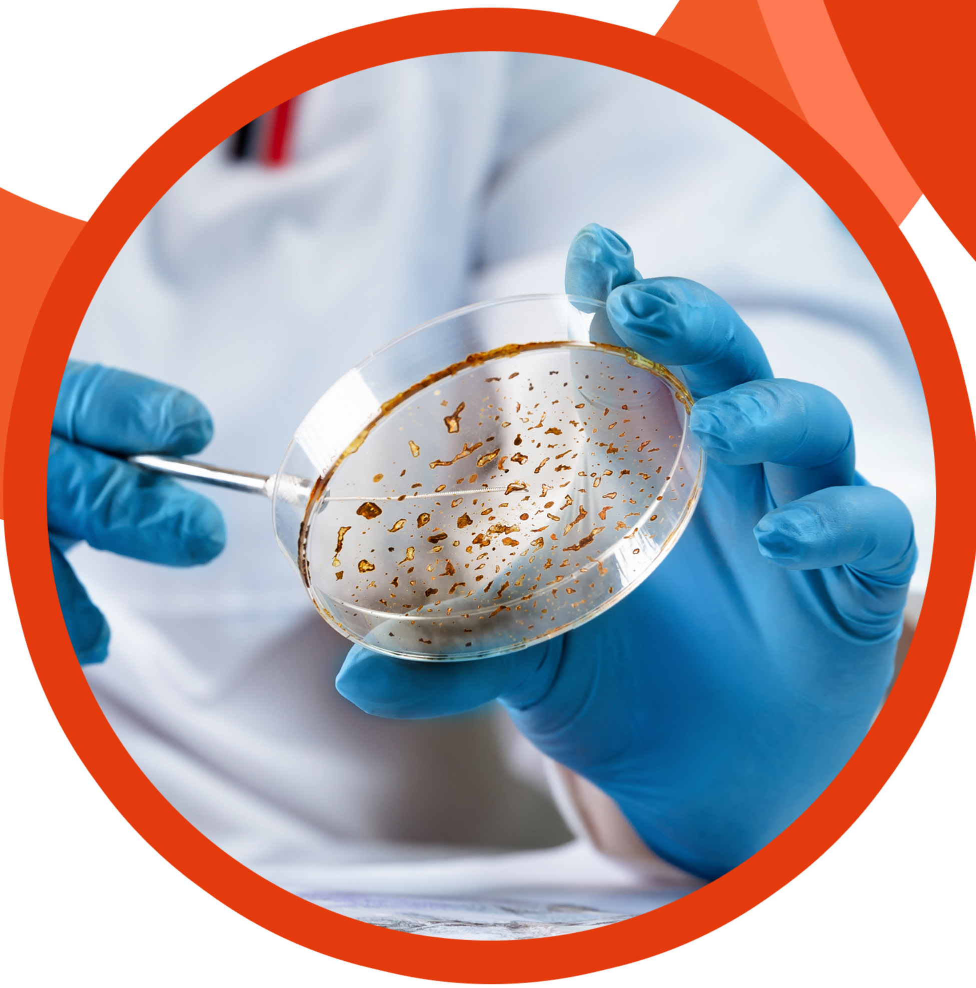
MIND MAPS IN CLINICAL BIOCHEMISTRY



Simmi Kharb

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Mind Maps in Clinical Biochemistry

Author by

Simmi Kharb
Pt.B.D. Sharma PGIMS
Rohtak, Haryana
India

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Author: Simmi Kharb

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PREFACE

The Biochemistry for Paramedical Courses textbook is prepared strictly according to the major university syllabus. This book is a concise easy-to-follow, and step-by-step format that will aid the paramedical course students to learn biochemistry concepts easily. There are sixteen chapters where biochemistry is discussed in terms of basics, chemistry, and metabolism of biomolecules; nutrition and vitamins; acid-base and electrolyte balance; basic principles of biochemistry investigations (in terms of instrumentation, methods, and applied aspects) and organ function tests are discussed in an easy-to-follow format with diagrams and tables.

This book would assist the students to acquire knowledge of the normal biochemical composition and functioning of the human body, and its alterations in disease conditions, and to apply this knowledge in their practice.

Simmi Kharb
Pt.B.D. Sharma PGIMS
Rohtak, Haryana
India

CHAPTER 1

Basic Biochemistry

Abstract: The eukaryotic cell is a complex structure capable of replication, carrying out reactions to maintain its intracellular environment and protect it from surrounding environmental hazards.

Keywords: Prokaryotic cells, Eukaryotic cells, Subcellular components, Plasma membrane, Active and passive transport.

INTRODUCTION

A cell is the smallest component of life that can exist independently. The cell is the structural and functional unit of life. The human body is composed of 100 million (1010) cells. Within the cell, most chemical reactions take place.

Molecular and functional organization of a cell and its subcellular components

1.1. CELL NUMBER, SHAPE, AND SIZE

A- Applied Aspects:

Disorders associated with cell number:		
Cell No.:	*WBC*	*RBC*
Decreased: Tissue degeneration	*Low white blood cell count:* *Causes:* Viral infections Congenital disorders Cancer	*Low red blood cell count:* *Causes:* Anemia: Vitamin B_6, B_{12} or folate deficiency Cancer and cancer treatment Sickle cell disease or thalassemia

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		Blood loss: internal bleeding, severe heart, liver disease or malnutrition, bleeding tumor
Increased: Cancer *Hyperplasia:* noncancerous growth: hyperplasia of the endometrium lining of uterus during pregnancy *Callus*	*High WBC count:* Infections, *Drug reactions, Bone marrow disease, Immune system disorder,* Autoimmune disorders, Leukemia, lymphoma, Drugs *e.g.*, corticosteroids and epinephrine	*High RBC count:* *Causes:* *Primary:* Polycythemia vera: due to Abnormally increased red cell production in the bone marrow *Secondary:* due to chronic hypoxia or tumors releasing erythropoietin
Multiple nuclei: Occur in cancer cells		
***Parasites**:* Plasmodium spp. or Babesia spp. seen on peripheral blood smear: they invade RBCs		
Loss of size control: Cancers, diabetes. *Defects in cell size are associated with diseases:* Lhermitte–Duclos disease where increased cerebellar granule cell size leads to seizures and eventual death.		

