I SEMESTER M. Sc. BIOCHEMISTRY

BCT – 101: Biophysical and Bioorganic Chemistry

4 units (52 hrs)

Properties of water

Ionization and ionic product of Water, structure of liquid water and ice. Physical and chemical properties of water.Effect of solutes on colligative properties of water. Importance of water in biological system with special reference to the maintenance of native structure of biological molecules. Biological relevance of pH and pKa, determination of pKa of weak acid.

Buffers, buffer action, and buffer capacity. Henderson–Hasselbalch equation, Preparation of buffers of Blood plasma and interstitial fluids-carbonic acid, phosphate, Hemoglobin and amino acids as buffers. Problems **6 hrs**

Thermodynamics:

Laws of thermodynamics basic concepts of entropy , enthalpy, and, free energy changes, standard free energy change and its relation to equilibrium constant. Thermodynamically controlled biological reactions.Redox potential, Redox potential value of high energy compounds. Oxidation – reduction reactions in biological systems. Experimental determination of emf of half cell.

5 hrs

Stereochemistry:

Optical isomerism, chirality, symmetry elements, enantiomers, diastereomers, DL and RS notations, ,stereoisomerism and geometrical isomerism, cis – trans and E – Z conventions. Importance of chirality in biological systems and Circular dichroism **4 hrs**

Mechanism of Bio-organic reactions:

Introduction, kinetic and non kinetic. Homo and heterolytic cleavage.Homolytic cleavage- **Free radicals**: formation – photolysis, thermolysis, redox reactions, radical reactions with biomolecules .

Heterolytic cleavage : structure and reactivity of carbocation (C^+) , carbanion (C^-) and carbon free radical ($C^{\cdot}$) characteristic aspects of ionic, radical and concerted reactions, substitution, nucleophilicity and basicity, leaving group effects, solvents effects. addition, elimination and rearrangements. Saytzeff and Hoffman eliminations, dehydration of alcohols, pyrolytic eliminations.Energy profiles of reactions, transition state theory, kinetically and thermodynamically controlled reactions. Reactions SN^1,SN^2, SN^ineighbouring group participation. E_2 ,E_i, Curtin Hammett principle. Electrophilic addition to C=O, aldol condensation,, Michael addition. Esterification and hydrolysis. **17 Hrs**

Rearrangements:

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Migration to electron deficient C, N and O; Wagne-Meerwein, Pinacol, Beckmann, Hoffmann, Bayer-Villiger reactions, allylic rearrangements. Benzilicacid rearrangement, Cannizaro and Manich reactions. **6 hrs**

Heterocyclic systems:
Occurrence in biological systems, structure and properties of furan, pyrrole. Indole, thiazole, imidazole, pyridine, pyrimidine, purine, quinone, pteridineand isoalloxazine containing biomolecules. **8 hrs**

Bioinorganic chemistry:
Crystal field and Ligand field theory of complexes, stability, kinetics and mechanism of reactions of complexions. Ligand replacement reactions and electron transfer reactions of organometallic moieties of biological macromolecules (cytochromes, chlorophyll and hemoglobin).
6 hrs

Self study Topics:

1. Antacids- a basic remedy
2. Acids and bases in daily Life
3. Relationship between the pH of a solution and its acidity.
4. Optical activity in Chiral compounds
5. Thermodynamics in relation to stability
6. Bonding in organic molecules
7. Supra Molecular chemistry
8. Behaviour of Transition metal complexes.
9. Oxidation reductions in biological systems
10. Transesterification in carboxylic acids

BCT- 102 : Biomolecules

4 units(52 hrs)

Carbohydrates:
Brief review of configurational and conformational aspects of carbohydrates. Structure, properties and importance of structural (cellulose and chitin) and storage polysaccharides (starch and glycogen), glycosaminoglycans, cardioglycosides and bacterial cell wall polysaccharides. Structure elucidation of polysaccharides (starch, glycogen and cellulose). Glycoproteins – structure and functions, blood group antigens, Methods of structural degradation of oligosaccharides Methylation, Periodate oxidation. Lectins – characteristics and functions in biological system. Glycoside-amygdalin **10 hrs**

Lipids:

Lipid classification, brief account of the chemical properties and structure of lipids (without structure elucidation) & biological role of the following: fatty acids, acyl glycerols, phospholipids, plasmalogens, sphingolipids, glycolipids, steroids, eicosanoids – prostaglandins, thromboxanes, &leukotrienes, leptin and visfatin. Types and structures of surfactants.**7 hrs**

Amino acids and Proteins:

Review of classification and structure of amino acids, acid – base properties of amino acids.
Non – standard, non–protein and biologically active amino acids. UV light absorption property of amino acids. Ionic properties of peptides and proteins.Naturally occurring peptides. Peptide synthesis– reactive ester method and modified Merrifield solid phase synthesis.
Primary structure: Elucidation of primary structure of proteins – Determination of amino acid composition, end group analysis, cleavage by enzymes and chemicals, separation of fragments.
Manual and modern methods of sequencing and reconstructing the protein sequence. Assignment of disulfide bonds.
Secondary structure: Peptide bond – structure and conformation, Regular secondary structure:
α – helix and other types of helices, β – pleated sheet, irregular, turns, loops and triple helical structures.Ramachandran plot.Helix stabilizing and destablizing amino acids. Structure of fibrous proteins: α keratin, silk fibroin and collagen. Motifs (super secondary structure – triose phosphate isomerase, concanavalin and Rossmann fold) and domain structure(glyceraldehydephosphate dehydrogenase). Secondary structure of insulin, ribonuclease, lysozyme, myoglobin and chymotrypsin.
Tertiary structure:Forces stabilizing tertiary structure of proteins. Protein denaturation and renaturation. – Anfinsen's experiment.
Quaternary structure and symmetrystructure and function of myoglobin and hemoglobin.
Cooperative mechanism of oxygen binding to hemoglobin.Abnormal hemoglobin– sickle cell hemoglobin. **22 hrs**

Protein folding pathways:

Protein dynamics – kinetics of protein folding and disulfide bond formation, molecular chaperones and protein disulfide isomerase. Disease related to protein folding – Alzheimer's and mad cow disease. **3 hrs**

Nucleic Acids:

Structure of nucleic acids– primary, secondary and tertiary structure of DNA. Isolation, fractionation and characterization of nucleic acids .Properties of nucleic acids in solution. Types of RNA.Secondary structure of tRNA and role of secondary structure in mRNA stability. Chemical synthesis of oligonucleotides (phosphate and phosphite method). Nucleic acid sequencing – Maxam and Gilbert and Sangers method. **10 hrs**

Self-study Topics

1. Biomolecular Interactions
2. Conformational analysis of proteins/DNA
3. Physico-chemical factors in protein folding
4. Applications of surfactants

