine. Such acidified urine keeps well for several hours.

If a 24-hour sample of urine is collected with acetic acid as preservative, hydrogen sulphide (H_2S) must be passed through the urine sample prior estimation with a view to reduce any dehydroascorbic acid present for its conversion to ascorbic acid.

Calculation:

Suppose, burette reading is 'X' ml.

'X' ml. of urine contains 0.1 mg ascorbic acid.

The daily excretion of ascorbic acid in urine.

$$= \frac{0.1 \times 1500}{X} \text{ mg}$$

[The average daily excretion of urine is assumed to be 1500 ml.]

Note: Besides ascorbic acid, the urine contains other substances which decolourise the dye. Although, the result obtained for ascorbic acid is not very accurate it is still sufficiently accurate for clinical purposes.

Discussion:

1. Food is the source of vitamin C in the body. Vitamin C is distributed in blood, tissue fluids and in all secretions and excretions of the body including urine. The excretion of vitamin C in urine depends upon the level in blood.

2. The daily urinary output of ascorbic acid is about half its intake. Normally, the daily excretion of it in urine ranges from 6 to 18 mg.

3. Urinary ascorbic acid is much reduced and may even disappear in the deficiency of vitamin C. The values may increase in urine when the intake is high.

CHAPTER 12

FOOD AND FUNCTION TEST

HYDROLYSIS OF STARCH BY SALIVA

(Rebert's achromatic point method)

Principle: A given amount of Saliva containing amylase is added to a measured amount of starch solution at 40°C and pH 6 8 The digested samples are removed and treated with dilute iodine at intervals until no further colouration is produced The time taken to reach this achromatic point is determined NaCl acts as an activator for amylase

Reagents:

(1) Starch (1%)

(2) NaCl (0 5%)

(3) **Buffer solution (pH 6 8):** Treat 50 ml of 0 2 N acid potassium phosphate with 21 ml of 0 2 N NaOH and dilute to 200 ml with distilled water

(4) Dilute iodine (0 01N)

Procedure:

1 Measure 5 ml of 1% starch into a clean test tube

2 Add 2 ml of buffer solution (pH 6 8) and 2 ml of 0 5% NaCl to it

3 The tube is placed in a water bath at 40°C to warm it and in the same water bath, diluted saliva (1 in 20) is also kept in a test tube

4 A series of test tubes are prepared each containing 2 ml of distilled water and 2 drops of diluted iodine (0 01N)

5 Add 2 ml of saliva to the warm starch solution Mix well and note time Transfer at intervals one to two drops of digested mixture to one of the iodine tubes by a glass tube taking care that no iodine is transferred back to the digested mixture The sample is shaken and note the colour which is blue at first

6 Note the time at which no colour is produced when a drop of the digested mixture is added to an iodine tube This is said to be "Chromic period" which is an inverse measure of the activity of the enzyme

Note: Accurate results can be obtained if the saliva is so diluted as to give chromic period of about 10 to 15 minutes and the digest is taken every 30 seconds towards the end of the period and compared with the blank iodine tube to serve as control

Discussion:

Salivary amylase acts upon boiled starch yielding maltose at the optimum temperature and pH The activity of this enzyme is increased in presence of an activator (Chloride) Its activity is decreased in absence of the suitable conditions

ESTIMATION OF REDUCING SUGAR IN MILK

(Benedict's method)

Principle: Lactose, the reducing sugar of milk, can be obtained by precipitating the protein of the milk by copper sulphate and sodium hydroxide solution It may be estimated by using Benedict's quantitative solution just like reducing sugar in urine

Reagents:

1 Zinc sulphate solution (10%)
2 Sodium hydroxide (1N)
3 Benedict's quantitative reagent
4 Sodium carbonate (anhydrous)
5 Pumice stone

Procedure:

Pipette 10 ml milk into a 100 ml Volumetric flask and add to it 2 ml of 10% $ZnSO_4$ solution and 1 ml of 1N NaOH solution Mix well Make up the volume upto *100 ml* by adding distilled water *Allow to stand for 1 to 2 minutes* and then put it in boiling water bath for a few minutes till the casein is precipitated Then filter Pour the filtrate into a burette

Titrate just like reducing sugar in urine using benedict's quantitative reagent As such take three burette readings and take the mean for calculation

In the case of Cow's milk, it is wise to take 20 ml of milk with 4 8 ml of $ZnSO_4$ and 2 4 ml of NaOH

Calculation:

10 ml of Benedict's quantitative solution is reduced by 0 027 gms of reducing sugar of milk.

Suppose, the mean burette reading is 'X' ml

Hence, 10 ml of Benedict's quantitative solution = 'X' ml filtrate

'X' ml of milk contain (0 027 × 10) gms of reducing sugar (10 being the dilution factor]

100 ml of milk contain $\frac{0\ 027 \times 10 \times 100}{x}$ gms of reducing sugar

100 ml of milk contain $\frac{27}{x}$ gms of reducing sugar

ESTIMATION OF FAT CONTENT OF MILK

(Babcock's Centrifugal method)

Principle: Concentrated sulphuric acid when added to the milk charrs organic matter and breaks emulsion of fat The centrifugation of the acid treated milk in a special tube (*Babcock tube*, fig 12 1) brings the fat in the narrow calibrated part of the tube from which fat content is directly red

Reagents.

1 Concentrated sulphuric acid (Sp gr 1 83—1 84)

2 Acid alcohol mixture Mix equal volumes of Conc HCl and amyl alcohol

Procedure:

1 Introduce milk into the Babcock tube upto 5 ml mark by means of a narrow pipette

2 Add conc H_2SO_4 to fill the body of the tube, 1 ml at a time. Mix well by rotation after each addition, Cool, and then add further acid to fill the body once again

3 Fill the neck of the tube to the Zero mark with an acid alcohol mixture

Fig 12 1
Babcock tube.

4 Centrifuge the tube for 2 to 3 minutes and read the Percentage of fat from the calibrated part of the neck If the top of fat column is not at Zero, it may be brought there by addition of hot water and then recentrifuge

5 In case, fat content is over 5 percent, the milk should be diluted with an equal volume of water and then proceed as above multiplying the observed reading by 2

Discussion:

Cow's milk and human milk contain 4 per cent fat Presence of less than 3 5 per cent fat in cow's milk indicates either dilution of milk or removal of fat from it

GASTRIC ANALYSIS

Collection of Gastric juice for fractional test meal·

1 The subject is advised not to eat or drink anything during the 12 hours preceeding the test

2 Ryle's tube lubricated with a little paraffin is passed to the stomach through oral or nasal passage upto the double mark on the tube Saliva should not be swallowed afterwards

3 The resting juice is removed with a 50 ml syringe The volume is noted and the contents are kept for further examination This is called 'Zero sample'

4 The person is given a pint of Oat meal to drink. The time is noted About 10 ml of the stomach contents are removed every 15 minutes for 2½ hours into different test tubes marked from 1 to 10 At the end of this period, the stomach is emptied completely and the volume of the residual contents are measured The tube is then withdrawn gently

Examination of the specimen:

1 **Colour:** Greenish colour indicates bile pigments
Red dish or brownish colour indicates blood

Confirm blood by benzidine test

2 Add a drop or two of dilute iodine solution to a drop of the contents from each tube on a white porcelain tile Blue colour indicates the presence of starch Starch test becomes negative when all the oat meal has passed out into duodenum

Acid titration:

Both free and total acids are determined in each specimen

Free acid· Pipette 1 ml of the filtered gastric content into a small beaker Add about 2 3 ml of distilled water and then a drop of Topfer's indicator It will turn Pink in the presence of free HCl Titrate it with *N/100 NaOH* until pink colour disappears and the colour ultimately becomes yellowish orange (pH 4 0) At this pH all free HCl is titrated Take the burette reading The volume of the alkali required for titration represents the free HCl present in 1 ml gastric content Calculate the free HCl as N/10 acid present in 100 ml of gastric juice

Total acid: Now add a drop of phenolphthalein to the above content and continue titration with the alkali until a definite red tinge reappears (pH 8 5) Take the second reading of the burette The difference between this reading and the initial reading represent the total acid present in 1 ml of the gastric contents

The difference between the total acid and free HCl is due to the combined acid (HCl neutralised by weak bases) and organic acid

Calculate the total acid present in 100 ml of gastric juice

In this way find out the free HCl and total acid for each specimen Draw the results of the gastric analysis on a graph paper

Abnormal acidity:

1 **Hyperchlorhydria:** If the free HCl is above the normal range is termed hyperchlorhydria This is commonly seen in cases of duodenal ulcer

2 **Hypochlorhydria:** If the free HCl does not exceed 20 ml N/10 HCl in any specimen is termed hypochlorhydria

3 **Achlorhydria:** It is a condition in which free HCl is absent from all specimens

Reagents:

1 N/100 NaOH

2 **Topfer's indicator:** Dissolve 0 5 gms of Topfer's indicator (dimethyl aminoazobenzene) in 100 ml of 95 % alcohol

3 Phenolphthalein indicator (1% solution in ethanol)

4 Dilute iodine solution (N/50)

A graphical representation of normal acidity:

In normal individuals there is found a small amount of free HCl in the resting contents This is usually between 0 and 30 ml. of N/10 acid per 100 ml.