Deposition of matter in cell: Amyloid accumulation, Hemosiderosis, Hemochromatosis

Hemosiderosis: iron deposition in reticuloendothelial cells of spleen and bone marrow and Kupffer cells of liver

Hemochromatosis: iron deposition in parenchymal cells of liver, pancreas, heart, *etc*.

B- Basics:

Key features of cell:

1. Cell: structural and functional unit of life

2. Most chemical reactions take place within cells.

3. Types of cells: Prokaryotic cell, Eukaryotic cell

The human body is composed of 10^{14} cells.

Prokaryotic cell: Lacks a well-defined nucleus, and contains a rigid cell wall.

Eukaryotic cell: Highly complex and organized with a well-defined nucleus.

Cells possess a genetic program and can produce more of themselves.

Cells acquire and utilize energy and carry out a variety of chemical reactions: *metabolism.*

Cells engage in *mechanical activity* able to respond to stimulants.

CHAPTER 2

ENZYMES

Abstract: Enzymes are catalysts of biological systems, and most enzymes are proteins in nature. They increase the rate of reaction and decrease the energy of activation of a reaction to speed up the reaction. They are specific for substrates and products and function within a moderate pH and temperature range.

Many human diseases result from a mutation in enzymes and enzymes are used as important diagnostic tools and therapeutic targets in a number of diseases.

Keywords: Alloenzyme, Activation energy, Coenzyme, Cofactors, Competitive inhibitor, Diagnostic enzymes, Enzyme kinetics, Enzymes, Enzyme inhibitors, Isoenzyme, Km, Mutation in enzymes, pH, Metalloenzymes, Michaelis- Menten constant, Turnover number, Substrate concentration, Non-competitive inhibitor, Temperature, Therapeutic enzymes.

INTRODUCTION

Enzymes are catalysts of biological systems, and most enzymes are proteins in nature. Enzymes increase the rate of reaction and decrease the energy of activation of a reaction and speed up the reaction. Specific for substrates and products. Function within a moderate pH and temperature range. Mutation in enzymes may result in many diseases. Also, enzymes are used as important diagnostic tools and therapeutic targets in a number of diseases.

2.1. CONCEPT OF ENZYME, ISOENZYME, ALLOENZYME, COENZYME, AND COFACTORS

A- Applied Aspects:

Allozymes: applied in many population genetics studies, evolutionary genetics, systematics and molecular phylogeny, human genetics, and forensics.

Isoenzymes: useful tools for clinical diagnosis of many diseases.

Serum enzymes and isoenzymes: can be used as molecular markers of tissue damage.

B- Basics:

Fundamental concepts

Enzymes:

Catalysts of biological systems

Most enzymes are protein in nature, *exception: ribozymes.*

Increase rate of reaction (10^6 – 10^{12} times) and decrease energy of activation of a reaction and speed up the reaction

Specific for substrates and products

Function within a moderate pH and temperature range

Many human diseases result from mutations in enzymes

Used as important diagnostic tools and therapeutic targets in a number of diseases.

Coenzymes:

Complex non-protein organic molecules

Participate in catalysis by providing additional reactive groups.

Have little activity in the absence of enzymes.

Examples:

Vitamin B complex group:

TPP, FMN, FAD, NAD, NADP, Lipoic acid, pyridoxal PO4 (PLP), CoA FH4, biotin coenzyme, methylcobalamin, deoxyadenosyl cobalamin.

Non-vitamin coenzyme:

ATP, CDP, UDP

Nucleotide coenzyme:

NAD, NADP, FMN, FAD, CoA, UDPG.

Cofactors:

Apoenzymes that require presence of certain metal ions for their activity

Examples: Calcium (SDH), Cobalt (arginase) Magnesium (enolase), Manganese (Cholinesterase), chloride (Salivary amylase).

Metalloenzymes:

Enzymes that require metal ions for their activity and metal ion forms an integral part of enzyme,

Examples: zinc (SOD), iron (Cytochrome), Copper (tyrosinase, cyt oxidase), Molybdenum (Xanthine oxidase), and Selenium (GSHPx).

Allozymes:

Multiple forms of enzyme *coded by different alleles of the same gene* present in *one locus.*

Have different structures and catalyze different reactions.

Exhibit high levels of functional evolutionary conservation throughout specific phyla and kingdoms

Isozymes:

Multiple forms of enzyme but *coded by different genes present in different loci*.

Differ in amino acid sequence but they catalyze the same chemical reaction.

CHAPTER 3

Chemistry and Metabolism of Carbohydrates

Abstract: Carbohydrates are hydrates of carbon and have empirical formula: (CH2O) n. Polyhydroxy aldehydes or ketones form major constituents of living systems. The primary source of energy, and provides a structural component of the cell membrane and participates in cell growth, signaling, lubrication, and immunity..

Keywords: Aldoses, Asymmetric carbon, Disaccharides, Epimers, Enantiomers, Glycosaminoglycans, Heteropolysaccharides, Isomerism, Ketoses, Mono-saccharides, Oligosaccharides, Polysaccharides, Reducing sugars.