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5. Relevance of steroids to biological system
6. Denaturation and renaturation kinetics in DNA
7. Receptor-ligand interaction
8. Unique properties of amino acids
9. Role of Glycoproteins in cellular functions
10. Lipid levels in normal and abnormal conditions

BCT-103 : Analytical Biochemistry I

4 Units (52 hrs)

Overview of Biochemical Investigations:
Introduction to biochemistry, outline of strategies in biochemical investigations employing whole animal studies, isolated organs, tissues, and cell cultures.Specific investigations with isolated organelles; mitochondria and ER. Investigations with microorganisms and their mutants yeast, Ceanorhabditiselegans, Arabidopsis thaliana and Drosophila melanogaster as model specimen for biochemical investigations.
Basic equipments and methods, and safety considerations in animal cell culture.Types of animal cells and their characteristics in culture, culture media and common animal cell lines for laboratory investigation. Plant cell culture, media for plant cell culture, potential of plant cell culture in biochemical investigations.
Extractions; Preparation of organic and aqueous extracts for biochemical investigations, physicochemical properties of metabolites and drugs extracts from biological materials. Physico-chemical properties of solvents, solubility and missibility, ionic bonds, and salting out. Partition, ionization, buffering and their effect on extraction.Choice of solvent for solvent extraction, mixed solvents, solid phase extraction. **12hrs**

Microscopic techniques:
Review of light microscope, resolution of microscopes, Optical contrast, phase contrast, and dark field microscopy, preparation of specimen for biochemical investigations.
Electron microscopy; Working principle and applications, specimens for electron microscopy, fixatives, immune-gold microscopy and its advantages.Metal shadowing, design and applications of Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), and cryo-electron microscopy. 3-D images, negative staining, single particle reconstruction.**5 hrs**

Centrifugation:
Principle of centrifugation, the Swedbergequation , types of centrifuges and rotors. Density gradient centrifugation.Caesium chloride and sucrose density gradients; examples of separations, Subcellular fractionation.
Design and working of analytical ultracentrifuges, sedimentation velocity and sedimentation equilibrium analyses.
Ultra-filtration; Principle, instrumentation and application. Dialysis, principle and uses of equilibrium dialysis,.Precipitation; methods andapplications.
Flow Cytometry; Principle and design of flow cytometer, cell sorting. Detection strategies in flow cytometry and parameters measured by flow cytometry. **7 hrs**

Biocalorimetry:

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Arrhenius equation, determination of energy of activation from Arrhenius plots. Main thermodynamic parameters; enthalpy, and entropy. Isothermal titration calorimetry, design of experiments, determination of change in heat capacity, eg.,oligomerization of valinomycin, DNA duplex. Determination of specific heat from enthalpy.Differential scanning calorimetry; design of experiment, application of DSC, microcalorimetry.Determination of thermodynamic parameters by noncalorimetric data. **5 hrs**

Manometry:
Instrumentation, types of manometry; Warburg constant volume manometer, Gilson'sdifferential respirometer, applications. **3 hrs**

Radioisotopic methods of analysis:
Atomic stability and radiation, types of decay, rate of radioactive decay, half life, units of radioactivity Detection and measurement of radioactivity.
Design and applications of Geiger Muller Counter, and types of scintillation counters.
Disadvantages of scintillation counters, quenching, Chemiluminescence and phospholuminescence counting efficiency, channel ratio, sample preparation, scintillation cocktails, Cerenkov counting. Autoradiography; types of emulsions and films for exposure to isotopes, suitable isotopes, times of exposure and processing films, direct autoradiography, fluorography, intensifying screens, quantification.
Radio tracer techniques; Supply storage and purity of radiolabeled compounds, specific activity, radiolabeled nucleotides, metabolites.Pulse chase experiments. **10 hrs**

Quantitative biochemical measurements:

Analytical considerations and experimental errors, nature of experimental error random and systemic errors. Identification of systemic errors, SOPs.
Performance of analytical methods, precision, accuracy, detection limit, analytical range ,specificity, sensitivity, and robustness. Gaussian distribution (normal) of data, quantification of precision by standard deviation, coefficient of variation and variance, (data to be provided for calculation of each parameter) ,Correlation and regression analyses. .
Assessment of accuracy; Population statistics confidence limits and confidence intervals, student's t test, standard error of mean, examples for calculation.
Q tests, examples and applications, Null hypothesis, use of t test to validate analytical methods unpaired, paired, one sample, two sample tests with examples. Calibration methods; Least mean square method of fitting straight line to data with example. ANOVA, one way and two way ANOVA.
Quality Assurance :Quality control, Quality assessment, External and Internal methods of Quality Assessment, Control Charts. **10 hrs**

Self-study Topics

1. Density gradient centrifugation as an analytic tool
2. Principles and applications of solvent extraction
3. Radio isotope techniques in Biological studies
4. Principles and applications of solid phase extraction

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BCT 104: General Physiology

4 units (52 hrs)

Tissues:

Formation of different kinds of tissues from primary germ layers.Types and functions of epithelial tissue, intercellular junctions. Connective tissue – extra cellular matrix, Collagens – types, composition, structure and synthesis, Elastin, fibronectins, and other proteins of the extra – cellular matrix. Basal lamina; laminins and associated proteins and their functions. **6 hrs**

Cytoskeleton and Cellular dynamics:

Microfilaments; Assembly and polymerization of Gactin, role of ThymosinB4, Profilin and Cofilininpolymerization, structural and functional property of Factin, Capping proteins and assembly of actin filaments, branched and unbranchedfilament assemblies, intracellular cellular movement and actin polymerization, use of toxins in study of actin dynamics. Role of cross-linking and adaptor proteins in actin bundlingand membrane association.

Structure and organization of microtubules; dynamics of microtubules, assembly by MTOC, dynamic instability, tubulin polymerization as target of drugs.Side and end-binding proteins, capping and severing proteins.*Kinesins and dyneins;* vesicular transport along microtubule, role of kinesin-1 and dynein motors in organelle transport. Role of microfilaments and microtubules in cell migration.