Concentration above 50 ml N/10 acid probably indicates hyperacidity The acid is also expressed in terms of unit (1 unit per litre=1 ml N/10 acid per 100 ml juice)

After taking Oat meal, acidity falls immediately and starts rising in a quarter of an hour In about 1½ hour, acidity reaches its maximum At this time, the

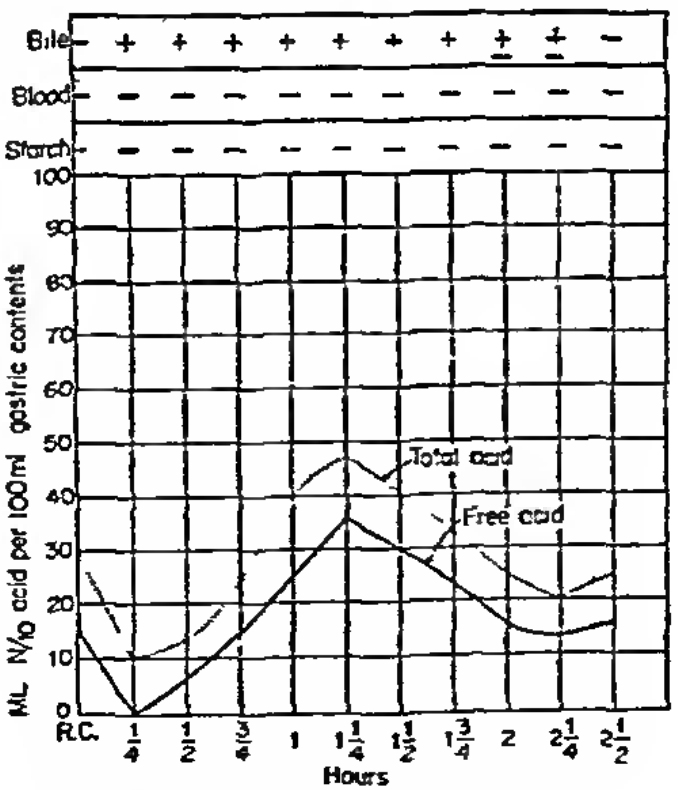


Fig 12.2 Fractional test meal analysis A typical normal curve.

free HCl in normal individuals is usually below 50 ml N/10 acid per 100 ml juice The total acidit, of the juice is about 10 ml higher than the free HCl at all times

In normal cases, the total acidity of the gastric contents is mainly due to the presence of free HCl and partly due to the presence of combined HCl and some organic acids such as lactic acid and butyric acid Organic acids and combined acid are usually about 10 ml of N/10 acid per 100 ml Higher amount of these organic acids are found in cases of pyloric obstruction and gastric carcinoma

Discussion

1 Gastric juice is collected after stimulating gastric secretion by giving oat gruel or by administration of histamine etc

2 The chemical examination of gastric contents gives us a valuable information regarding the secretory and motor functions of stomach The important secretory products of stomach are hydrochloric acid and the enzyme pepsin

3 *Hyperchlorhydria is associated with duodenal or gastric ulcer*

4 Hypochlorhydria is associated with pernicious anemia or gastric carcinoma

5 Sometimes no hydrochloric acid is secreted in cases of gastric carcinoma and pernicious anemia even after histamine injection (achylia gastrica)

Liver function tests.

Van den Bergh Reaction

This consists of two parts—the direct (Qualitative) and the Indirect (Quantitative) reaction

Reagents:

The diazo reagent is prepared freshly before use by adding 0 8 ml of solution B to 10 ml of solution A

Solution A: Dissolve 1 gm of sulphanilic acid in 15 ml of Conc HCl and make upto 1 litre with water

Solution B: Dissolve 0 5 gm of sodium nitrite in water and make upto 100 ml Prepare freshly at frequent intervals

The Direct Reaction.

Procedure: Pipette 0 3 ml of serum in a test tube and add 0 3 ml of diazo reagent Mix and observe the colour change

Results:

Direct immediate Positive	Pink or Permanganate colour develops at the junction of two fluids, within *one minute*
Direct delayed Positive	Pink or Permanganate colour develops at the junction of two fluids, between 1 *and* 10 minutes
Indirect Positive	The above colour does not develop in 10 minutes but the colour develops after addition of 1 ml alcohol
Negative	No colour develops even after the addition of alcohol

The indirect reaction is done for the estimation serum bilirubin

Thymol Turbidity Test.

Principle: When serum mixes with thymol buffer, the degree of turbidity is measured colorimetrically

Reagent:

Thymol buffer: Add 1 38 gms of barbitone, 1 03 gms of sodium barbitone and 3 gms of thymol to 500 ml of distilled water Heat the mixture, shake well and cool thoroughly It will be turbid Add a small amount of powdered thymol crystals, shake, and allow to stand overnight at a temperature of 20 to 25°C. Shake well and filter Store at about 20°C.

Powdered thymol crystals, shake, and allow to stand overnight at a temperature of 20 to 25°C. Shake well and filter Store at about 20°C

Procedure Pipette 0 1 ml of serum into a dry test tube and add 6 ml of thymol buffer Allow to stand for ½ hour Compare with the set of Protein standards containing 10, 20, 30, 40 100 mg per 100 ml

Result:

A turbidity equivalent to 10 mg per 100 ml protein =1 unit
,, ,, ,, ,, 20 mg ,, ,, ,, ,, =2 units
,, ,, ,, ,, 100 mg ,, ,, ,, ,, =10 units

The result is better obtained from a graph prepared by the Protein standards For photoelectric colorimetric reading use red filter at 680 m/μ

Discussion

1 The normal range by this test is 0-4 units In infective hepatitis, the thymol turbidity is usually highest soon after the onset of the jaundice.

2 The development of turbidity is due to liver disease γ globulin is responsible for the turbidity rise β globulin also takes part in the degree of turbidity Normal serum albumin inhibits the effect of the γ globulins

Flocculation test

This may be either qualitative or quantitative and involves one or more of the globulin fractions

Procedure Place 1 ml of 0 9% NaCl solution into eight small tubes Add 1 ml of serum to the first, mix, transfer 1 ml to the second tube, mix and proceed similarly along the row of eight tubes so that a series of dilution of serum from $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{256}$ is obtained

To each tube add 0 25 ml of 10% Na_2CO_3 solution and 0 3 ml of a solution made by mixing equal volumes of 0 5 per cent mercuric chloride and 0 025 per cent *fuchsin in water*

Results Normal serum only precipitates from tube 4 onwards A precipitate in tube 3 or before that constitutes a positive reaction

Discussion: The test is positive in Cirrhosis of liver and is not specific for liver dysfunction

PROTHROMBIN TIME

(Quick, 1939)

Principle Prothrombin time is the time required for clotting to take place in citrated plasma to which optimum amourts of thromboplastin and calcium has been added

Reagents

1 Sodium Citrate solution (3 8 per cent)

2 Thromboplastin (Commercial preparation available in the market) Add 0 3 gms of the dried powder in 5 ml of 0 85% Saline containing 0 5%

Phenol and incubate for 15 mts at 37°C Centrifuge & remove the supernatant Prepare serial dilutions 1 in 2, 1 in 4, 1 in 8 with the saline

3 Calcium chloride (0 025 M) Solution Dissolve about 3 gms of the anhydrous salt in water and make upto a litre Standardize

Procedure: Collect Venous blood in a siliconized syringe and add 9 volumes of blood to one of citrate in a plastic container Mix by inverting several times and centrifuge for 10 minutes

Add 0 1 ml of plasma to 0 1 ml of thromboplastin prepared by the method mentioned above Warm the mixture for 15 30 seconds in a water bath to 37°C Add 0 1 ml of the 0 025M $CaCl_2$ solution, also warm to 37°C using a graduated pipette and start stop watch

Note the time required for Coagulation

Precaution: Glass containers should be avoided since contact activtation of Factor VII shortens the prothrombin time

Calculation: Prothrombin time is expressed in seconds A dilution 1 in 2 has an activity of 50% and of 1 in 4 of 25%

Discussion:

1 The normal prothrombin time is 10 seconds

2 Prothrombin is formed by the liver cells and vitamin K being required When bile salts are not present in the intestine, the absorption of vitamin K is impaired So in jaundice and liver cell disease, the prothrombin time is prolonged

3 In obstructive jaundice, the prothrombin time is more If adequate vitamin K is given parenterally, the prothrombin time returns to normal rapidly in uncomplicated obstructive jaundice but in liver disease, the response is less

4 Determination of Prothrombin time is also used to decide whether there is a danger of bleeding at operation in biliary tract disease *In* normal persons, the prothrombin activity is within the range of 70 to 100 per cent Below this level (at 60%), bleeding may occur

CHAPTER 13

BLOOD

ESTIMATION OF SERUM ALKALINE PHOSPHATASE AND ESTIMATION OF SERUM INORGANIC PHOSPHATES

(Method of King, Abul Fadl, and Walker, 1951)

Principle: The enzyme phosphatases catalyse the splitting of phosphate group from certain monophosphoric esters The enzyme activity is measured by the inorganic phosphate liberated in a given time at a particular temperature.

King and Armstrong have defined 1 unit (K A Unit) as the activity of the enzyme which will liberate 1 mg of phenol or equivalent amount of phosphate (1/3 mg) from phenyl phosphate substrate in 15 minutes at 37°C

The inorganic phosphate liberated is estimated by reacting it with molybdic acid to form phosphomolybdate which on reduction by reducing agent such as 1 2 4 aminonaphthol sulphonic acid forms a blue coloured soluble compound (molybdenum blue) The blue colour thus produced is proportional to the amount of inorganic phosphate present

Reagents·

1 **Sodium Carbonate bicarbonate buffer (0.1 N., p^H10):** Dissolve 0 636 gm of anhydrous sodium carbonate and 0 336 gm of sodium bicarbonate in distilled water and make upto 100 ml with distilled water Store in a refri gerator

2 **Substrate disodium phenyl phosphate (0 01 M.):** Dissolve 0 218 gm. of disodium phenyl phosphate in distilled water and make upto 100 ml Bring it quickly to boil, cool and add about 2 ml chloroform and store in a refrigerator

3 Trichloracetic acid, 20% (W/V)

4 Molybdic acid Dissolve 5 gms of ammonium molybdate in 100 ml of 5N H_2SO_4 (Add 14 ml Cone H_2SO_4 slowly to 86 ml H_2O to prepare 5N H_2SO_4)