INTRODUCTION

Carbohydrates present in food are polysaccharides, disaccharides, and very small amounts of monosaccharides. During digestion, the carbohydrate is broken down into simple, soluble sugars (monosaccharides) that get transported across the intestinal wall into the circulatory system to be finally transported throughout the body.

3.1. CHEMISTRY OF CARBOHYDRATES

A- Applied Aspects

*Importance***:**

Major fuel for all tissues

Structural element

Storage

Structural component of membrane

Signaling

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Diagnostic Value of Carbohydrates:

Inulin (*Fructosan):* used to carry out: Inulin clearance test checks glomerular filtration rate of kidney.

Carbohydrate antigen 19-9 (CA19-9): widely reported to play a role in diagnosis of cancer

Carbohydrate deficient transferrin (CDT): most specific serum biomarker of heavy alcohol consumption

Carbohydrate-specific antibodies: widespread among all classes of immunoglobulins and include antibodies to ABO blood group antigens.

Therapeutic Value of Carbohydrates:

Carbohydrate-based or -modified therapeutics: used extensively in cardiovascular and hematological conditions, inflammatory diseases, anti-thrombotic treatments, wound healing.

Examples:

Heparin is a well-known and widely used.

Carbohydrate-based drugs available:

- *Hyaluronic acid* used in sepsis and to treat osteoarthritis
- *Cardiac Glycosides (*Digoxin) used in cardiac insufficiency
- *Glycosides (Ovabain and Phlorizin)* in treatment of Diabetes mellitus
- *Glycosides (Streptomycin, Erythromycin*) to treat bacterial infections
- *Mannitol* as osmotic diuretic used in acute renal failure
- *Lactulose*: relives hyperammonaemia in hepatic encephalopathy
- *Dextran:* as plasma substitute in hemorrhagic cases to prevent hypovolemic shock.

Vaccination strategies that utilize carbohydrate antigens:

Polysaccharide vaccines for childhood meningitis

Glycoconjugate vaccines have also been approved for use:

Pneumococcal 7-valent vaccine (Prevnar)

Haemophilus B

Typhoid Vi

Glycosidase inhibitors:

Acarbose to slow down the digestion of carbohydrates, useful in DM

Carbasugar AO-128 shown to exert anti-obesity activity

Miglitol in treatment of non-insulin-dependent diabetes mellitus.

Carbohydrates to replace deficient enzymes:

Aldurazyme (Laronidase): glycoprotein: enzyme replacement therapy in Hurler's disease

Imino sugar: Zavesca: potent inhibitor of glucosylceramide

Glucosyltransferase for the treatment of Gaucher disease

Polyglucosan bodies: Accumulation of branched polysaccharides into cytoplasmic inclusions: *corpora amylacea and Lafora bodies*.

CHAPTER 4

Chemistry and Metabolism of Lipids

Abstract: Lipids of major physiologic significance are fatty acids, their ester, cholesterol, and steroids. They constitute an important dietary constituent, are hydrophobic, insoluble in water, and soluble in polar solvents..

Keywords: Alpha and Omega oxidation, Beta-oxidation, Cholesterol, Ceramides, Eicosanoids, Fatty acids, Glycosphingolipids, Ketone Bodies, Lipid absorption, Lipoproteins, Lipid digestion, Lipid Metabolism, Phospholipids, Sphingolipids, Sphingomyelin, Saturated and unsaturated fats, Triacylglycerols.

INTRODUCTION

Major function of lipoproteins is transport of energy-rich triglyceride from intestine and liver to the sites of storage and utilization.

Serum cholesterol correlates with the incidence of atherosclerosis and coronary heart disease. Lifestyle affects serum cholesterol levels.

4.1 MAIN CLASSES OF LIPIDS

A- Applied Aspects:

Zellweger syndrome:

Defective biogenesis of peroxisomes

Accumulation of VLCFA in peroxisomes

Demyelination of nerve axons.

Trans Fatty Acids: Formed in some bacteria and ruminant animals: their butter, milk, cheese, and meat

Artificial trans-fats: Produced by partial hydrogenation of polyunsaturated FA.

Fried foods, margarine, and baked goods contain trans-fats

Diets high in trans-FA raise plasma LDL cholesterol and triglyceride levels and lower high-density lipoprotein (HDL) levels.

Can contribute to cardiovascular risk

Diseases due to defects in lipid:

Cholesterol: Abetalipoproteinemia: hypocholesterolemia, can be associated with increased risk of hemorrhagic stroke, cancer, respiratory disease, aortic dissection

Eicosanoids: COX-1 deficiency: bleeding disorders

Sphingomyelin: lysosomal storage disease

Cerebroside: Cerebroside sulfatidase deficiency: metachromatic leukodystrophy

Ganglioside: Neuraminidase deficiency: neurodegenerative disease

Tumor formation induces synthesis of new complement of gangliosides: Absence of enzymes required for degradation of GSL results in genetically transmitted diseases, *e.g.*, *Tay Sachs disease* (GM2 involved).

APL syndrome (APS):

Presence of antiphospholipid (aPL) antibodies or abnormalities in phospholipid-dependent tests of coagulation.

Cardiolipin being the dominant antigen used in most serologic tests for syphilis: *these patients may have a false-positive test result for syphilis.*

Hypercoagulability: occurs due to production of antibodies against coagulation factors, activation of platelets and vascular endothelium.

Reaction of antibodies to oxidized low-density lipoprotein predisposes to atherosclerosis and myocardial infarction.

B- Basics:

Lipids:

Hydrophobic in nature

Composed of saturated or unsaturated long chain hydrocarbons

Constitute important dietary constituent.

Functions:

Fuel: *Source of energy, energy reserve*: neutral fats, TAG stored in adipocytes

Mechanical and electrical function

Insulation: thermal insulator

Building Block: of cell membrane: contain phospholipids, glycolipids, cholesterol.