Intermediate filaments; Assembly and tissue specific expression, dynamic nature of intermediate filaments, diseases associated with Lamins and Keratins defects **7 hrs**

Nervous System:

Types and structure of neuron.Myelin sheath; composition and function.Resting membrane and action potential.Nernst and Goldman equations. Mechanism of initiation and propagation of action potential – voltage gated ion channels, ionophores and toxins in study membrane transport. Design and use of Patch Clamp in measuring membrane potential.Neurotransmitters and receptors; synaptic transmission, post synaptic potentials. Outline and functions of autonomic and central nervous systems. **6 hrs**

Muscular System:

Ultra structure of smooth, skeletal and cardiac muscle fibers.Contractile and other proteins of muscle.Energy metabolism in muscle; Phosphagens, neuromuscular junctions, excitation of striated muscles.Organization of sarcolemma, transverse6tubular system and sarcoplasmic reticulum, mechanism of muscle contraction. Regulation of contraction in striated and smooth muscle. Calmodulin and its regulatory role, muscular dystrophies. **6 hrs**

Digestive System:

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Secretion, regulation of secretion, composition and functions of saliva, gastric, pancreatic and intestinal juices and bile.Gastrointestinal hormones. Digestion, absorption and transport of carbohydrates, proteins , lipids, nucleic acids and vitamins. Liver – structure and functions. Detoxification mechanisms .Liver function tests. **5 hrs**

Cardio – vascular System:
Systemic and pulmonary circulation.Structure of blood vessels.Regulation of cardiac activity.Blood volume, blood pressure.Plasma composition and functions of plasma liproproteins.Mechanism of blood clotting, role of role of vitamin K, clot dissolution, anticlotting factors, Formation, counting and functions of erythrocytes, leukocytes and thrombocytes.Lymph, Cerebro spinal fluid (CSF); composition and analysis in diagnosis. **6 hrs**

Respiratory System:
Mechanics and regulation of respiration, pulmonary and alveolar ventilation and its control, transport of respiratory gases, respiratory mechanism of acid-base balance. Anion Gap, Pasteur effect. **5 hrs**

Excretory System:
Mechanism of urine formation and composition of urine.Urine analysis for abnormal constituents, tubular function tests.Nephritis and nephrosis.Kidney hormones.Regulation of acid -base electrolyte and water balance.Respiratory and metabolic acidosis and alkalosis.**5 hrs**

Endocrine system:
Hormones, feedback regulation, biosynthesis, storage, secretion, Circulation in blood. Degradation and peripheral transformation .Receptors and the mechanism of hormone action.Measurement of hormones, and receptors. Disorders of endocrine system.
6 hrs

Self-study topics

1. Homeostasis in Physiology
2. Principle and application of gaseous transport
3. Biochemistry of synaptic function
4. Biochemistry of Aging and age related disorders
5. Principles underlying organogenesis
6. Alterations in respiration under different physiological conditions
7. Hormonal regulation of blood sugar
8. Biological rhythms and Human system:
   Different types of physiological rhythms
   ultradian, circadian, infradian. Different zeitgebers and their relation with circadian clock. Hormonal biorhythms and their significance. Body temperature rhythm. Neural basis of biological clock . Sleep wakefulness cycle. Time keeping genes. Jet lag and shift work.
9. Application of patch clamp method
10. Multiple sclerosis
11. Parkinson's disease

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BCSCT105: Nutrition

4 units (39 hrs)

Carbohydrates:
Occurrence and physiological functions, factors influencing metabolism.Lactose intolerance.Dental caries.Artificial sweeteners.Role of dietary fiber in health and disease.Disorders related to carbohydrate metabolism. Glycemic index of foods and its uses.

Lipids:
Concepts of visible and invisible fats. EFA, SFA, MUFA, PUFA sources and physiological functions. Role of lipoproteins and cholesterol, triglycerides in health and disease.

Proteins
Concepts of essential and non essential amino acids and their role in growth and development. Physiological functions of proteins. Requirements, nitrogen balance concept. Protein efficiency ratio.Methods for evaluating protein quality.Protein energy malnutrition and clinical features and biochemical changes. **12 hrs**

Macro-minerals:
Calcium, Phosphorus Magnesium, Sodium, Cobalt, Potassium, Chloride.
Micro minerals:
Iron, Zinc, copper, selenium, chromium, iodine, manganese, Molybdenum and fluoride.
Ultra trace minerals:
Arsenic, Boron, Nickel, Silicon, Vanadium & cobalt: Digestion &absorption, Functions, Toxicity, interaction with other nutrients. RDA and food sources **6 hrs**

Vitamins and Energy metabolism: Fat soluble vitamins: RDA. Vitamin- A, vitamin- D, E & K. Water soluble vitamins: Vitamin-C, Thiamine, Riboflavin, Niacin, Pantothenic acid, Biotin, Folic acid, Vitamin-B12, Vitamin-B6 (Digestion, absorption and transport and excretion, functions, interaction with other nutrients (if any), Deficiency and toxicity, major sources, Assessment of nutritive value and analysis in food material.

Energy metabolism: Basal and resting metabolism- influencing factors. Methods to determine energy requirements & expenditure. Thermo genesis, adaptation to altered energy intake, latest concepts in energy requirements and recommendations for different age groups. BMR and methods of BMR determination.Factors affecting BMR.

Energy requirements for different physical activities.Specific dynamic action (SDA) of food.

Regulation of food intake: role of hunger and satiety centers, effect of nutrients. Basis for computing nutrient requirements: latest concepts in dietary recommendations, RDAICMR and WHO: their uses and limitations.Food Fortification and government policies and its implications Nutraceuticals and functional foods:Scope, sources and future prospectus of Nutraceuticals. Role of isoprenoids, flavonoids, carotenoids, tocotrienols, PUFA, Lecithin and terpenoids as functional foods. **10 hrs**

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Nutrition in various age groups: Physiological adjustments, Nutritional requk8irements, Effect of malnutrition, and special needs and nutritional problems in Pregnancy, Lactation, infancy, preschool, adolescent, young adults and elderly adults. **4 hrs**

Oxidative stress and Antioxidants: Free radicals: definition, formation in biological Systems. Natural anti-oxidants, defense against free radicals. Role of free radicals and antioxidants in health and disease.Determination of free radicals, lipid peroxides and antioxidants.Antioxidant enzymes and their role. **7 hrs**

Self Study:

1. Basic food groups-Energy giving foods, Body building foods and Protective foods
2. Basis of Nutrigenomics
3. Dietary charts for various physiological conditions
4. Protein sparing action of carbohydrates.
5. Mutual supplementation of food
6. Dietary fibre

BCP – 106: Gen. Biochemistry – I (4 Credits)

1. Preparation of buffers; Acetate, phosphate and trisbuffer.
2. Determination of saponification number and acid value of oils and fats.
3. Determination of iodine number and peroxide value of oils and fats.
4. Determination of pka of weak acids and amino acids by pH metric titration.
5. Determination of alkali content in antacid tablets by titration with acid.
6. Hydrolysis of starch / glycogen and estimation of its purity by H.J. method.
7. Hydrolysis of starch / glycogen and estimation of its purity by Somogy's method.
8. Analysis of water: estimation of calcium and magnesium by EDTA method.
9. Determination of pI of casein from milk.
10. Estimation of total Phenolic content of biological samples
11. Determination of phytic acid.