5 **Aminonaphthol sulphonic acid solution (0.25 per cent):** Add 0 25 gm of 1 2 4 aminonaphthol sulphonic acid to 97 5 ml of 15% sodium bisulphite and 2 5 ml of the 20% sodium sulphite Stopper and shake until dis solved If the solid remains undissolved, add sodium sulphite solution, 0 5 ml at a time shaking well after each addition until complete solution is made Store in the cold in a brown bottle Renew every month

6 **Stock phosphate standard (1 mg. per ml):** Dissolve 0 439 gm. of Pure potassium dihydrogen phosphate (KH_2PO_4) in water and make to 100 ml

7 Phosphate standard for use (0 004 mg per ml) Dilute 0 4 ml of stock to 100 ml 5 ml of standard contains 0 02 mg phosphate

Procedure:

	Test (T)	Control (C)	Blank (B)
Buffer + Substrate	(3 ml +3 ml)	(3 ml +3 ml)	(3 ml +3 ml)

Put all the test tubes in a water bath at 37°C for about 5 minutes to attain the Temp Then add 0 3 ml serum to 'T' mix for 30 seconds and place it into the bath After exactly 15 minutes stop the reaction of the enzyme by adding 1 2 ml tri chloracetic acid which will cause the precipitation of all proteins

Now add 0 3 ml *serum* to 'C' and *immediately* add to it 1 2 ml trichloracetic acid Mix well

After a few minutes, filter both 'T' and 'C to have a clear filtrate

Add 0 3 ml water to 'B' and add 1 2 ml trichloracetic acid to it and mix

	Test (T)	Control(C)	Blank (B)	Standard (S)
Filtrate	5 ml	5 ml	5 ml	
Standard solution				5 ml
Molybdic acid	0 8 ml	0 8 ml	0 8 ml	0 8 ml
Aminonaphthol Sulphonic acid	0 2 ml	0 2 ml	0 2 ml	0 2 ml

Mix well and keep these four tubes in the dark for 10 minutes to develop colour Read the optical densities in a photoelectric colorimeter using red filter at 680 mμ

Calculation

Serum alkaline phosphatase per 100 ml Serum

$$= \left(\frac{T-B}{S} \times \text{amount of inorganic phosphate in standard} \times \frac{100}{\text{ml of serum used}} \times 3\right) \text{K A Units}$$

[3 is the factor Because 1 mg of phosphate is liberated by 3 K A Units of the enzyme]

$$= \left(\frac{T-B}{S} \times 0\,02 \times \frac{100}{0\,2} \times 3\right) \text{K A Units}$$

$$= \left(\frac{T-B}{S} \times 30\right) \text{K A Units}$$

Serum inorganic phosphate per 100 ml Serum

$$= \left(\frac{C-B}{S} \times \text{amount of inorganic phosphate in standard} \times \frac{10}{\text{ml of serum used}}\right) \text{mg}$$

$$= \left(\frac{C-B}{S} \times 0\,02 \times \frac{100}{0\,2}\right) \text{mg}$$

$$= \left(\frac{C-B}{S} \times 10\right) \text{mg}$$

Note 5 ml filtrate contains 0 2 ml Serum and 1 ml standard contains 0 02 mg of inorganic phosphate

Discussion

1 Serum contains acid and alkaline phosphatases depending upon whether they are active in acid (p^H_5) or alkaline (p^H_{10}) medium respectively Alkaline phosphatase is found in a number of organs such as small intestine, bones, liver and kidneys

2 The normal range for serum alkaline phosphatase by the King Armstrong method is from 3 to 13 units per 100 ml (23 92 I U /litre) With higher values for children during periods of most active growth, usually upto 20 units, occasionally between 20 and 30 in infants

3 In hypophosphatasia, serum alkaline phosphatase value is low even 3 units per 100 ml

4 There is an increase in alkaline phosphatase in rickets, osteomalacia and obstructive jaundice but a marked increase in rickets in children

5 A moderate increase in alkaline phosphatase has been found in the impaired absorption of Vitamin D and of Calcium

6 The normal serum inorganic phosphate ranges from 2 5 to 5 mg /100 ml in adults and 4 to 6 mg /100 ml in children

7 Serum inorganic phosphate level is low in rickets, Osteomalacia and in hyperparathyroidism

8 An increase in serum inorganic phosphate is found in hypoparathyroidism and in renal failure

ESTIMATION OF BLOOD SUGAR

Besides blood glucose other reducing substances such as glutathione, ergathione and glucuronic acid and its compounds are present in red cells These non glucose reducing substances are equivalent to 30 mg glucose per 100 ml

Several workers found that if the blood is put into an isotonic solution of sodium sulphate in which the cells are not hemolysed diffusible substances pass into solution and the cell envelopes which contain much of the non glucose reducing substances are taken down with the precipitate

Asatoor and King used isotonic sodium sulphate-copper sulphate with the addition of sodium tungstate to have the product copper tungstate which gives results near to the true glucose

The high degree of accuracy in results very close to the true glucose has been obtained by the use of glucose oxidase which oxidizes glucose to gluconic acid without affecting any other sugar

Various methods have been adopted for the estimation of blood sugar The titrimetric method gives also better results The original Folin WU method still appears to be widely used This technique gives results nearer to the true glucose content When the blood sugar is abnormally low, it is better to use a more specific technique Accordingly, a glucose oxidase method is preferred Some of the titrimetric methods are well suited but they are not so sensitive as the colorimetric

Preservation of blood· Blood is to be collected in a tube containing 6 mg of Potassium oxalate as anticoagulant and 2 mg of NaF as antiglycolytic agent per ml of blood Mix throughly

Glucose Oxidase method (Method of Marks, 1959)

Principle Glucose is oxidized by glucose oxidase to gluconic acid

$$\text{Glucose} + H_2O + O_2 \longrightarrow \text{Gluconic acid} + H_2O_2$$

The hydrogen peroxide formed is broken down to water and oxygen by a peroxidase in presence of an oxygen acceptor which is converted to a coloured compound which is read colorimetrically

Reagents

1 NaCl Solution (0 9 per cent)

2 $ZnSO_4$ Solution (5 per cent)

3 NaOH Solution (0 3 N)

4 O Tolidine solution (1 per cent in absolute ethanol)

5 "Ferm Cozyme ', a stable liquid preparation of glucose oxidase containing 750 units per ml from Hughes and Hughes Ltd London

6 **Acetate buffer (0 15M. pH_5):** Add approximately 3 volumes of 0 15M acetic acid to 7 volumes of 0 15M Sodium acetate (17 7 gms sodium acetate per litre) Adjust to pH_5

7 **Peroxidase·** 20 mg Per 100 ml of acetate buffer This is refrigerated for months

8 **Glucose oxidase reagent:** Add 0 5 ml of Fermcozyme to about 80 ml of acetate buffer Then add 5 ml of the peroxidase solution Mix and add 1 ml of the O-Tolidine Make to 100 ml with buffer Keep in the refrigerator in a dark bottle

9 **Standard glucose solution:** Prepare a solution containing 100 mg glucose per 100 ml saturated benzoic acid and dilute with the acid to obtain solution containing 2 5, 5, 7 5 and 10 mg per 100 ml These are equivalent to 50, 100, 150, 200 mg glucose per 100 ml

Procedure: Add 0 4 ml of 5% Zinc sulphate solution, 0 4 ml of 0 3N NaOH, 1 1 ml of 0 9% NaCl and 0 1 ml blood to a test tube For low values use 1 ml of saline and 0 2 ml blood and for high values use 0 05 ml blood and 1 5 ml saline

Mix well, centrifuge and transfer 1 ml of the supernatant to a test tube Measure 1 ml water as blanks and 1 ml of standard glucose solution equivalent to 200 mg glucose per 100 ml

Add 3 ml glucose oxidase reagent to each at ½ minutes intervals, mix gently for not more than ten seconds, and read the colour developed exactly 10 minutes later at 625 mμ or using an orange filter

Notes:

1 Colour development reaches a maximum in about 10 minutes and then declines slowly Therefore, reading should be taken immediately after 10 minutes

2 This technique is the same for cerebrospinal fluid glucose estimation

Calculation·

$$\text{Blood glucose per 100 ml} = \left(\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 200 \text{ mg}\right)$$

Method of Folin and WU

Principle: The protein free blood filtrate is heated with alkaline copper sulphate solution, using a special tube to prevent reoxidation The cuprous oxide thus formed is treated with a phosphomolybdic acid solution, a blue colour formed is read in a photoelectric colorimeter and compared with the standard

Reagents:

1 Sodium tungstate solution (10%)

2 Sulphuric acid (2/3N)

3 **Alkaline Copper sulphate** Dissolve 40 grams of anhydrous Na_2Co_3 in 400 ml of water in a litre flask Add 7 5 grams of tartaric acid and dissolve Add 4 5 grams of crystalline copper sulphate, dissolve and dilute the solution to 1000 ml

4 **Phosphomolybdic acid·** Place 35 grams of molybdic acid and 5 grams of sodium tungstate in a 500 ml beaker Add 200 ml of 10% NaOH and about 200 ml of water Boil for about 30 minutes (Volume should be reduced to about 350 ml) Cool and add 125 ml of 85% phosphoric acid Make upto 500 ml with distilled water

observed in severe nephritis, Pancreatic disease, hyperthyroidism, and in certain hepatic disorders Low blood sugar values are found in insulin administration, Addison's disease, hypopituitarism, cretinism, myxedema

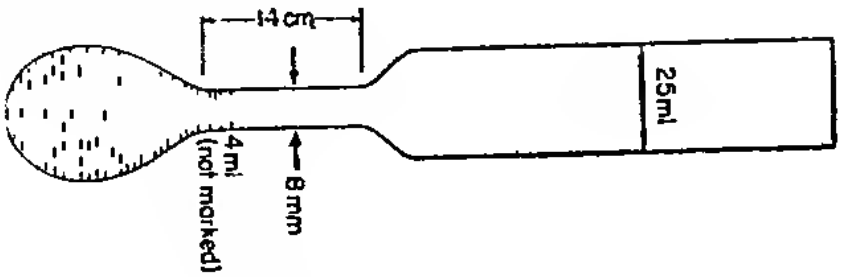


Fig 13 1 Folin WU Sugar tube

ESTIMATION OF BLOOD UREA

(Urease Nesslerization method)