Transport function: *e.g.*, lipoproteins

Precursor for steroid hormones, eicosanoids, phospholipids, steroids

Absorption of: fat soluble vitamin and essential fatty acids.

Classification:

Simple lipids: Fats & waxes

Precursor and derived lipids: FA, glycerol, steroids, and alcohol.

Derived lipids: ketone bodies, steroids, fatty aldehydes, Prostaglandins, lipid soluble vitamins, hormones.

Neutral Lipids.

Complex lipids: Esters of FA with alcohol and contain an additional group:

Phospholipids

CHAPTER 5

Chemistry and Metabolism of Proteins

Abstract: Proteins are complex macromolecules composed of amino acids. Each body protein has a unique sequence pattern of amino acids. Amino acid sequence of a protein dictates its 3D arrangement, and the three-dimensional structure of a protein defines its size, shape, and function. Any change in the amino acid sequence of a protein can lead to structural changes to proteins or enzymes.

Keywords: Proteins, Amino acids, Optical activity, Amphoteric nature, Essential, Nonessential, Glycogenic, Ketogenic amino acids, Fibrous and globular proteins, Alpha-helix, Beta-sheet, Protein misfolding, Diseases related with structural anomalies in proteins, Digestion of proteins, Zymogen, Proteolytic enzyme, Amino acid metabolism, Inherited metabolic disorders and other diseases of protein metabolism.

INTRODUCTION

Dietary proteins are broken down to individual amino acids by various enzymes in the gastrointestinal tract. These amino acids are further broken down to α-keto acids that can be recycled in the body for generation of energy, and production of glucose or fat or other amino acids or ketone bodies. Amino acid decomposition results in carbon skeletons and nitrogenous waste and high concentrations of nitrogen are toxic. The urea cycle processes nitrogen and facilitates its excretion from the body. Several inherited disorders are associated with amino acid metabolism

5.1 STRUCTURAL ORGANIZATION OF PROTEINS: FUNCTIONS, STRUCTURE-FUNCTION RELATIONSHIPS

A- Applied Aspects:

Alteration in amino acid sequence can change folding and stability of the protein:

Human missense mutations change secondary structure and are more likely to cause disease processes.

Protein folding is a probabilistic, trial-and-error process where *lowest- energy conformations are favored.*

Protein misfolding:

If protein is not folding properly: a chaperone may send it directly for degradation

5.1.1 STRUCTURAL ORGANIZATION OF PROTEINS

A- Applied Aspects:

Alteration in amino acid sequence can change folding and stability of the protein:

Misfolded proteins (or toxic conformations):

Typically insoluble

Tend to form long linear or fibrillar aggregates known as *amyloid deposits*.

Implicated in many diseases:

E.g., Alzheimer disease, Parkinson's disease, Huntington's disease, Creutzfeldt-Jakob disease, cystic fibrosis, Gaucher's disease, and many other degenerative and neurodegenerative disorders and even cancers

Clinical relevance:

Cystic fibrosis:

Defect: 3 nucleotide deletion on chromosome 7:

ΔF508 mutation in chloride channel (CFTR)

↓ stability of protein and ↑ folding time

Instead of getting inserted into plasma membrane:

Mutated protein is degraded in Golgi apparatus

Result: ↓ chloride conductance → ↓ Na^+ & Cl reabsorption in sweat glands

Presentation: ↓ *water content of mucus → thick mucus → cannot be cleared →*

Respiratory infections

Malabsorption

Meconium ileus

Biliary cirrhosis

Therapeutic strategies for the treatment of protein-misfolding diseases:

1.By inhibiting protein aggregation with peptides or small molecules identified via structure-based drug design or high-throughput screening

2.By interfering with post-translational modifications that stimulate protein misfolding and aggregation

3.By upregulating molecular chaperones or aggregate- clearance mechanisms.

Defects in destruction of misfolded proteins:

Inability to send degraded proteins to proteasome results in accumulation in ER

Example: α1-antitrypsin (AAT) deficiency:

AAT: Synthesized by hepatocytes and exocytosed into circulation, inhibits proteases. *In AAT deficiency:* Misfolded α1-antitrypsin accumulates in ER and damages hepatocytes: *PAS+ granules.*

Folding process is difficult and potentially dangerous. Biological health depends on its success and disease on its failure..

Protein denaturation: occurs at *secondary, tertiary & quaternary level but not at primary level*.

Significance of Protein Precipitation

CHAPTER 6

Metabolism and Homeostasis

Abstract: The central interconnecting metabolic pathway (pathways of synthesis, degradation, and interconversion of important metabolites) common to most cells and organisms is referred to as intermediary metabolism. Co-ordination of metabolic activities of different organs serves to support glucose homeostasis and provides a steady supply of glucose to meet the needs of the brain and RBCs. This also helps in the storage of fuel when available in plenty. The liver supplies glucose and ketone bodies to other tissues. Adipocytes make FA available to other tissues. The circulatory system transports metabolic fuels, intermediates, and waste products among tissues. Also, certain metabolic pathways occur in multiple tissues, namely, Cori cycle, glucose alanine cycle..

Keywords: Intermediary metabolism, Sugar phosphates, Alpha-keto acids, Coenzyme A derivatives, Phosphoenol pyruvate, Obesity, Fasting, Starvation, Fed state.

INTRODUCTION

The central interconnecting metabolic pathway (pathways of synthesis, degradation, and interconversion of important metabolites) common to most cells and organisms are referred to as intermediary metabolism. All metabolic pathways are under precise regulation to adjust the synthesis and degradation of metabolites to physiological requirements. This is mainly determined by the activity of key enzymes.