BCP_107: General Biochemistry – II (4 Credits)

1. Verification of beer lamberts law.
2. Absorption spectra of proteins and nucleic acids and determination of molar extinction coefficient.
3. Estimation of reducing sugars (lactose in milk) by DNS method.
4. Estimation of total sugars by Phenol – Sulfuric acid / Anthrone method.
5. Estimation of total lipids by Folch method.
6. Estimation of protein by Lowry's method.
7. Estimation of inorganic phosphate by Fiske-Subbarao method.
8. Estimation of Iron using ammonium thiocyanate by Colorimetric method.
9. Estimation of Lysine.

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10. Estimation of Tryptophan.
11. Estimation of DNA by Diphenylamine method.
12. Estimation of RNA by Orcinol method.

I sem. M.Sc Biochemistry

BCT 201 Enzymology

4 Units (52 Hrs)

Introduction to Enzymes
Nomenclature and classification of enzymes.Specificity and active site. Fundamentals of enzyme assay – enzyme units, coupled kinetic assay, Enzyme localisation. Zymogen activation, Criteria of purity of enzymes.**4hrs**

The investigation of active site structure: The identification of binding sites and catalytic sites –trapping the E-S complex, use of substrate analogs, enzyme modification by treatment with proteolytic enzymes, photo – oxidation and chemical modification of amino acid side chains (cys, met, his, ser, asp, glu, lys, and tyr). Affinity labeling studies (chymotrypsin triosephosphateisomerase) an and super reactive amino
acid chains (chymotrypsin and glutamatedehydrogenase). Site directed mutagenesis. The 3-D structural features of active sites of chymotrypsin trypsin, elastase and triose phosphate isomerase.**6 hrs**

Enzyme catalysis: Chemical nature of enzyme catalysisand mechanism of -General acid-base catalysis, electrostatic catalysis, covalent catalysis, intramolecular catalysis and enzyme catalysis. Mechanisms of action of the following enzymes- Monomeric enzymes; serine proteases (chymotrypsin, trypsin, elastase), sulphydryl enzymes (papain, lysozyme, ribonuclease, oligomeric enzymes lactate dehydrogenase) and alcohol dehydrogenase multi- enzyme complexes.
(pyruvatedehdrogenase complex). Metal– activated and metallo-enzymes (mechanism of action of pyruvate kinase, creatine kinase, superoxide dismutase &carboxypeptidase – A) and multifunctional enzymes.Artificial enzymes, Abyzmes- Catalytic antibodies. **7 hrs**

Coenzymes: The mechanistic role of the following coenzymes in enzyme catalyzed reactions – nicotinamide nucleotides, flavin nucleotides, pyridoxal phosphate, coenzyme-A, lipoic acid, thiamine pyrophosphate, biotin, tetrahydrofolate and coenzyme B12. **5 hrs**

Kinetics of enzyme-catalyzed reactions: Methods used in the investigation of the kinetics of enzyme-catalyzed reactions, initial velocity studies, rapid reaction techniques and relaxation technique. Enzyme kinetics of single substrate reactions – Michaelis-Menten and Briggs and Haldane theory (rapid equilibrium and steady state theory). Kinetic data evaluation-linear

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transformation of Michaelis-Menten equation. Pre-steady state kinetics.Haldane equation.King-Altman procedure for deriving the rate equation.Effect of pH & temperature on enzymatic reactions, Arrhenius plot, determination of activation energy.
8hrs

Enzyme Inhibition:
Types of reversible inhibitors; competitive, non-competitive,
uncompetitive, and mixed inhibitors. Partial inhibition, substrate inhibition and allosteric inhibition.Irreversible inhibition.**6 hrs**

Kinetics of bi- substrate reactions: Sequential mechanism, compulsory order and random order mechanism, non-sequential mechanism, ping pong mechanism, distinction between different kinetic pathways using primary and secondary plots. Inhibition studies in the characterisation of bisubstrate reactions. Investigations of reaction mechanisms using isotopic exchange at equilibrium.**5 hrs**

Allostery of enzyme action: Binding of ligands to proteins, Co-operativity, the Hill equation, Adair equation, Scatchard plot and equilibrium dialysis techniques. *Sigmoidal kinetics:* MWC and KNF models. Significance of sigmoidal behavior.Allosteric enzymes and metabolic regulation taking ATCase as an example.**6 hrs**

BCT202: Analytical Biochemistry II

4 units (52hrs)

Unit 1
Chromatography:
Introduction, partition coefficient phase systems, liquid and solid phases, principle procedure and application of paper chromatography, parameters employed in column
chromatography, retention, resolution, physical basis of peak broadening , plate height equation, capacity factors, peak symmetry, standard systems of chromatography and its components, stationary phase, elution.
Types of chromatography: Ion exchange, major ion exchange matrices, elution in ion exchange chromatography. Examples of cation and anion exchangers, chromate-focusing.
Gel filtration: matrix used fractionation range and matrices, determination of native mass of proteinbygelfiltration. Reverse phase principle and procedure.
Hydrophobic interactions and affinity chromatograpy:
Affinity ligands immobilization of ligands.Activation of matrices, coupling affinity ligands (example–GSH). Metal affinity chromatography, His tag, open column chromatography, hydroxyl apatite chromatography.
Thin layer chromatography:
Introduction; phases used in TLC preparative TLC, metabolic profiling, solvent systems for TLC. Detection of compounds on TLC plates.**12hrs**

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Gas chromatography:

Principle and design of instrument. Factors affecting GC, stationary phase, mobile phase, column length, diameter, film thickness, flow rate temperature, sample introduction. Detectors: flame ionization, thermal ionization, electron capture, mass selective detection. G.L.C; principle and application.

HPLC: Instrumentation, column, pumps, plumbing, injectors, mobile phases in HPLC, two dimensional HPLC, factors affecting resolution in HPLC chromatography, flow rate and linear velocity. Separate modes: normal and reverse, gradient reverse phase, ion suppression and ion pairing. Chiral-HPLC, chiral columns. Detectors: types, UV, visible fluorescence, electrochemical detectors. Fast protein liquid chromatography (FPLC).**6 hrs**

Electrophoresis: Historical developments, principle, non-denaturing PAGE, activity staining for enzymes, zymogram, denaturing electrophoresis (PAGE), SDS-PAGE, 2D, chemical cross linking of proteins urea electrophoresis,.pulse field electrophoresis
Immuno-electrophoresis: Dot blotting and immune-diffusion tests with antibodies, zone electrophoresis/immune-electrophoresis. Rocket electrophoresis, counter immunoelectrophoresis, Agarose gel electrophoresis of nucleic acids, , physical basis, equipment and applications.
Electroblotting: western, southern, northern blot analysis **12 hrs**

Spectroscopic techniques: Wave particle duality of light, electromagnetic spectrum, transition in spectroscopy. Principle, design and application of UV-Vis spectrophotometry.Principle, design and application of fluorescence spectroscopy. Measurement of fluorescence and chemiluminiscence, use of fluorescence in binding studies. Spectroscopy techniques using plane polarized light, circular dichroism (CD), equipment for CD measurement, CD of biomolecules (proteins) and LD (linear dichroism) of biomolecules. *IR spectroscopy:* Physical basis of IR spectroscopy. Instrumentation, use of IR in structure determination, Fourier transfer,

NMR: Principle, effect of atomic, identity on NMR, chemical shift, spin coupling NMR, measurement of NMR spectra, biochemical application of NMR.