Principle: The enzyme urease present in Soyabean splits urea into ammonia and carbondioxide which combine to form ammonium carbonate at an optimum temperature This produces a compound after reacting with Nessler s reagent developing yellow colour

Reagents·

1 Soyabean powder

2 **Isotonic sodium sulphate solution·** Dissolve 3 gms of Na_2SO_4, $10H_2O$ in distilled water and make upto 100 ml

3 Sodium tungstate solution (10 per cent)

4 Sulphuric acid (2/3 N)

5 Gum Ghatti solution Suspend 1 gm of Gum ghatti, loosely tied in muslin, for twenty four hours in a litre of distilled water Preserve the solution with a few drops of chloroform Discard the residue in the muslin

6 **Nessler's reagent·**

(a) Koch (1924)

A Dissolve 22 5 gms of iodine in a solution of 30 grams of potassium iodide in 20 ml of water When solution is complete, add 30 grams of mercury Shake well, keeping cool by immersing in cold water from time to time, until the supernatant has lost all yellow colour due to iodine Decant the supernatant and test a few drops with starch for the presence of iodine If starch gives no colour, add an iodine solution in small quantities Dilute to 200 ml and mix

B Sodium hydroxide solution (10 per cent)

Mix 200 ml of A with 975 ml of B Allow any precipitate to settle and use the clear supernatant

(b) **Harrison** (1947) Mix 150 ml of A, 700 ml of B and 150 ml of water

A Dissolve 150 grams of Potassium iodide in about 100 ml of distilled water Add 200 grams of mercuric iodide, when dissolved dilute to about a litre Filter and make to 2 litres

B Sodium hydroxide solution (10 per cent)

7. **Stock ammonium sulphate solution standard·** Dissolve 2 2 grams of the pure dry salt in distilled water and make upto 1 litre

Standard ammonium sulphate for use· Dilute 5 ml of the stock standard to 100 ml with water 1 ml of this is equivalent to 0 05 mg urea

Procedure·

Add 0 2 ml of blood to 3 2 ml of isotonic sodium sulphate solution Then add about 20 mg of powdered soya bean, stopper the tube and incubate for at least 1ɔ minutes at 40° to ɔ0 C. Add 0 3 ml. of 10 per cent sodium tungstate and 0 3 ml. of $\frac{2}{3}$ N H_2SO_4 Mix well, stand for a few minutes and centrifuge.

	Test (T)	Standard (S)	Blank (B)
	2 ml. Supernatant	2 ml. Standard	7 ml. water
Water (ammonia free) or Gum ghatti	5 ml.	5 ml.	
Nessler's reagent	1 ml.	1 ml.	1 ml.

Take Reading using blue filter at 480 mμ.

Calculation 2 ml supernatant is equivalent to 0 1 ml. blood.

$$\text{Mg of urea per 100 ml. of blood} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} 0\,1 \times \frac{100}{0\,1}$$

$$= \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 100$$

For accurate results, a curve should be drawn with different concentration of standard solution. From this unknown can be determined.

Discussion·

1 The normal range of urea on a full ordinary diet is from 20 to 40 mg per 100 ml. of blood. The urea content is influenced by the amount of Protein in the diet and tends to be lower in people on low protein diets.

2 Blood urea is lower in pregnancy, it is commonly between 15 and 20 mg per 100 ml. Blood urea decrease is rare. In some cases of severe liver disease, the value is decreased.

3 Blood urea level rises in conditions in which excretary functions of kidneys are impaired such as in kidney diseases, in pre-renal conditions such as dehydration, shock etc., and in post renal conditions such as obstruction in urine flow, enlarged prostrate gland, stricture of urethra due to stone in ureter etc.

ESTIMATION OF SERUM URIC ACID

(Method of Caraway)

Principle Uric acid in blood reduces phosphotungstic acid in presence of Sodium Carbonate to blue coloured reduced phosphotungstate. This colour has been found to be proportional to the amount of uric acid present.

Reagents·

1 **Tungstic acid** Add 2ɔ ml. of 10% sodium tungstate, 2ɔ ml. of 2/3 N H_2SO_4 and a drop of phosphoric acid with mixing to 400 ml. of distilled water Keep in a brown bottle and store in refrigerator Discard when cloudy

2. **Phosphotungstic acid (Stock) solution** Dissolve 50 grams of sodium tungstate in about 400 ml. of water Add 40 ml. of 8ɔ% phosphoric acid and reflux gently for 2 hours. Cool, transfer to 500 ml. flask and make to the mark with water Keep this in a brown bottle.

Dilute solution for use· Dilute 10 ml. of the stock solution to 100 ml. with water and store in a brown bottle. This keeps well for months.

3 Sodium Carbonate solution (10 per cent) Keep in a well stoppered polythene bottle.

4 **Uric acid standard solution (Stock):** 10 mg per 100 ml Dissolve 60 mg of lithium Carbonate in 15 to 20 ml of water in a test tube Heat the solution to about 60°C and pour on to 100 mg of uric acid taken in a small beaker Stir until dissolved, heat further if necessary When dissolved, transfer with washing to a 100 ml flask Add 2 ml of 40% formalin and then slowly with shaking, add 1 ml of 50% (W/V) acetic acid Make to the mark with water and mix Keep in a well stoppered brown bottle away from light This keeps at least for a year

Uric acid standard for use: Dilute 1 ml of the stock to 200 ml with water Store in a brown bottle Renew every week This contains 0 005 mg Uric acid per ml

Procedure: Add 9 ml of dilute tungstic acid to 1 ml of serum in a centrifuge tube and mix well by inversion Centrifuge for 5 to 10 minutes

	Test (T)	Standard (S)	Blank (B)
	5 ml of Supernatant	5 ml standard	5 ml distilled water
Sodium Carbonate solution	1 ml	1 ml	1 ml
Dilute Phosphotungstic acid	1 ml	1 ml	1 ml

Mix and place them in a water bath at 25°C for 30 minutes Take readings in a photoelectric colorimeter using a red filter at 700 mμ

Calculation: 5 ml of the supernatant is equivalent to 0 5 ml of serum

$$\text{Mg of uric acid per 100 ml of serum} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 0\,025 \times \frac{100}{0\,5}$$

$$= \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 5$$

Discussion:

1 Uric acid is formed endogenously from nucleoprotein metabolism and exogenously from the metabolism of purines taken in the food The normal range of uric acid in serum is **2 to 6.5 mg.** per 100 ml

2 The most important condition in which uric acid level is raised in blood is gout, a disease of the joints Urates are deposited as crystals in the solid form in and around the joints causing swelling and pain The increase in this disease is from 6 5 to 12 mg per 100 ml serum In impaired renal function in which blood urea level is raised, the uric acid level ranges from 4 to 20 mg per 100 ml of Serum In gout only, no blood urea level will rise

3 Increases in serum uric acid are also found in leukemia, toxemia of Pregnancy, lobar Pneumonia, and large abscesses In these conditions, there is excessive breakdown of cells resulting in increased metabolism of nucleoproteins which are oxidized to uric acid in the body

4 In rheumatic conditions in which uric acid is normal, Cortisone tends to produce an increase in serum uric acid

5 Low values are found in Wilson's disease and other cases of the Fanconi Syndrome

6 Uric acid estimations give little information to the Clinician except in cases of gout

ESTIMATION OF SERUM (PLASMA) PROTEINS

(Biuret method)

Principle: This method is based on the sensitive biuret reaction given by all proteins including polypeptides The purple colour produced by reacting protein with dilute copper sulphate in alkaline medium is proportional to the amount of Protein present

Reagents

1 **Sodium sulphate solution (23 68 per cent)**· Dissolve 23 68 grams of anhydrous Na_2SO_4 in 80 ml. water and make upto 100 ml.

2 **Stock biuret reagent**· Dissolve 22 5 grams of Rochelle salt (Potassium sodium tartarate) in about 200 ml of 0 2N NaOH and add 7 5 grams of Copper sulphate stirring continuously until solution is complete. Add 2 5 grams of Potassium iodide and make upto 500 ml. with 0 2N Sodium hydroxide

3 **Buiret solution for use:** Dilute 50 ml of stock buret reagent to 250 ml with 0 2 N NaOH containing 5 grams of Potassium iodide per litre.

4 **Standard solution of Protein** Prepare it from bovine albumin which will contain 0 5 gram protein per 100 ml Measure accurately 2 ml. portions into tubes and store at —20°C until required Take out one tube when required Thaw it and then add 3 ml of buret reagent directly

Procedure·

1 For albumin estimation, precipitate the globulin with sodium sulphate solution and remove it by filtration

To 6 ml. of 23 68 per cent solution of sodium sulphate add 0 4 ml of serum (plasma), cork and gently mix by inversion several times Final sodium sulphate concentration becomes 22 2% At this stage, white turbidity is seen due to precipitation of globulin Keep the test tube at 37°C in a water bath for at least 15 minutes (longer time is preferable) to allow formation of larger aggregate of globulin precipitates Filter through whatman filter paper No 1 to get a clear filtrate which contains albumin

2 For total protein estimation, dilute the serum (plasma) with sodium chloride solution

Take 0 2 ml. serum into 3 ml. of 0 9 per cent NaCl solution, cork and mix gently by inversion several times

3 Dilute 0 2 ml. of the standard with 3 ml of Saline solution.

	Total Protein (T)	Albumin (A)	Standard (S)	Blank (B)
	2 ml. dilute total protein	2 ml. Albumin filtrate	2 ml. diluted standard	2 ml water
Working buret reagent	2 ml	2 ml	2 ml.	2 ml.