6.1 METABOLIC PROCESSES IN BODY IN FED AND FASTING STATES

A- Applied Aspects:

Biochemical metabolic pathways are central to pathophysiology, clinical presentation, and management of human diseases:

Diabetes, myocardial ischemia, hepatic dysfunction, and rare inherited metabolic diseases.

➢*Reader should be able to appreciate intimate interrelationship of metabolic pathways and basis of rational diagnostics and therapeutic decisions after reviewing the chapter.*

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Disturbances in fuel metabolism occur in:

Starvation

Diabetes mellitus

Obesity

Inherited diseases of intermediary metabolism:

Include: Defects in enzymes of metabolism of amino acids, carbohydrates, FA, or mitochondrial energy metabolism:

Amino acidopathies, organic acidurias, amino acid transport disorders, ammonia detoxification, peptide metabolism (GSH)

Carbohydrate metabolism and transport

FA oxidation and ketogenesis

Mitochondrial disorders

Disorders of cobalamin and folate metabolism

Disorders of transport or utilization of copper, manganese, iron, zinc.

Mature onset diabetes of the young (MODY):

Patients with MODY are typically lean, and they have good pancreatic function shown by normal or high C-peptide levels.

Patients with MODY typically have strong family histories of diabetes, which stretch across generations, but they are usually diagnosed in childhood or early young age.

The majority of patients with MODY can be successfully treated with diet or sulfonylureas. If they require insulin it is usually small amounts to cover basal needs and not necessarily prandial needs.

Most patients with MODY do not develop diabetes complications except in rare types like MODY-5, where there can be progressive loss of renal function independent of diabetic nephropathy.

B- Basics:

Metabolic processes in Fed- Fast State:	
Absorptive phase (after eating): *Hormone: Insulin* *Insulin acts on:* Adipocytes Hepatocytes Myocytes *Insulin stimulates/activates:* Glucose uptake in liver, muscle, fat cell Glycogen synthesis FA synthesis Protein synthesis	*Post- Absorptive state (in between meals):* *Hormone: Glucagon, epinephrine* (*Does not act on RBCs and Brain)* *Glucagon acts on:* Adipocytes Hepatocytes Myocytes *Glucagon stimulates/activates:* Glycogen breakdown GNG FA oxidation Protein breakdown
Starvation: *1-3 days*: energy source: TAG breakdown *In brain:* glucose from GNG (lactate, alanine)	

CHAPTER 7

Molecular Biology

Abstract: DNA is a double-stranded helix with major and minor grooves. It is composed of two polynucleotide chains joined by hydrogen bonds between bases. DNA is composed of a nitrogen base (A, G, C, T), deoxyribose, and phosphates. Four nitrogenous bases include purines (A, G), and pyrimidines (C, T). Adenine base pairs with thymine (two bonds) and Guanine base pairs with cytosine (three bonds). RNA is usually a single-stranded molecule. Three types of RNA are mRNA, rRNA, and tRNA. RNA differs from DNA as RNA contains ribose sugar instead of deoxyribose and uracil (U) rather than thymine..

Keywords: DNA, RNA, Chargaff's Rule, Replication, Cell cycle, Polymerases, Reverse transcriptase, Transcription, Ribosomes, Genes, Translation, Mutation, Operons, Cancer, Recombinant DNA, Technology, PCR, Taq polymerase, Electrophoresis, Cloning, Vectors, Xenobiotics, Antioxidants.

INTROCUCTION

Semi-conservative replication is a process of making an identical copy of a portion of DNA using existing DNA as a template for the synthesis of a new complementary DNA strand. DNA damage can be caused by chemicals or radiation exposure, and incorporation of incorrect base pairs during replication. Multiple DNA repair systems in prokaryotes and eukaryotes repair damaged DNA before mutants become fixed by replication. If cells are allowed to replicate using a damaged template, there is a risk of introducing a stable mutation in new DNA or developing cancer if the repair mechanism is defective.

Transcription is the process of RNA synthesis. RNA synthesis is controlled by the interaction of promoters and enhancers and several types of RNA are produced.

The translation is a process where a mature mRNA molecule is used as a template to assemble a series of amino acids to produce a polypeptide with a specific amino acid sequence according to the message's instructions.

Mutations are permanent changes in a DNA sequence and create new alleles. Depending on the location and type of mutation can be beneficial, neutral, or detrimental.

Gene expression is the orderly use of genomic information during development and for responding to changes in both internal as well as external environments.

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Regulation of prokaryotic gene expression occurs mainly at the transcriptional level.

Regulation of eukaryotic gene expression is complex and includes chromatin-modifying activities, response elements binding to DNA and upstream promoter elements.

Xenobiotics are chemical compounds foreign to the body. At least thirty different enzymes catalyze reactions of xenobiotic metabolism. Liver is main organ involved in their metabolism. Other organs active in xenobiotic metabolism are kidney, GIT, gonads, adrenals, placenta, and nasal epithelium.

Free Radical is any species capable of independent existence that contains one or more unpaired electrons. Free radicals are formed in the body under normal conditions. They cause damage to nucleic acids, proteins, and lipids in cell membranes and plasma lipoproteins.

Mechanisms of protection against radical damage include antioxidants. Antioxidants are compound that can delay the start or slow the rate of lipid oxidation reaction. Impaired endogenous antioxidant system results in accumulation of free radicals, which not only induces lipid peroxidation but also imposes severe stress on the body leading to many diseases.