ESR: Principle, measurement of ESR spectra uses of ESR in chemistry.
Mass spectroscopy: Principle, overview of MS- experiment, ionization modes, equipments in MS analysis (Identification of metabolites) MS of protein/ peptides.
Interfacing MS with other methods; MS/MS, LC/MS, GC/MS, electrophoresis/MS.
Uses of MS in Biochemistry: MS and heterogeneity in proteins, peptide mapping, post translation modification analysis, determination of disulfide bridges, analysis of DNA compounds. **22 hrs**

BCT 203 Immunology & Toxicology

4 units (52 hrs)

Unit 1:Overview of Immune system

Typesofinfectionandnatureofinfectiveagents.Cells and organs of the immune system, Structure and functions of immune cells- T cells, B cells, Macrophages, NK cells and dendritic cells, Eosinophils, Neutrophils, Mast cells. Primary and secondary lymphoid organs. Types of immunity-Innate and acquired immunity, Primary and secondary immune response. **6hrs**

Unit 2:Antigens and Antibodies

Structure and properties of antigens –Iso and alloantigens-antigen specificity, Haptens and adjuvants- structure and properties.
Immunoglobulins-Structure and properties, types and subtypes.

Methods of raising antibodies, Purification of polyclonal antibodies.Monoclonal antibodies; Principle of monoclonal generation and limiting dilution cloning, hybrid antibodies and purification of antibodies.**7 hrs**

Unit 3:Complimentsystem

Introduction,alternateandclassicalpathway and regulation. **4hrs**

Unit 4 :TheImmuneSystem

Recognitionofselfandnonself,themajorhistocompatibilityantigens, H-2andHLAantigens,Antigenecity; humoralandcell-mediatedimmunity. TandBlymphocytes;origin,differentiation,characteristicsandfunctions,natureofsurfacereceptors . Antigen processing and presentation.Role of MHC. T cell and B cell interaction. Cytokines, monokines, lymphokines and their functions.

Unit 5:MolecularImmunology

Theories of antibodyformation; clonal selection and network, Geneticsofantibodydiversity,germlineandsomaticmutationtheories, immunoglobulin,MHCaTCRgeneorganizationandtheirrecombination,classswitchofIggenes. Salient features of TCR mediated signalling. **5hrs**

Unit 6 :Clinical Immunology

Immunedisorders;hypersensitivity,autoimmuneandimmunodeficiencydiseases.Tissuetransplantation; auto–iso-,allo-,andxenografts,tissuematching. Transplantation rejection: mechanismandcontrol. Tumorimmunology.**7hrs**

Unit 7:Immunoassaymethods

Antigen–antigeninteraction: affinityandavidity. Determination of affinityandavidityconstants.Principle,procedureandapplicationsofImmunoprecipitation, neutralization,agglutination,complimentfixation,immunodiffusion,immunefluorescence,FACS, RIA,ELISA and microELISATechniques. **5hrs**

Unit 8: Immunization
Vaccines- conventional, peptide vaccines, subunit, DNA vaccines.

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Toxoids, antisera, edible vaccines, plantibodies, ISCOMs, recombinant antibodies, Immune stimulatory complexes.Common immunization programmes- BCG, small pox, DPT, polio, measles, Hepatitis-B.
Applications of immunologically relevant antigens and T cell subtypes in vaccine development.**3 hrs**

Unit 9:Toxicology

Toxicological chemistry,factorsinfluencingtoxicity;Doseresponserelationship–LD50,ED50,NOEL.Reversibility andsensivity.Xenobioticsandendogeneoussubstances. Detoxificationenzymes.Mutations-genotoxicity, Amestest.Carcinogenictoxins.Cytotoxicity: methodstotesttoxicogens.Diagnosisoftoxiceffectsinliver and kidney.Metaltoxicity–Arsenic andlead. **8hrs**

BCT204: Membrane Biochemistry

4 units (52 hrs)

Introduction:
Review of **s**tructure, nomenclature and properties of glycerolipids, sphingolipids, glycolipids and sterols. Properties of lipids in solution, hydrophobic and hydrophilic interactions, Polar lipids and their ability to form mono, bi-layers and micelles, Langmuir trough.
Cell and organelle membranes; Physical properties of bi-layers, Polymorphic phases and molecular shapes exhibited by lipids, use of differential scanning calorimetry (DSC) and P NMR to study transition in phases. Effect of lipid composition and modification on viscosity and fluidity; role of cholesterol, cardiolpin, engineering membrane lipid composition.Models of membranes; Metamorphic mosaic model, Singer-Nicolson fluid mosaic model, Isolation and characterization of membrane lipids.Composition of plasma- and organelle membranes; transbilayer asymmetry; methods to determine membrane sidedness.Asymmetry of lipid distribution in bacterial, plant, and animal membranes, Lateral heterogeneity of membrane lipids; lipid domains, lipid rafts, caveoli, Non bilayer lipids and their role in membranes.
Physical organization of bilayers; human erythrocyte membrane as a prototype plasma membrane, role of cytoskeleton in organization of bilayers.*Liposomes;* preparation, properties and application in membrane biochemistry.

13 hrs

Membrane proteins:
Isolation and characterization of cell membranes.Detergent solubilisation of membrane proteins.Purification and reconstitution of membrane proteins.Erythrocyte ghosts; proteins of RBC membrane and their interaction with cytoskeleton. Classification of membrane proteins based on membrane-protein interaction. Types of integral membrane protein, forces responsible for holding integral proteins in membranes, secondary structure of membrane spanning portions of integral membrane proteins; transmembrane K-helices and L-barrels, hydropathic plots. 3-D structures of typical integral membrane proteins: glycophorin, bacteriorhodopsin, photosynthetic reaction centre. Role of integral membrane proteins in cell-cell interaction and adhesion; selectins, integrins, cadherins.Lipid-anchored membrane protein-acylprenyl-and GPI-anchors.

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Techniques for determination of membrane protein topology: *Biophysical methods:* X-ray crystallography, Freeze-fracture electron microscopy, Spin labeled ESR, NMR. *Biochemical and molecular biological methods*: Membrane protein dynamics. Lateral and rotational diffusion of integral membrane proteins. Fluorescence photobleaching recovery (FRAP). Single particle tracking.Lipid-protein interactions.Atomic force microscopy.