Mix and put all the tubes in a water bath at 37°C for 10 minutes Take readings in the photoelectric colorimeter using a green filter at 540 mμ

Calculation·

Grams of total protein in 100 ml of serum (plasma)

$$= \frac{\text{Reading of total protein} - \text{Reading of Blank}}{\text{Reading of Standard} - \text{Reading of Blank}} \times \text{Grams of total protein in 100 ml. of Standard protein.}$$

$$\text{Grams of albumin in 100 ml serum} = \frac{\text{Reading of Albumin} - \text{Reading of Blank}}{\text{Reading of standard} - \text{Reading of Blank}} \times \text{Gram of total protein in 100 ml standard protein}$$

$$\text{Grams of Globulin in 100 ml serum} = [\text{Grams of total protein per 100 ml} - \text{grams of albumin per 100 ml}]$$

$$\text{A G ratio} = \frac{\text{Grams of albumin Per 100 ml}}{\text{Grams of Globulin Per 100 ml}}$$

Discussion·

1 The chief proteins of serum (plasma) are albumin and globulin Fibrinogen is also an additional fraction of the protein of plasma (about 300 mg/100 ml) The total protein in normal serum (plasma) varies from 6 to 8 grms per 100 ml

Albumin	3 5 to 5 1 grams per 100 ml
Globulin	1 8 to 3 1 grams per 100 ml

Normally, the ratio of albumin to globulin lies between 2 1 to 1 2 1

2 Liver cells synthesize albumin A low serum albumin may be due to heavy loss of albumin in the urine, malabsorption of protein from the alimentary canal or decreased formation in the liver owing to liver diseases

3 A decrease in total proteins is always due to a low albumin level accompanied by no increase in globulin Therefore, there is a fall in albumin-globulin ratios

4 An increase in total proteins may occur in dehydration Both albumin and globulin are increased due to hemoconcentration and the albumin—globulin ratio remains unaltered

5 A reduction in total protein occurs in edema in which more albumin is excreted in urine The total protein is reduced with low albumin values in severe hemorrhage, shock, extensive burns, negative nitrogen balance due to increased protein breakdown, acute infectious diseases, untreated diabetes mellitus hyperthyroidism

6 In severe liver disease, although albumin is found to be low with a low total protein, it is more common for the globulin to be increased so that the total protein is normal or increased

7 An increase in globulins may be observed in many chronic infections, rheumatoid arthritis, tuberculosis, Kala azar, etc

ESTIMATION OF SERUM CALCIUM

(Method of Clark and Collip, 1925).

Principle: Serum Calcium is precipitated as Calcium oxalate on the addition of ammonium oxalate Calcium oxalate, on treatment with Sulphuric acid, liberates equivalent amount of free oxalic acid which is then titrated against a standard Potassium permanganate solution in presence of H_2SO_4 The oxalic acid is oxidized to CO_2 and H_2O

$$2KMnO_4 + 3H_2SO_4 + 5(COOH)_2 \rightarrow K_2SO_4 + 2MnSO_4 + 10\,CO_2 + 8H_2O$$

Reagents:

1 Ammonium oxalate solution (4 Per cent)

2 Ammonia solution (2 per cent, V/V) Dilute 2 ml of ammonia to 100 ml with water

3 Stock 0 1 N. Potassium permanganate solution Dissolve 0 3162 grams of $KmNO_4$ in 100 ml distilled water Store in a brown bottle at 4°C

N/100 $KmNO_4$ solution Dilute the stock before use

4 Stock N/10 oxalic acid Dissolve 0 6304 grams of dry oxalic acid in 100 ml. of distilled water

N/100 oxalic acid Dilute the stock ten times before use

5 Sulphuric acid (1N)

Procedure Take 2 ml of serum, 2 ml of distilled water, and 1 ml. of ammonium oxalate solution in a centrifuge tube Mix with the help of a thin glass rod Allow the tube to stand for at least 30 minutes Centrifuge the tube for 10 minutes A small amount of Calcium oxalate can be found deposited at the bottom of the tube Pour away the clear supernatant by inverting the tube and stand it on a pad of filter paper in the inverted position for 5 minutes for the liquid to drain completely

Then wipe the mouth of the tube and run in 5 ml of ammonia solution by the side of the tube Stir the precipitate using the same rod Centrifuge again. Pour off the supernatant and repeat the draining on the filter paper

Add 2 ml of 1 N H_2SO_4 Mix by the same glass rod. Put the tube in water to 70° — 75°C in a beaker to have free oxalic acid completely and titrate the contents with N/100 $KMnO_4$ keeping the tube in the water bath A faint Pink develops at the end point which persists for about a minute Use a microburette for the titration.

For standard, take 1 ml of N/100 oxalic acid and 2 ml of 1N H_2SO_4 and titrate with $KMnO_4$ solution keeping the tube in the water bath at 70°C — 75°C.

Calculation

1 ml of N/100 oxalic acid = 1 mL of N/100 Calcium.

$$= 20 \times \frac{1}{100} = 0\,2 \text{ mg Calcium.}$$

If X ml of Permanganate is required to titrate the oxalic acid liberated from Calcium, and 'Y' ml for 1 ml of N/100 oxalic acid,

$$\text{Calcium present in 2 ml Serum} = \left(\frac{X}{Y} \times 0\,2\right) \text{mg}$$

$$\text{Calcium present in 100 ml Serum} = \left(\frac{X}{Y} \times 0\,2 \times \frac{100}{2}\right) \text{mg}$$

$$= \left(\frac{X}{Y} \times 10\right) \text{mg}$$

Discussion

1 Blood calcium exists in three forms—ionized (diffusible), Protein bound, and complexed with citrate (non-diffusible) Total calcium is required for bone and teeth formation but ionic calcium is important for neuro-muscular irritability, blood clotting and tissue permeability

2 Normal serum calcium ranges from 9 to 11 mg per 100 ml. (4 5 to 5 5 m Eq/L) In children, the normal value tends to be towards the upper limit of the range

3 Serum calcium level is influenced by the level of its absorption from the intestine, by alterations in the amount of Parathyroid hormone, and changes in serum inorganic phosphate and serum proteins

4 Lower values are found in hypoparathyroidism, rickets, osteomalacia, steatorrheas and renal failure

5 Higher values are observed in hyperparathyroidism, hypervitaminosis D, and in myeloma (raised plasma protein level)

ESTIMATION OF SERUM CHOLESTEROL

(Sackett's method)

Principle: Cholesterol of serum is extracted by ether alcohol mixture The extract is evaporated and cholesterol is dissolved in chloroform Cholesterol then reacts with acetic anhydride and sulphuric acid developing a green colour (Libermann—Burchard reaction) which is proportional to the amount of cholesterol present

Reagents:

1 Alcohol (Rectified spirit)

2 Ether

3 Chloroform

4. **Stock Standard solution of Cholesterol:** Dissolve 50 mg of Pure cholesterol in chloroform and make upto 25 ml with chloroform Standard cholesterol for use(5 ml = 0 4 mg of cholesterol) Dilute 1 ml of the stock standard to 25 ml with chloroform

5 Ethanol—ether mixture Mix 3 volumes of ethanol and 1 volume of ether

6 Acetic anhydride sulphuric acid mixture Mix 20 ml of acetic anhydride with 1 ml of concentrated sulphuric acid Make freshly just before use

Procedure: Take 10 ml of ethanol ether mixture in a centrifuge tube and add 0 2 ml serum to it Cork the tube tightly and shake vigorously Allow the tube to lie horizontally so that the precipitate is fairly distributed evenly along the tube Stand for 30 minutes and then centrifuge for a few minutes to get a firm deposit Decant the supernatant completely into a suitable test tube Evaporate to dryness in a water bath

Dissolve the residue in 5 ml of chloroform Into another tube take 5 ml of the standard cholesterol Add 2 ml of acetic anhydride sulphuric acid mixture to each of the tubes Mix and stand in the dark at 25°C for 15 minutes In the blank, take 7 ml chloroform

Take reading using a red filter at 680 *Mμ*

Calculation:

$$\text{Mg of cholesterol per 100 ml serum} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 0\,4 \times \frac{100}{0\,2}$$

$$\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 200$$

Discussion:

1 Cholesterol, a derived lipid, is an important constituent of blood It exists in free and esterified form in blood

2 The normal range of cholesterol in adults is from 150 to 240 mg per 100 ml The value rises with age more in men than in women In middle age, the value is raised The value does not alter after meals In pregnancy, the value rises by 20 to 25 per cent of the normal value

3 Hypercholesterolemia is found most characteristically in nephrosis, diabetes mellitus, obstructive jaundice, myxoedema, and in Xanthomatosis Values upto 400 and 500 mg Per 100 ml are commonly found in diabetes melli-

tus when treatment is inadequate In myxoedema, values upto 500 to 700 mg per 100 ml are seen which gives a valuable help in diagnosis Thyroxine administration causes a marked fall in serum cholesterol

4 The effect of hyperthyroidism is to reduce the serum cholesterol as low as 80 to 100 mg per 100 ml Sometimes in hemolytic jaundice, the value falls below 100 mg per 100 ml in some cases Similar findings may be seen in malabsorption syndrome, in severe wasting, in acute infections

ESTIMATION OF SERUM BILIRUBIN

(Lathe and Ruthven, 1958)

(*Method of Malloy and Evelyn modified)

Principle: Serum is diluted with water and methanol added in an amount insufficient to precipitate the proteins, yet sufficient to permit all the bilirubin to react with the diazo-reagent developing colour This colour is proportional to the amount of bilirubin present

Reagents

1 Absolute methanol

2 Hydrochloric acid (1 5 per cent, V/V with water)

3 **Diazo-reagent**· Prepare freshly before use by adding 0 3 ml of solution B to 10 ml of solution A

Solution A: Dissolve 1 gram of sulphanilic acid in 15 ml of 0 2 N HCl and make upto 100 ml with water

Solution B: Dissolve 0 5 gm of sodium nitrite in water and make upto 100 ml Prepare freshly at frequent intervals

4 Standard solution of Bilirubin Prepare a solution containing 10 mg per 100 ml chloroform It may be necessary to reflux the mixture gently to dissolve the bilirubin.