7.1 STRUCTURE AND FUNCTIONS OF DNA, RNA AND CELL CYCLE

A- Applied Aspects:

Double helix distorted by intercalating agents:

Actinomycin D, ethidium bromide, Acridine orange

Application of renaturation in techniques:

Southern blot (DNA/cDNA)

Northern blot (DNA/RNA)

Allows specific identification of hybrids from mixtures of DNA or RNA

Topoisomerases:

Important therapeutic agents

Doxorubicin, etoposide, teniposide: inhibit topoisomerase I, used in cancer treatment

Nalidixic acid, norfloxacin: act on bacterial DNA gyrase, used in treatment of UTI.

Ciprofloxacin, novabiocin: inhibit DNA gyrase, used as anticancer drugs.

Telomerase:

Telomerase activity:

Present only in *embryonic cells, germ (reproductive) cells, and stem cells, not active in somatic cells*.

Contributes to aging of cells.

Cancer cells: Have relatively high levels of telomerase, preventing telomeres from becoming shortened and contributing to immortality of malignant cells.

Telomeres reactivation: may be of help in treatment age- related diseases in humans in future.

DNA methylation imprints: can be disturbed in number of rare human genetic disorders.

Dysfunction of imprinted gene causes genetic defects; *e.g.*, Prader Willi syndrome occurs due to failure to express genes from paternal chromosome.

Loss of imprinting of IF-2 gene in 10% population has been linked to colorectal cancer.

CHAPTER 8

Nutrition

Abstract: Nutrition is the study of understanding the effects of food on the human body in terms of health and disease. The major dietary components are carbohydrates, proteins, fats and dietary fiber. A healthy diet comprises important elements of fruits and vegetables. If consumed daily in sufficient amounts, it could help prevent major diseases such as CVDs and certain cancers

The human body requires energy for each physical activity and the amount depends on the duration and type of activity. This chapter deals with different components of diet and their importance to the human body and disorders associated with their poor or increased intake.

Nutritional diseases occur when dietary intake does not contain the right amount of nutrients, or when one cannot absorb nutrients from food correctly. Kwashiorkor and marasmus are the two major types of protein calorie malnutrition seen in malnourished children. Eating a diet high in saturated fats, trans fat, and cholesterol: linked to heart disease and related conditions, *e.g.,* atherosclerosis

Keywords: Dietary components, Calories, Proteins, Fats, Carbohydrates, Respiratory quotient, Complementary proteins, Trans fat, Dietary fiber, Basal metabolic rate, Biological value of a protein, Nitrogen balance, Balanced diet, Kwashiorkor, Marasmus.

INTRODUCTION

Nutrition is the process by which living beings acquire nutrient for growth, repair, and energy. Dietary carbohydrates are the chief source of energy, contributing to 60-70% of total caloric requirement of body. Among carbohydrate utilized by the body starch is most abundant and glucose is the major source of fuel for most organs and tissues.

Dietary fiber is another important component of our diet which plays a major role in protecting humans from diseases. Fruit and vegetables are an important component of a healthy diet and, if consumed daily in sufficient amounts, could help prevent major diseases such as CVDs and certain cancers.

8.1 IMPORTANCE OF VARIOUS DIETARY COMPONENTS AND DIETARY FIBER

A- Applied Aspects:

Dietary related diseases:

Eating too much or too little of certain foods and nutrients:

Can raise risk of dying of *heart disease, stroke, and type 2 diabetes*

Nutritional diseases:

Nutrition disorders: Caused by undernutrition, overnutrition or an incorrect balance of nutrients.

Nutritional diseases occur when:

Dietary intake *does not contain* right amount of nutrients, *or*

When one cannot nutrients from food are not absorbed correctly.

Examples:

Obesity and eating disorders

Chronic diseases: protein-energy malnutrition: Kwashiorkor and marasmus

Cardiovascular disease, atherosclerosis, hypertension

Cancer: colorectal cancer, prostate cancer, breast cancer

Diabetes mellitus

Dental caries

Eating a diet high in saturated fats, trans fat, and cholesterol: linked to heart disease and related conditions *e.g., atherosclerosis*

B- Basics:

Dietary Components:

Major dietary components: carbohydrates, proteins, fats and dietary fiber.

Healthy diet:

Comprises important elements of fruits and vegetables

If consumed daily in sufficient amounts*: can prevent major diseases*: *CVDs, cancers.*

Balanced diet key nutrient groups:

Proteins

Carbohydrates

Fats

Vitamins

Minerals

Deficiency of any one type of nutrient can lead to disease, starvation.

Dietary fibre:

Fibre or roughage is non-digestible carbohydrate

Role:

Play regulate gut transit & motility and aid water retention adding bulk to faeces. Beneficial for colonic function, lowering cholesterol and in diabetes.

CHAPTER 9

Extracellular Matrix

Abstract: This chapter discusses the functions of ECM, its structure, function and disorders of collagen, glycosaminoglycans (GAGs) and proteoglycans. For the proper functioning of the cell, it is important that each of its proteins must be localized to the correct organelle, such as mitochondria and lysosome. Also, the process of protein targeting, sorting and its biomedical importance are discussed here. Mislocalized proteins have been associated with human diseases. Therapeutic manipulation of protein localization in human diseases is promising.

Keywords: Collagen, Extracellular matrix, Glycosaminoglycan, Proteoglycan, Protein sorting, Protein targeting, Mislocalized proteins, Ehlers Danlos syndrome, Marfan syndrome, Osteogenesis imperfecta, Menkes syndrome, Tumor metastasis, Cathepsin.

INTRODUCTION

Connective tissue is composed of cells and extracellular matrix. Extracellular matrix consists of a network of proteins and carbohydrates. It consists of ground substance and fibers.