10 hrs

Membrane transport:

Relative permeability of pure phospholipid bilayer to various molecules. Fick's Law of Membrane Transport

Diffusion across the plasma membrane.Partition coefficient and hydrophobicity.Energetics of moving non polar and polar molecules across lipid bilayer (PM). Experimental methods for study of membrane transport: Assay of membrane transport, use of liposomes to study single types of transporters. Mechanism for transport**:** Properties of passive diffusion**,** facilitated diffusion, active transport and co-transport. Electrically neutral and electrogenic transport, Kinetics and model of Glut-1 uniport ATP–driven pumps; classification, and working mechanism. ABC-transporters; MDR1, CFTR Channels and pores. Transport across organelle membranes. Ion channels; working and voltage gating, ionselectivity, electrochemical gradients,

Nernst Equation, working of bacterial K-channels, aquaporinns, ionophores. *Bacterial transport systems;* Lactose permease, Phosphotransferase and sugar binding proteins.

Donnan membrane equilibrium

7 hrs

Intracellular compartments: Proteins sorting; Membrane enclosed organelles of eukaryotic cells, evolutionary origin and topological relationships. Protein trafficking: Sorting signals.

Mechanisms: Gated transport, transmembrane transport, vesicular transport. Signal sequences: Experimental evidence, Genetic experiment to demonstrate protein translocation.Transport of molecules between nucleus and cytosol. Nuclear pore, nuclear localization signals, nuclear transport receptors, nuclear export: Ras-GTPases- directionality. Regulation of nuclear import and export.Transport of protein into mitochondria and chloroplast, signal sequence, experimental setup to study protein translocators, working of TOM & TIM complexes.Energy requirement in protein import. Signal sequence for thylakoid membranes, peroxisomes. Endoplasmic reticulum, structural and functional diversity of endoplasmic reticulum, isolation of rough ER and signal sequence for protein import.

Signal Hypothesis: Signal Sequence, SRP-receptors for protein import to ER. Organization of translocation pore, Sec61 complex.**8 hrs**

Topology of membrane protein: Protein translocation, cotranslational, post translational translocation. Start transfer and stop transfer signals for single pass transmembrane protein and multipasstransmembrane proteins. ER retention signals, Glycosylation in ER. N-linked oligosaccharide, Dolichol-linked oligosaccharides. Folding of proteins in ER, role of chaperons Calnexin and Calreticulin. N-linked oligosaccharides as timers for protein turnover, ubiquitination and protein degradation (improperly folded proteins), GPI-anchored proteins.

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5 hrs

Biogenesis of lipid bilayers: Intracellular vesicular trafficking: Maintenance of compartmental diversity, pathways of endocytosis and secretory. Vesicles: types, study of cell free system, genetic approach, use of GFP. Clathrin coated- Assembly and disassembly, Structure and function. significance. Coatemer coated-Assembly and disassembly, Structure and function.Retromer assembly on endosomal membrane.Coat assembly control by monomeric GTPases.Role of Rab proteins in vesicular targeting.SNARE proteins and their role in vesicular transport and membrane fusion.Experimental proof for SNARE requirement in vesicular fusion.Entry of enveloped viruses into cell.Coatemer coated vesicles: COP-II-coated transport vesicles, transport of cargo from ER to Golgi, homotypic membrane fusion. Retrieval pathway to ER.
Compartments of Golgi: processing of oligosaccharide chain in Golgi, N-linked, proteoglycan assembly in Golgi. Transport through *trans*Golgi network to lysosomes. Mannose-6-phosphate receptors. Signal patch for mannose-6-phosphate lysosomal storage disease. Endocytosis: Phagocytosis, Pinocytosis- vesicles, receptor mediated endocytosis. Retrieval of proteins in endosomes, multivesicular bodies sequestration of endogenous proteins. **9 hrs**

BCSCT – 205: Microbiology

3 units (39 hrs)

Microbial classification:
Taxonomy- definition, phenetic and phylogenetic classification.binomial nomenclature, international code of nomenclature of prokaryotes. Taxon, species, strain.
Molecular methods in taxonomy- nucleic acid base composition, DNA homology, DNA-RNA homology, rRNA sequencing, DNA fingerprinting, Flow cytometry
Chemotaxonomy: cell wall components, lipid composition, isoprenoid-quinones, cytochrome composition, fatty acid profiles; Numerical taxonomy.
Classification of bacteria according to Bergey's Manual of systematic Bacteriology, Dichotomous keys, Cladograms, dendrograms, universal phylogenetic tree. **6 hrs**

Eukaryotic micro organisms: distribution and occurrence, morphology, structure, mode of reproduction, economic importance of protozoans, algae and fungi.Cultivation of protozoans, algae and fungi. **5 hrs**

Morphology and ultrastructure of bacteria:
Different cell morphology, flagella, pili, capsule, cell wall, cell membrane, cytoplasm. Inclusion bodies, nucleoid, plasmids, reserved food materials (metachromatic granules,polysaccharide granules, poly β hydroxybutyrate granules, cyanophycean granules and sulphur globules), endospores and exospores.
General properties of coliform, spore formers, lactic acid bacteria, actinomycetes, rickettsiae, spirochetes, cyanobacteria and Archaea.Gram, Acid fast &flagellar.**6 hrs**

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Viruses, Viroids and Prions (Acellular entities)
General characters, Structure, nomenclature and classification, Isolation, cultivation and identification of Viruses (Growing in Bacteria, animal inoculation, embryonated eggs, Cell Cultures).Inactivation of viruses by physical and chemical agents.Virioids and Prions - General properties and diseases caused by viroids and prions.**5 hrs**

Nutrition, Cultivation and control: Micro and macro nutrients, Nutritional types of bacteria. Culture media**,** classification of media (Simple,complex and special media with example).
Growth- Growth curve, factors affecting growth. batch, continuous.
Microbial growth control- Physical methods (Heat, Pasteurization, Filtration, Radiation, Dessication, Low Temperature, High Pressure, Osmotic Pressure) and Chemical Methods (Phenols, Halogens, Alcohols, quaternary ammonium compounds). **5 hrs**

Microbiological methods:
Enumeration methods-DMC, SPC, MPN, MFT and turbidometric.Evaluation of antiseptics and disinfectants.Antibiotic assay. Determination of MIC- disc and well diffusion method **3 hrs**

Fluorescence Microscopy:
Fluorophores, principle and applications of fluorescence microscopy, design and uses of Epifluorescencemicroscoy, and immunofuorescence microscopy. Imaging live cells and tissues; time lapse imaging, fluorescence stains of living cells, reporter molecules, multidimensional imaging . Measuring cellular dynamics; calcium imaging in live cells, fluorescence recovery after photo bleaching (FRAP), Fluorescence resonance energy transfer (FRET). **4 hrs**

Industrial microbial Technology:
Antibiotic production- Penicillin, biotransformation of steroids. Production of biofuel- biogas biodiesel, microbial fuel cells and Bioleaching **5 hrs**

BCP206: Biochemical and Immunochemical Techniques (4 Credits)