Procedure: Add 0 2 ml serum into 5 4 ml of water in a test tube and mix. Pipette 2 8 ml of this into a second test tube for use as the blank.

To the test add 0 7 ml of diazo-reagent and to the blank 0 7 ml of Sulphanilic acid solution Mix, stand for 5 minutes and read using green filter at 540 *mμ* This gives the *conjugated bilirubin.*

To obtain the *total bilirubin* add 3 5 ml of methanol to each tube and read again after standing 5 minutes

For *standard* add 0 2 ml of bilirubin standard to 3 5 ml methanol Then add 0 7 ml of diazo-reagent and after mixing 2 6 ml of water and read against a water blank after 5 minutes at 540 mμ

Note· For values above 15 mg per 100 ml , 0 1 ml of serum and 5.5 ml of water can be used

Calculation

$$\text{Mg conjugated bilirubin per 100 ml serum} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 10 \times 1\,05$$

conjugated bilirubin has a lower extinction in water, so the factor 10 5 has to be inserted

$$\text{Total bilirubin} = \left(\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 20\right) \text{mg}$$

The difference of the two gives the indirect bilirubin

Discussion:

1 The serum bilirubin of most normal persons appears to be in the range of 0 2 — 0 6 mg per 100 ml serum

2 Serum bilirubin determination gives a measure of the intensity of the jaundice Higher values are found in obstructive jaundice than in hemolytic jaundice In obstructive jaundice when the obstruction is complete, the serum may reach about 20 mg per 100 ml Values between 20 and 30 mg Per 100 ml may sometimes be reached in acute infective and toxic hepatitis

3 In pernicious anemia, the serum bilirubin rarely exceeds 3 mg per cent In the chromic hemolytic anemias, increases are also small and reaches upto 10 mg Per 100 ml

4 In sub-clinical jaundice, serum bilirubin increases between 1 and 3 mg per 100 ml which is of diagnostic value

5 In obstructive jaundice, if the obstruction is due to some malignant growth, the serum bilirubin rises to a high level and remains almost constant at that level

6 Determination of serum bilirubin is useful in jaundice of the newly born High values upto 30 to 40 mg Per 100 ml may be reached especially in babies who have developed Rh antibodies

ESTIMATION OF CREATININE IN BLOOD

Principle Same as for creatinine in urine

Reagents·

Working standard: 1 ml of the stock solution (mentioned in urine creatinine) to 500 ml (1 ml — 0 002 mg creatinine), other reagents are the same as in creatinine in urine

Procedure·

	Unknown (U)	Standard (S)	Blank(B)
	5 ml Folin-WU filtrate	5 ml Standard	5 ml water
Picric acid	2 ml	2 ml	2 ml
NaOH	0 5 ml	0 5 ml	0 5 ml

Allow to stand for 15 minutes and take readings in a photoelectric colorimeter using green filter at 540 mμ

Folin WU filtrate is prepared by taking 1ml of oxalated blood, 7 ml water, 1 ml sodium tungstate (10%), and 1 ml of 2/3 N H_2SO_4

Calculation:

$$\text{Mg of Creatinine present in 100 ml serum} = \frac{\text{Reading of Unknown}}{\text{Reading of standard}} \times 0\,01 \times \frac{100}{0\,5}$$

$$= \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 2$$

Discussion:

1 Creatinine is the least variable nitrogenous constituent of the blood The normal range varies from 1 to 2 mg per 100 ml serum (90 to 175 m mol/L)

2 Values from 2 to 4 mg per 100 ml are noted in early nephritis, and in chronic hemorrhagic nephritis with uremia, values from 4 to 35 mg Per 100 ml serum are seen

3 Creatinine is more readily excreted by the kidneys than urea or uric acid and an increase of creatinine to 4 or 5 mg or over per 100 ml is the evidence of marked impairment of kidney function Such high creatinine values in chronic hemorrhagic nephritis indicate an unfavourable prognosis

Questions to answer

1 Discuss the principle for photoelectric colorimeter

2 Why is photoelectric colorimeter termed as absorptiometer and how will you select filters?

3 State the operation of photoelectric colorimeter, the importance of blank, and name the types of photoelectric colorimeter

4 What precautions will you take to avoid hemolysis of blood

5 Name the method and state the principle and procedure of estimation of creatinine in urine

6 State the conditions in which creatinine excretion is increased

7 Discuss the principle for the estimation of phosphate in urine and the conditions in which phosphate excretion is increased.

8 Name the method and state the principle of estimation of urea in urine

9 State the composition of Benedict's quantitative solution and why is pumice stone added in the procedure of the estimation of reducing sugar in urine? Discuss the principle of Benedict's method for estimation of reducing sugar in urine

10 What precautions should be taken in the estimation of chloride in urine? Name the method and state the principle for the chloride estimation in urine

11 Discuss the precautions to be taken in the estimation of Vitamin C in urine

12 State the procedure for the gastric analysis What do you mean by hyperchlorhydria hypochlorhydria and achlorhydria?

13 Discuss the liver function tests

14 Name the method for the estimation of true glucose in blood What is the normal level of fasting true glucose in blood? Under what condition blood glucose is increased and decreased?

CHAPTER 14

Kidney function test

Kidney functions in the elimination of waste products and toxic substances from the body It regulates water balance and maintains normal crystalloid and colloid osmotic equilibrium between plasma and tissues It also maintains normal acid base equilibrium of the body

The following tests are required to be done to assess the kidney function under diseased conditions

1 **Concentration test:** This is based on the determination of the concentrating power of the kidney

The patient is given high protein content meal with 200 ml of fluids at 6 P M in the previous day No food or drink is allowed till the test is completed Empty the bladder at the bed time Discard this urine and any other sample passed in the night Collect the urine sample at 8 a m and 10 a m separately Determine the specific gravity of each sample

In case, the kidneys are normal , at least one specimen shows a sp gr, above 1022 often reaching 1032 In hypofunction, the sp gr is nearer 1010

2 **Dilution test (Water test)**· In presence of edema this test should not be done

The bladder is emptied early in the morning and urine discarded and breakfast not allowed The patient is advised to drink 1200 ml of water in 20 to 30 minutes (at 8 a m) The hourly urine samples are collected from 9 a m to 12 noon Measure the sp gr of each sample

In normal persons, the water elimination is 1200 ml in 4 hours The sp gr of at least one sample is 1002 to 1003 Water elimination is significantly decreased and the sp gr falls below 1010 in impaired state of kidney Renal or extra renal (edema or cardiac failure) may be the cause of the decreased output If the concentrating power is normal, the cause must be extra renal

3 **Urea clearance test**· This test is a sensitive index of renal function in children as well as in adults Under normal conditions urea excretion is constant and the blood cleared of urea per minute serves as a measure of the kidney function

In the normal adult, the urea excretion is directly proportional to the level of urea in blood when 2 ml or more is excreted per minute This is called maximum clearance (Cm) If the urine output is less than 2 ml per minute, it is clear that reabsorption of urea by the tubules is increased When the urea excretion is diminished and varies with the square root of output of urine per minute, it is called standard clearance (Cs)

Procedure: A light breakfast without tea or coffee is given on the day of test Drug therapy is stopped a day before The patient is advised to empty his bladder Note the time Allow him to drink 500 ml of water to have free flow of urine Collect blood for urea estimation at about 50 minutes At 60 minutes collect all the urine Measure the volume Ask him to drink about 500 ml of water more to drink At 120 minutes collect separately all the urine and measure the volume Estimate the blood urea nitrogen (urea) and urine urea nitrogen (urea) of the two urine samples

Calculation

$$Cm = \frac{U \times V}{B} \text{ ml} \qquad Cs = \frac{U \times \sqrt{V}}{B} \text{ ml}$$

whereas, U = mg. of urine urea nitrogen Per 100 ml.

V = urine volume per minute in ml.

B = Mg. of blood urea nitrogen per 100 ml.

The clearance may be expressed in ml. but generally it is expressed in percentage of the normal clearance. The values for average normal Cm is 75 ml. (100%) and for Cs is 54 ml. (100%).

$$\therefore \text{ Percent of Cm} = \frac{U \times V}{B} \times \frac{100}{75} = \frac{1{\cdot}33UV}{B}$$

$$\therefore \text{ Percent of Cs} = \frac{U \times \sqrt{V}}{B} \times \frac{100}{54} = \frac{1{\cdot}85U\sqrt{V}}{B}$$

If the surface area of the patient varies widely from the normal (1.73 sq m.), then the true 'V' is obtained by multiplying the observed 'V' by 1.73/A. Where A is the surface area in sq. m. This correction is necessary particularly in case of children.

Discussion: The clearance test is of great help in following the progress of advanced cases The elimination or deposition of edema fluid does not affect the test, so it is done in such cases.

Normal clearance in unimpaired kidney function is 75% or above (in Indians 40—70%). If all extra renal causes are excluded, a clearance of 10% or less indicates severe kidney damage.

The urea clearance test serves as an indicator of glomerular function. If the clearance value falls, it is the indication that the glomeruli is destroyed. The concentration tests are very sensitive to slight degree of renal damage but they fail to show differences between moderate and severe renal impairment. If the concentration test is normal, further studies are not necessary. Urea clearance test assures whether the decreased excreting power is serious or not. Excretory power and clinical condition follow the clearance test but not the specific gravity.