9.1 FUNCTIONS AND COMPONENTS OF THE EXTRACELLULAR MATRIX (ECM)

A- Applied Aspects:

MULTITASKING ROLES FOR ECM IN DISEASES

ECM molecular composition and structure:

Varies, markedly modified during normal tissue repair & diseases: *osteoarthritis, fibrosis, cancer*

Mutations in matrix genes → *genetic disorders* with diverse clinical phenotypes

ECM: novel *diagnostic tools and therapeutic targets": e.g.,* glucocorticoids, infliximab, cyclosporine A

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Abnormal remodeling of tissues:

Results in *alterations in ECM structure and function*

This promotes *development of common diseases: fibrosis, osteoarthritis, cancer*

ECM may be a major actor for emergence of *metabolic syndrome*

During aging & related processes: several alterations in elastin network → elastin-derived peptides

Elastin-based pharmacological strategies in future may fight cardiovascular and metabolic diseases.

Aberrant expression of proteoglycans (PGs) and glycosaminoglycans (GAGs):

In cancer cells and *tumor microenvironment* promotes *tumor progression* and *development of resistance against therapeutic strategies.*

Defects in GAG chain formation and attachment to PG core protein:

Lead to onset of several rare disorders, involving connective tissues and skeleton.

E.g., Ehlers–Danlos syndrome; XYLT1 deficiency or Desbuquois dysplasia type 2; Joint dislocations, skeletal dysplasia; multiple osteochondromas; Achondrogenesis; Chondrodysplasia with joint dislocations.

Ion–ECM interactions:

Chelation of endogenous transition metal ions such as Cu^{2+}, Zn^{2+} and Fe^{2+} can affect *tumor migration and angiogenic processes.*

Potential new avenues for therapeutic intervention: metal ions and GAGs present great potential to be used in diagnosis and treatment of cancer patients: MnSOD *liposomes administered by inhalation to protect murine lung from irradiation, might be used in cancer patients as adjuvant to chemo- & radiotherapies.*

Play important role in tissue regeneration after injury:

Tissue (muscles, nerves, epithelia) damage: basal lamina *provides scaffold* for *regenerating cells to migrate.*

In skin or cornea after injury:

Basal lamina becomes chemically altered after injury, *addition of fibronectin promotes cell migration.*

After nerve or muscle injury:

Basal lamina at synapse has a central role in *reconstructing synapse*.

Localized degradation of matrix components allows cells to migrate through basal lamina:

Occurs in response to infection or injury (WBCs migrate across basal lamina) into tissues

Cancer metastasis: Cancer cells migrate to distant organs via bloodstream or lymphatic vessels.

Tissue-type plasminogen activator (tPA): given to patients with heart attack or thrombotic stroke to dissolve arterial clot and restore blood flow to the tissue.

Urokinase-type plasminogen activator (uPA): Type of plasminogen activator. *Receptor-bound uPA* may also help some *cancer cells metastasize: e.g., Human prostate cancer cells* make and secrete serine protease uPA, that binds cell-surface uPA receptor proteins & cells metastasize.

CHAPTER 10

Oncogenesis and Immunity

Abstract: Genes involved in carcinogenesis include tumor suppressor genes and oncogenes. They produce products involved in the control of cell cycle, intercellular adhesion and DNA repair. Oncogenes arise from protooncogenes that encode proteins having a role in cell's normal activities. Most tumors contain alterations in both tumor suppressor genes and oncogenes They are derived from protooncogenes and activation of oncogenes can occur by mutation of the gene, duplication of the gene causing gene amplification to produce an excess of encoded protein; or chromosomal rearrangement that causes or alters expression of the gene leading to aberrant cell cycle control. Loss of function of one or more tumor suppressor genes or getting silenced epigenetically can cause cancer. Cells with irreparable genomic damage are destroyed by apoptosis normally. Apoptosis is involved in neurodegenerative disorders (Alzheimer, Parkinson's, Huntington's). The immune system is a unique adaptive defensive system to protect from invading pathogenic microorganisms and cancer. The immune response depends on pathogens and can be categorized as innate or adaptive and is produced in response to foreign substances (antigens).

Disorders of immune regulation can be aberrations of quantity, quality or direction of response. Autoimmune diseases can arise from a malfunctioning immune system. Deficiency in antibody production results in agammaglobulinemia, hypogammaglobulinemia and specific immunoglobin deficiency.

Vaccination induces a protective immune response against target pathogens without the risk of acquiring the disease and its potential complications..

Keywords: Oncogenes, Cancer, Protooncogenes, Tumour suppressor gene, Apoptosis, Immunity, Innate, Adaptive, Antibodies, Agammaglobinemia, Immune deficiency, Hypersensitivity, Vaccines.

INTRODUCTION

Cells proceed through multiple rounds of division during the growth and maintenance of an organism. Cells may be subjected to various insults *e.g.*, chemical, radiant energy or viruses. If the normal DNA repair mechanisms become overwhelmed, they may cause chemical changes, resulting in mutations.

Cancer development. is a multi-step process and the three main phases of cancer development include initiation, promotion and progression.

Protooncogenes are the normal cellular proteins that work to regulate normal growth and development and if they are mutated or mis expressed, then they become oncogenes and lead to aberrant cell cycle control.

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Aberrant expression of oncogene causes entry of cell into cell cycle with abnormal cell growth and gain of function. They function in growth or signaling pathways.

Tumor suppressor genes are cellular proteins whose activity if reduced, results in uncontrolled cell growth. Common tumor suppressor genes include RB, TP53, APC, BRCA 1 and BRCA 2. Transformation of a normal cell to a cancer cell is accompanied by loss of function of one or more tumor suppressor genes.

p53 gene is gate keeper of cellular proliferation and its mutation is common alteration in cancer. RB gene is responsible for retinoblastoma – rare, familial retina tumor.