1. Ascending descending and circular paper chromatography of amino acids / carbohydrates
2. Two-dimensional chromatography of amino acid / carbohydrates.
3. Thin layer chromatography of carbohydrates / amino acids.
4. 4. Gel permeation chromatography of pigments/proteins.
5. Separation of proteins by non-denaturing PAGE.
6. Determination of molecular weight of Proteins by SDS PAGE
7. Separation of isoenzymes by isoelectric focusing
8. Ion exchange chromatography of nucleic acids / proteins.
9. Demonstration of Ag-Ab interaction: Radial immuno-diffusion and ODD.
10. Demonstration of direct agglutination reaction using human blood group antigens.
11. Demonstration of indirect agglutination reaction-latex agglutination.
12. Bacterial agglutination (WIDAL)

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13. Antibody titration – ELISA; Direct, Indirect, sandwich, and micro ELISA.
14. Purification of antibodies; conventional (isolation of IgY from Egg yolk).
15. Rocket electrophoresis.
16. Western blotting of proteins and Immuno-detection.

BCP – 207: Enzymology (4 Credits)

1. Determination of total activity of pea esterase.
2. Determination of KM and Vmax of pea esterase.
3. Determination of optimum pH of pea esterase.
4. Determination of pH stability of pea esterase.
5. Determination of optimum temperature and activation energy of pea esterase.
6. Determination of temperature stability of pea esterase.
7. Determination of type of inhibition (reversible or irreversible) of pea esterase.
8. Determination of I50 of pea esterase using organophosphate inhibitor.
9. Determination of total activity of salivary K-amylase /L-amylase (sweet potato or germinated ragi).
10. Determination of Km and Vmax of K-amylase / L-amylase.
11. Determination of Km and Vmax of alkaline phosphatase (potato).
12. Determination of type of inhibition (reversible or irreversible) of alkaline phosphatase.
13. Determination of I50 of alkaline phosphatase.
14. Determination of inhibitor constant, Ki of alkaline phosphatase.
15. Determination of optimum temperature and activation energy of urease (horsegram).

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III Semester MSc Biochemistry

BCT 301 Molecular Biology I

4 UNITS (52 hrs)

Introduction: Historical account of DNA discovery. Relationship between genes and proteins, central dogma of molecular biology. Review of physical chemistry of DNA. RNA as genetic material.Variation in size and shape of genomes; ultracentrifugaion and electron microscopic methods to study the shape and size of genomes.Relationship between size of genome and genetic capacity; C-value paradox. Organelle genomes, Genome sequence and gene numbers, measurement of expressed genes. **4 hrs**

Clusters and repeats; tandem repeats, mini- micro- satellites and interspersed genome-wide repeats and their significance. Pseudo genes and transposable elements. Globin gene clusters, gene duplication and gene evolution, biases in mutations, gene conversion and codon usage. **3 hrs**

Prokaryotic DNA Replication: Replicon, single and multi copy replicons, linear and circular replicons, unidirectional and bidirectional replication, experimental methods, mapping origin of replication, semi-conservative and semi-discontinuous replication; experimental demonstrations. Topological problems in DNA replication; topoisomerases, helicase and gyrase. Mechanism and classification of topoisomerases, assay of topoisomerases Priming DNA synthesis in bacteria; experimental evidence, components of primosome,. Initiation at origin (oriC) of E. Coli. Creation of replication forks.Regulation of initiation at origins, sequesteration of origins after replication, role of helicase, assay of helicase.

6 hrs

Enzymology of DNA replication; DNA polymerases, chemistry of nucleotide polymerization and in vitro assay, Hand-palm structure of DNA polymerases.Use of conditional lethal mutants and in-vitro complementation methods for identification of replicative polymerase.Properties and functions of DNA polymerase-I, Korenberg enzyme.Subunit composition of polymerase – III holoenzyme, identification of functions of individual subunits by complementation and mutational studies mechanism of replication of E. coli DNA-trombone model, termination of replication.Processivity and fidelity of replication. Bacterial replication and its connection to cell cycle **5 hrs**

Eukaryotic DNA replication; Replicative and repair enzymes of eukaryotes.Initiation, elongation by eukaryotic DNA polymerases.ARS of yeast, ORC, Licensing factors and control of eukaryotic DNA replication, role of MCM proteins.Replication of organelle genomes, maintenance of ends of linear DNAs; telomeric DNA and telomerase.Regulation of eukaryotic DNA replication and inhibitors of DNA replication. **5 hrs**

Extra chromosomal replication: Replication of phage DNA φX174, SV-40,rolling circle model of replication. Linear DNA-ends, terminal proteins, replication of plasmid DNA.**2 hrs**

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Replication of RNA viruses: ss +RNA viruses; Picarna (Polio) and ss-RNA viruses, orthomixovirus (influenza virus). dsRNA- reovirus (Rota virus), Structure and mechanism of RDR pol. Retroviruses; Structure and mechanism of reverse transcriptase and integrase (HIV), replication of tumor virus (RSV).Replication of Qβ virus. **4 hrs**

DNA repair: experimental demonstration of repair in prokaryotes, damaging agents and damage recognition, direct repair, Mis-match repair assay for mismatch repair, Base excision repair (BER), Nucleotide excision repair (NER) systems; components and mechanism of repair, error prone repair, SOS and Rec-A. Eukaryotic BER and NER, controlling direction of mismatch repair, DNA damage in chromatin. **5 hrs**

Transcription in prokaryotes: The transcriptome, prokaryotic RNA polymerase; molecular composition, and mechanism of transcription. Initiation of prokaryotic transcription; Structure of bacterial promoters.Effect of sigma factor on binding of RNA pol. to promoters. Structure and function of sigma factor, reuse of sigma factor (sigma cycle). Sigma movement relative to DNA: FRET assay. DNA melting at promoters, promoter clearance.Role of α-subunit in upstream element recognition.Foot-printing of upstream elements with α-subunit. Elongation: Role of β-subunit in phosphodiester bond formation. Structure of elongation complex and core polymerase. Termination of transcription: Rho- dependent and independent, termination, RNA product under Rho dependent termination. **6 hrs**

Transcription in eukaryotes: Separation of nuclear RNA polymerases- rat liver RNA pol. Roles of the RNA polymerases. Sensitivity to α-amanitin. Subunits of RNA pol-II (yeast polII). Heterogeneity of Rpb1 subunit. Formation and maintenance of transcription bubble. Eukaryotic promoters: Class-II core promoter, modular organization, SV40 early promoter. Linker-scanning mutagenesis, TATA Box, downstream promoter elements, proximal promoter elements, TATA-less promoters and initiators.Class-I and Class-III promoters, Enhancers and silencers.Class-II pre-initiation complex, foot-printing DAB Structure and function of TFIID, TBP and associated factors (TAFs).Phosphorylation of CTD of RNA pol-II, Mediator complex and RNA pol-II. Elongation factors: Effect of TFIIS, reversal of transcription arrest, proof reading of transcripts. Composition and working of transcription units at class-I and class-III promoters. **7 hrs**