CHAPTER 15

Electrophoresis, Chromatography and p^H

Paper electrophoresis of serum protein

Introduction: Electrophoresis is a method by which electrically charged particles of a mixture of substances are separated under an electric field The positively charged particles migrate towards cathode, and the negatively charged particles towards anode The rate of migration depends on the number of charges each particle carries As a result of different rates of migration, a mixture of proteins (such as plasma) can be separated into a number of fractions having similar mobility

Tiselius (1937) first performed electrophoresis in a fluid electrolyte In this method, the differently charged particles moved inside the buffer This method was called "moving boundary electrophoresis' Few workers only used this method because of the costly and complicated apparatus

Consden, Gordon, and Martin (1946) introduced filter paper to hold the electrolyte in the electrophoretic technique Filter paper was the first anticonvection medium to be widely used Later on, other media such as cellulose acetate, agar gel, starch gel, starch block and acrylamide gel have been used in place of paper with better results

Principle: A small amount of serum is applied on a whatmann filter paper No 1 strip previously soaked in buffer solution, the two ends of the strip drip in two compartments of an electrophoresis tank containing the buffer solution A direct current is applied to the ends of the paper for a desired period During this time different fractions of the protein mixture separate into bands depending upon the amount of charge, the shape and size of the protein particles After that electric current is disconnected, paper is removed from the tank, dried and stained with a protein stain to demonstrate different protein bands The fastest band is albumin, behind it are α_1-globulin, α_2 globulin, β globulin and γ globulin (shown in fig 15 2) The stained fractions are eluted in suitable solvent and the eluted colour is measured in a colorimeter

Two types of electrophoretic tanks are available

1 Vertical and 2 Horizontal

1 In the vertical type, there are five compartments The electrodes are present in two outermost compartments, which is connected to adjoining compartments by wicks By this arrangement the p^H change occuring in the electrode

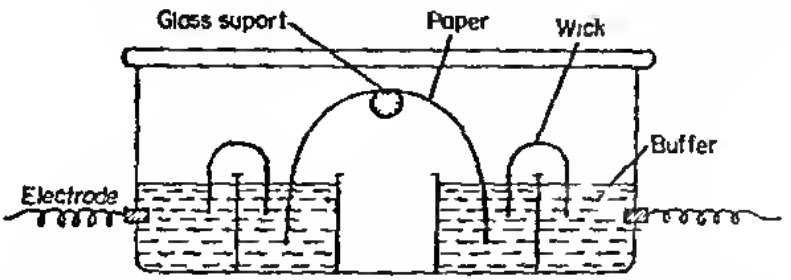


Fig 15 1 A Vertical electrophoretic tank

compartment is prevented from affecting the adjoining compartments in which the ends of the paper dip In the middle, there is a central compartment The arrangement is shown in fig 15 1 All compartments excepting the central one are filled by buffer

2 In the horizontal type, paper lies in horizontal position supported by strings The paper ends dip in two compartments on the two sides of the tank These compartments are connected by means of wicks to adjoining compartments containing electrodes *Buffer* is kept in all compartments to equal levels.

Procedure. Pour sufficient buffer into the four compartments upto the same level, leaving the central compartment empty Cut Whatmann No 1 paper into strips of 35 cm long and 5 cm. wide. Mark the centre of the strip with a pencil and mark the point of application of sample by another line 2 5 cm. in length parallel to and 5 cm. from the mid line (for the vertical tank) or 7 5 cm. away from the mid line for the horizontal tank. Now apply the paper in such a way that the two ends of the paper dips in the two buffer compartments adjoining the electrode compartments Replace the lid of the tank. In about 2 hours time the whole paper is soaked with the buffer Now apply 10μl of the serum (or plasma) by a micropipette on the line marked for sample application. Replace the paper in its place and cover the tank At a time, several such strips can be set up for different samples Now connect one electrode with the cathode and the other with anode of a direct current with constant voltage. The electrode nearer the site of application is connected to the cathode. Switch on the current and allow the electrophoresis to run for 16 to 18 hours A voltage gradient of 3 2 volts per cm length of the paper, or a current of 1 5 to 2 0 milli amps for each 5 cm wide strip is suitable At the end of the desired length of time, first disconnect the instrument from the mains, remove the strip from the tank, and dry it at 100 C to 110°C for 30 minutes

Staining Pour sufficient stain in a large size glass or enamel tray and put the dried paper strips in it for at least 6 hours (overnight staining is always preferable) After removing from staining tray, wash for 6 minutes each time in two changes of wash solution, then in the fixative solution for a further 6 minutes. Dry the paper in air or in an oven at 100°—110°C.

After staining 5 bands (fig 15 2) corresponding to albumin, α_1-, α_2 , β, and γ-globulins are noticed It is possible to guess from the electrophoretogram if some protein fraction is absent, present in decreased or increased amount, or some extra band such as myeloma protein is present

However, the relative amount of different fractions can be accurately estimated by scanning the paper under photoelectric scanner or different bands are cut and eluted in 6 ml. of 0 01 N NaOH for 30 minutes These eluted coloured solutions are read in a photoelectric colorimeter at 540 mμ

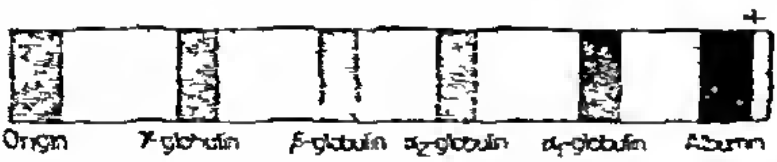


Fig 15.2 Serum protein electrophoretogram

Reagents·

1 **Buffer (p^H 8 6, ionic strength 0 083 M)** Dissolve 3 12 grams of diethylbarbiturate in water and make upto 1 litre.

2 **Dye solution:** Add 25 ml. of 95 per cent ethanol to 0 1 gram of bromphenol blue and 50 grams of $ZnSO_4$. Mix well, add 5 per cent acetic acid (V/V), mix to dissolve and make upto a litre with further 5 per cent acetic acid

3 Wash solution Acetic acid (5 per cent, V/V)

4 Fixation solution Acetic acid (5 per cent) containing 0 3 per cent of sodium acetate (CH_3COONa, $3H_2O$)

TABLE

Electrophoretic pattern changes in some conditions

'—' mark indicate no change

Disease	Albumin	α_1 globulin	α_2 globulin	β globulin	γ globulin
Normal (gm/100ml)	3 5 to 5 5	0 1 to 0 4	0 4 to 0 8	0 5 to 1 0	0 7 to 1 5
Infancy	—	high	high	—	—
Pregnancy	Low	high	high	high	—
Acute infection	—	high	high	—	—
Chronic infection	low	high	high	—	high
Cirrhosis	low	high	—	—	high
Nephrotic syndrome	low	high	very high	—	low
Myeloma tosis	—	—	—	—	high
Hypogamma globulinemia	—	—	—	—	Very low

Separate band occassionally seen in α and β positions

PAPER CHROMATOGRAPHY OF AMINO ACID MIXTURE

Introduction In biology and chemistry it is most often necessary to separate components of a mixture which are very similar and are difficult to separate by chemical or physical methods Chromatography and electrophoresis are two powerful modern methods utilised for such purpose

Tswett (1906) the Russian biologist, first appreciated the possibilities of chromatography and he put the term 'Chromatography Consden Corden and Martin (1944) described paper chromatography in which separations were done mainly by partition

Principle The separation of components of a mixture by a chromatographic system depends on multiple partition process Small differences in partitioning of each component of a mixture are multiplied many folds The greater such differences, the greater is the ease of separation

A small drop of solution containing the mixture of compounds is put on a strip of filter paper and allowed to dry A suitable solvent (mixture of two solvents) is allowed to flow along the filter paper over this spot The substances in the initial spot are extracted by the flowing solvent and carried forward along the filter paper to a distance which appears related to their partition coefficient between the free and bound solvent phases of the filter paper After the solvent has run for a suitable distance along the paper, the paper is removed dried and subjected to suitable tests to locate the various compounds

Rf value is defined as the ratio of the distance travelled by the component to the distance covered by the solvent Rf value depends on the nature of the solvent the temperature, and the presence of other substances

Procedure Whatmann filter paper is cut into 35×15 cm sheet A pencil line is drawn about 3 cm above the shorter edge of the paper and 5 points are

marked at equal spacing leaving 2 5 cm from the two edges On the middle point, a mixture of four amino acids is applied with the help of a fine capillary It is dried with the help of hot air blown by a hair dryer Again, another small quantity of the mixture is spotted at the same place and dried This process is repeated 2 to 3 times more On the other points, the individual amino acid of the mixture are similarly applied The positions of these amino acids are marked with a pencil

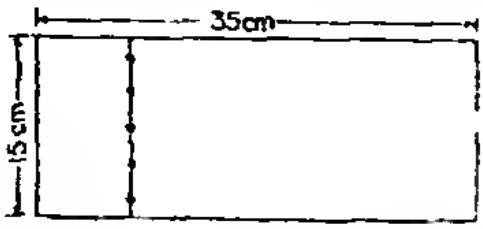


Fig 15 3 Serum protein electrophoretogram

The solvent for developing the chromatogram is a mixture of n butanol acetic acid water (12 3 5) respectively This mixture is freshly prepared

If a chromatographic tank is not available, specimen jar (about 40 × 15 cm) with fitting lid can very well serve the purpose In such a jar, put about 100 to 150 ml of the solvent mixture and replace the lid so that the lid is air tight In case of doubts, apply vaseline to the lid to avoid leakage In an hour, the inside atmos phere will be saturated with the solvent vapour Now fold the paper in which sample has been applied in the shape of a cylinder and tie the opposing ends of the paper with staples or thread Open the lid and place this folded paper in upright position in the jar, the pencil line lower most and about a centimeter above the solvent (fig 15 4) Replace the lid The paper should stand absolutely vertically Leave the chromatogram to develop for 10 to 15 hours or earlier if the solvent has ascended quite near the upper margin of the paper

Take out the paper at the desired time, cut the stitches and let it dry com pletely in the air

After the paper has dried thoroughly, the location reagent (0 2 per cent ninhydrin in acetone) is sprayed uniformly on the paper with the help of an all glass sprayer The paper is then allowed to dry first in the air and then in a hot air oven at 105°C for 3 minutes Purple coloured amino acid spots are seen on the chromatogram

Identify the amino acids in the mixture with the help of spots produced by known amino acids The R_f values of the amino acids can be calculated as

$$R_f = \frac{\text{Distance travelled by particular amino acid}}{\text{Distance covered by the solvent from the point of origin}}$$