Apoptosis Programmed destruction of cells occurs in cells with irreparable genomic damage are destroyed by apoptosis normally. The fate of a cell depends on whether survival or death- depends on a delicate balance between proapoptotic and antiapoptotic signals.

Innate immunity or native immunity is resistance to infections that an individual possesses due to his genetic and constitutional makeup. It is unaffected by prior contact with microorganisms or immunisation

Adaptive immunity or acquired immunity is the resistance that an individual acquires during life, as distinct from the inborn innate immunity.

The immune system's activities are mostly beneficial; however, there are several situations where they can have deleterious effects.

Vaccines act by initiating an innate immune response, which in turn activates an antigen-specific adaptive immune response.

10.1. CANCER INITIATION, PROMOTION AND ONCOGENE ACTIVATION

A- Applied Aspects:

Ways of activating (proto-) oncogenes

	Mechanism	*Oncogene*	*Tumor*
1.	*Amplification*	ERB B2(HER 2)	Breast, ovary, lung, gastric, colon cancer
		NYCN	Neuroblastoma
2.	*Point mutation*	HRAS	Bladder, lung, colon cancer
		NIT	GIT tumor, mastocytosis
3.	*Chromosomal rearrangement*	BCR=ABL 1	Chronic myeloid leukemia
4.	*Transduction to a region of transcriptionally active chromatin*	MYC	Burkitt's lymphoma

Protooncogenes and cancers

Protooncogene	*Neoplasm*	*Lesion*
ABL	Chronic myelogenous leukemia	*Translocation*
BCL2	B-cell lymphoma	*Translocation*
ERB B	Squamous cell carcinoma	*Amplification*
NEU/HER 2	Adenocarcinoma Breast, ovary, stomach	*Amplification*
MYC	Burkitt's lymphoma, Carcinoma lung, breast, cervix	*Translocation, amplification*

CHAPTER 11

Biochemical Laboratory Tests

Abstract: This chapter gives an overview of the basic principles, components, types, and applications of equipment such as a spectrophotometer, colorimeter, ELISA reader, radioimmunoassay (RIA) analyzer, electrophoresis and chromatography and centrifugation. This chapter also makes the learner know about the basic principles, different types of laboratory tests, and their applications. At the end of this chapter, the diagnostic approaches are being discussed.

Keywords: Spectrophotometry, colorimetry, Lambert's law, instrumentation, techniques, monochromator, centrifugation, electrophoresis, radioimmunoassay, chromatography, partition chromatography adsorption chromatography, gas-liquid chromatography, ion-exchange chromatography gel filtration chromatography, affinity chromatography, radioimmunoassay, enzyme-linked immunoassay.

INTRODUCTION

The biochemical laboratory tests are a series of blood tests used for the evaluation of the functional capacity of various critical organs and systems, such as the liver, heart, gastrointestinal tract, endocrinal organs, and kidneys and monitoring of disease processes. They require various equipments and safe lab practices for the analysis. The different biochemical tests allow the clinician to specify different diagnostic options that can subsequently be confirmed through additional tests.

11.1 LABORATORY APPARATUS AND EQUIPMENTS, GOOD SAFE LABORATORY PRACTICE AND WASTE DISPOSAL

B- Basics:

Instruments:

Lab supplies for clinical chemistry lab:

1. *Chemicals and related substances:*

i. Reagent grade chemicals: Laboratory grade LR

ii. Analytical grade AR

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iii. HPLC grade or ultrapure

iv. Reagent grade water: Distilled water, Deionized water, Reverse osmosis

2. *Laboratory supplies:*

i. Glassware: Tubes, flasks, cylinders, funnels

Thermal resistant, borosilicate glassware: Pyrex, corning

ii. Plasticware

i. Polyethylene and polypropylene: used in disposable plastic-ware

ii. Polycarbonate: used in centrifuge tubes

iii. Fluorocarbon resin (Teflon): inert, high corrosion resistance at extreme temperatures

iv. Synthetic and rubber tubing: Tygon

3. Volumetric equipments:

Pipettes

Dispensers

Burette

Flasks

4. Centrifuges

5. Mixers and homogenizers

6. Balance and weighing instruments

7. Thermometers

8. Analytical techniques and instrumentation:

i. Colorimeter

ii. Spectrophotometer

iii. Spectrophotofluorometer

iv. Flame emission spectrophotometer

v. Atomic absorption spectrophotometer

vi. Nephelometer and turbidimeter

vii. Scintillation (counting detectors)

viii. *Potentiometry:*

Electrodes: inert metal: Hydrogen; Ion – selective; Ion – exchange; gas electrode: pCO_2

Biosensors

ix. Electrophoresis

x. Chromatography/Mass spectrometry/ HPLC

Commonly used laboratory apparatus & equipment:

Laboratory balances for measurements of weight of samples and chemicals:

Digital laboratory balances

Top-loading balances: for range of several grams to several hundred grams.

Analytical balances: for the precise measurement of small weights

Centrifuges:

Simple bench-top centrifuges: to preparative devices with much higher capacities (volumes up to several litres, accelerations around $10^4 g$)

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Simmi Kharb

She has teaching experience of 30 years, currently working as Sr. Professor & Head, Department of Biochemistry, Pt. B. D. Sharma PGIMS, University of Health Sciences, Rohtak. She has extensive teaching and research experience (published >200 papers in Indexed Journals and presented >75 papers in national, international conferences). Her h-index-28, i10-index- 58, Citations- 2444. She has handled many research projects and received research grants. She is trained from EMBO and Wellcome Trust and Sangers Institute. Her research on fluoride toxicity has been appreciated at several national and international platforms. She has published twelve books and her book "Mind Maps in Clinical chemistry is collaborated with Texidium for inclusion in their e-learning resource platform.