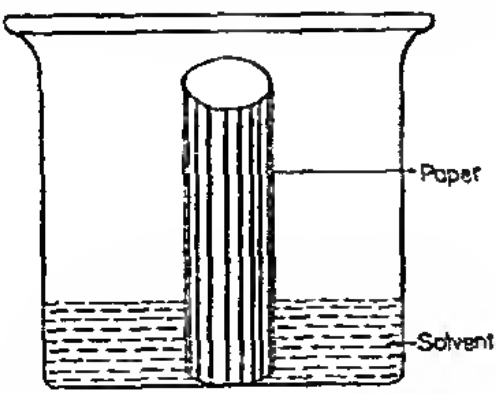


Fig 15 4 The chromatographic jar containing the solvent and the paper

Reagents:

1 N butanol (Chromatographic grade)

2 Glacial acetic acid

3 Solvent mixture of butanol, acetic acid, and distilled water in the proportion of (12 3 5)

4 Ninhydrin 0 2 per cent solution of ninhydrin in acetone It is prepared just before use

THIN LAYER CHROMATOGRAPHY(TLC)

Introduction: In recent years, thin layer chromatography has been developed This technique consists of a thin layer of absorbents (silica gel, alumina, Kiselguhr or cellulose) on a glass plate or plastic sheet Since absorbent is used, it is also termed as absorption chromatograpby This technique also provides superior results than that of paper chromatography The spots are more compact with better resolution and the run is comparatively of shorter duration Therefore, quicker run is possible

Preparation of plates: Chromatographic plates (20×20 cm) of 200μ thickness are prepared by using a suspension of 30 grams of silica gel G in 63 ml of 0 1 M Na_2CO_3 solution by shaking vigorously for 90 seconds Only these plates are used which appear to be uniform in both transmitted and reflected light These plates are activated at 110°C for 30 minutes immediately prior to use

Procedure Samples (5—100μL) are applied as a spot of less than 5 mm diameter on the lower right corner of the plates under a stream of warm air Plates are first developed in a standard Brinkmann developing chamber previously saturated with the vapour of the solvent mixture with chloroform methanol acetic acid water (250 74 19 3, V/V) When the solvent front migrates about 15 cm , plates are dried in air for 15 minutes and develop in the second dimension (90° rotation clockwise) with chloroform methanol 7 M ammonium hydroxide (230 90 15 , V/V) The solvent front is again allowed to move about 15 cm

Developed plates are then dried in air for 5 minutes and exposed to iodine vapour in a sealed chamber for 30 to 60 seconds The pale yellow areas are quickly outlined using a dental probe and the plates are exposed to air until the iodine has evaporated from the spots When a permanent record of developed plates is desired, plates are sprayed lightly with 10 N H_2SO_4 and then heated at 110°C for 15 minutes

The silica gel in each spot is scraped with the aid of a sharp edged poly ethylene blade on paper The weighing papers are then transferred to a 12 ml conical centrifuge tube and eluted by different solvents for estimation by photoelectric colorimeter

Discussion: The constituents of the mixture of amino acids, and the constituents of neutral lipids and phospholipids are separated and estimated in a short time

DETERMINATION OF P^H

The electrometric method is the most accurate method for the determination of P^H of a solution This method measures the electromotive force set up due to the existence of H^+ and OH^- in the solution The instrument which measures P^H in this manner is known as P^H meter

The other method is the colorimetric method which is also fairly accurate and can be used in the absence of a P^H meter The basis of it is that certain organic dyes (indicators) change colour with change in P^H Each indicator in its ionised state gives one colour and in unionised state gives another colour

Each indicator has its useful range of P^H within which it can indicate change in P^H by change of in colour and beyond which there is no further change in colour

It is possible to prepare an indicator whose p^H range covers almost the entire p^H scale Such an indicator is known as 'Universal indicator' The universal indicator enables us to determine the approximate p^H of a solution and thus helps us to choose an appropriate indicator and buffers for subsequent more exact determination of p^H of the solution

Principle: When equal volumes of the unknown solution and a series of standard buffer solutions with closely varying p^H values are treated with a suitable indicator, the colour of the unknown solution will be found to match with one of the standard buffer solution The p^H of the unknown is, therefore, the same as the p^H of the buffer with which its colour matches

Permanent standard made from glass discs of the appropriate colour and shade for each indicator may also be used in place of the standard buffer and indicator Hellige's comparator with its permanent standard discs made of col oured glass is a very convenient instrument for this purpose

p^H determination of a colourless solution: First determine the appro ximate p^H of the solution with the help of a universal indicator and colour chart Take a small piece of universal indicator paper and just dip its one end in the given solution After about 30 seconds compare the colour of the paper with the standard colour chart provided and know the approximate p^H If universal in dicator solution is to be used in place of paper, put a drop of it in about 2 ml of the solution and compare the colour of the solution with that of the colour chart After having known the approximate p^H of the solution, choose from the set of indicators an indicator which more closely covers the p^H range of the solution under examination Then add a drop of this indicator to approximately 2 ml of the solution, and match the shade of the colour produced against the series of coloured standard buffers The standard colour solutions are prepared by adding one drop of the same indicator to 2 ml of each of the buffer solutions (chosen in the p^H range of the unknown) The p^H of the unknown solution is that p^H of the buffer with which the colour matches In case, the colour of the unknown is intermediate between two colours produced by two consecutive buffers, the p^H of the unknown lies between the two known standards The accuracy of this method can be in creased by employing set of buffer solutions with closely differing p^H values

p^H determination of coloured solution (or Urine): Place a test ube containing plain urine or coloured solution in front of the coloured buffer standards View the two test tubes together and then match this against the urine or coloured solution containing the same indicator in front of which a test tube containing water is to be placed The use of a comparator makes the matching easier, otherwise, a double rowed test tube rack is also convenient

Reagents

1 Set of indicators

2 Standard buffer solutions

Electrometric method of p^H determination·

The switch of the instrument is put on and after a few minutes when the instrument is warm enough, the pointer is adjusted to 0mv or 7 0 p^H position by *set zero* control A standard buffer solution is taken in a clean glass or polythene beaker Electrodes are lowered so that they are immersed in the solution to a depth of about one inch The temperature of the buffer solution is measured by a thermometer and the instrument is again adjusted by *temperature compensate* control to this value The pointer is again set at 7 0 p^H by means of *set control* The *Selector* switch is now turned to proper range i e , 0—7 or 7—14 Pointer will now move to

show the p^H of the buffer and by the help *set buffer* control, the pointer is set to the exact p^H value of the buffer Now the p^H meter is standardized and is ready for determination of p^H of any given solution

To measure the p^H of a given solution, the *selector* is brought back to *Zero* position and the electrodes are cleaned by distilled water The given solution is taken in a clean beaker and the electrodes are immersed in it *Selector* switch is now put on expected p^H range and the reading of the pointer on the p^H scale is noted This value is the p^H of the given solution

Qualitative tests of some substances

Aldehyde

1. **Silver mirror test:** To 1 ml of $AgNO_3$ add 4 drops of NaOH and then ammonia drop by drop until the precipitate dissolves Then add 1 ml of acetaldehyde and shake briskly Bright mirror, due to the reduction of $AgNO_3$ develops on the side of this test tube

2 **Reduction of Fehling's solution:** To 3 ml of acetaldehyde solution add an equal volume of Fehling's solution Heat, brick red ppt of Cu_2O is formed due to the reduction of alkaline copper sulphate

(Formaldehyde gives both of these tests positive)

Acetone

1 **Iodoform test:** To 1 ml of acetone add about 5 grams of Na_2CO_3 Add 5 ml of iodine solution (10%) The iodine solution should be added till no further decolouration occurs Crystals of iodine separates out even in cold

2 **Rothera's test:** Mentioned in acetone detection in abnormal urine

Formic acid

1 **Silver mirror test** Same as in aldehyde

2 To 3 ml of formic acid solution add little mercuric chloride solution and warm white precipitate is formed On boiling the precipitate turns grey

Acetic acid

1 Add a few drops of 1% $FeCl_3$ to dilute acetic acid solution Light red colour develops

2 To 1 ml glacial acetic acid add 4 drops of conc H_2SO_4 and 1 ml of absolute aochol Boil carefully Observe the fruity, fragrant odour of the exter ethyl acetate

Glycerol

1 Mix together 1 ml of $CuSO_4$ and 5 drops of glycerol Add 5 drops of NaOH A clear blue solution is obtained Boil the solution, no reduction occurs, because glycerol is soluble in the mixture

2 Heat 2 drops of glycerol with a pinch of sodium hydrogen sulphate Acrolein is formed Note its characteristic pungent odour

3 To 5 ml of Borax solution (0 5%) add 4 drops of phenolphthalein indicator A bright pink colour is produced Add 2% glycerol solution drop by drop until the pink colour just disappears Boil the solution, the pink colour re appears

Cholesterol

1 Salkowski's test
2 Leibermann—Burchard reaction } See both tests in lipid chapter 2 in cholesterol detection

Urea

1 Specific urease test
2 Buiret test

{ See both tests in detection of urea of normal urine

Uric acid

1 Murexide test
2 Benedict's uric acid test

{ See both test in detection of uric acid of normal urine.

3 Silver reduction test Moisten a strip of filter paper with $AgNO_3$ solution and add to it a drop of dilute uric acid solution in 2% Na_2CO_3 A black or yellowish brown stain of reduced silver is immediately formed.

4 To 5 ml of uric acid solution in NaOH or KOH add 0 5 ml of Nylander's reagent and heat to boiling No reduction takes place.

Ethyl alcohol

1 To 1 ml. of absolute alcohol add 0 5 gram of anhydrous Na_2CO_3 and 5 ml of iodine solution (10%) Warm in a water bath at 70°—80°C until it is decolourised Iodoform separates out as a yellow precipitate on cooling

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PRACTICAL