RNA processing: split genes, RNA splicing: R-looping experiments, splicing signals, effect of splicing on gene expression. Splicing of nuclear mRNA precursors.Branched intermediate, mechanism of RNase T_1 and T_2, direct evidence for a branched nucleotide. Signal at branch. Spliceosomes: snRNPs, U1snRNP, detection of spliced product by RNase protection assay. U6snRNP, U2snRNP and U4snRNP.Spliceosome assembly and function.Alternative splicing, exon-intron definition.Commitment of precursor RNA to splicing, role of sr protein. Yeast two hybrid assay. Role of RNA pol-II in splicing, control of splicing.Self splicing RNase.Group-I introns, demonstration of exon ligation, Group-II introns. Post transcriptional modification of mRNA: Structure of cap, purification of caps, capping substrate, functions of cap. Polyadenylation: Function of poly A, mechanism and signals for polyadenylation. Cleavage and Polyadenylation for mRNA elongation of poly-A, poly-A binding protein (PABP),

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turnover of poly-A. Coordination of mRNA processing with Coupling termination and mRNA 3' end processing. **5 hrs**

References:

1. Biochemistry and Molecular Biology of Plant; Buchanan, Gruissum and Jones, (2000),ASPP, USA.
2. Biochemistry; David Rawn, Panima Publishers (2012).
3. The Bacteriophages; Richard Calendar, 2nd Edition, Oxford University Press (2005).
4. Basic Virology; Wagner and Hewlett; Blackwell Science, (2004)
5. LEWINS Gene XI; J.E. Krebs, E.S. Goldstein, and S.T. Kilpatrick, Jones and Barttlett Publishers (2012).
6. Molecular Biology of the Cell, Alberts et al., Garland Publications, (2012).
7. Molecular Biology, David Freifelder, Narosa Publishers, (1997).
8. Molecular Biology Robert F. Weaver, McGraw Hill (2012).
9. Microbial Genetics; Maloy et al., Jones and Bartlett Publishers, (1994).
10. Modern Microbial Genetics; Uldies N. Streips and Ronals E. Yasbin, Wiley Leis Inc. New York, (2002).
11. Molecular biology and Biotechnology; 4th Edn., J.M. Walker and R. Rapley, RSC (2000).
12. Molecular Biology of Gene; Watson, J.D. et al., 5th Edn. Pearson Education; (2004).
13. Principles of Virology; S.J. Flint et al., ASM Press (2000).
14. Chromatin structure and Gene Expression; 2nd Edn. Sarah Elgin, Jerry Workman, OxfordUniversity Press (2000)
15. Molecular Cell Biology; Harvey Lodish 5th Edn. (2010)
16. Biochemistry 5th Edn. Jeremy M. Berg, John L. Tymoczko, Lubert Stryer (2011).
17. Viruses: Biology, Applications, and Control; David Harper, Garland Science (2011).

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BCT 302 – MOLECULAR PHYSIOLOGY

4 Units (52hrs)

Nervesignaling:originandmechanismofactionsofneurotransmitters (Acetylcholine, catecholamine,serotonin;aminoacids(glutamate,aspartate, GABA,and glycine)and neuropeptides(somatostatin/enkephalins).
Trafficking proteinsofsynapticvesicles,vesiclecycle–exo–andendocytosisofsynaptic vesicles.

Structure,subtypesand functionsof receptorsofACh,GABA,Glycine,Serotoninandglutamate and peptideneurotransmitters,activation by ligands & interactionwith effectors.Role of agonists &antagonists of neurotransmitters. Biochemical basis of neurological diseases. Natural,geneticandenvironmentalfactorsaffectingthedevelopmentofCNS,Co–ordination betweennervousand endocrinesystems. **13hrs**

Endocrine signaling: Introduction, hormones as signal molecules – peptide, amino acid derivatives, steroid, ecosanoids; regulation of hormone secretion; Overview of signal transduction, signalling cascades, Intracellular signaling proteins (adaptors, activators, bifurcators, integrators, effectors, etc.).signal amplification.
Signal transduction pathways of – 1) G P– protein-coupled Receptors: Overview of signalling, second messengers (cAMP, cGMP, phosphoinositides), effectors (Adenylate cyclase, guanylate cyclase, Phospholipase- C), transcriptional activation by CREB, regulation of GPCRs, examples (epinephrine, glucagon), GPCRs in sensory perception .
Biochemistry of vision: Structure of an eye, lens and retina, perception of light, rods and cones, rhodopsin, primary events in visual excitation, cGMP and transduction in generation of nerve impulse, colour vision)

2) Ion–channel (ACh), 3) Receptor Tyrosine kinase {(RTK): Ras-MAP Kinase pathway [EGF, IGF, insulin]}, transcriptional activation byMAP kinases (Erk – fos – jun – cyclin – D), IP3 and DAG in RTK signaling pathways, examples (EGF, insulin)3) Intrinsic enzyme / cytokine receptors and 4) specificity of protein kinases
Arachidonic acid, Ca^{2+}, and NO as second messengers and their regulation;
Nuclearsignaling:Steroid,thyroid,Vitamin-Dandretinoicacidreceptorsandtranscriptional activation.Transcriptionalactivationbyphosphorylationcascade;CREB. **19hrs**

Cell Cycle: Overview of cell cycle, Growth factors and cytokines, growth phases and check points of cell cycle (DNA replication and spindle- attachment checkpoint) and their regulation. Cyclins and cyclin-dependent kinases.Promotion of G1/S by growth factors, cell cycle arrest at G1, role of Rb proteins in cell cycle arrest, entry of cell from G2 to M – phase Role of M – Cdk, MPF. Regulation of M- phase (role of mitogen, survival factor and TGF- β). Role of ubiquitin.
Stem Cells: Embryonic and adult stem cells; unique properties, and potential applications.
Apoptosis: Discovery, intrinsic and extrinsic pathway,morphologicalchanges,signal transduction via TNFpathway,Fas pathway. Role of caspases,.Distinguishingapoptoticcellsfromnecroticcells.Hyperactiveapoptosis and treatment in HeLacells.
Cancer: Introduction, causesandsymptoms,pathophysiology, diagnosis,preventionand treatment. Etiologyofbreast,colonand prostatecancerSignalingcascadesincancer (MAPkinases, Raspathways,JAK-STAT and TGF-βpathways). **20hrs**

References

1. Signal transduction and human disease; Toren Finkel, and J. Silvio Gutkind, John Wiley & Sons, Inc. (2003)
2. Greenspan's Basic and Clinical Endocrinology; 9th Edn. David Gardner and Dolores Shoback Lange Clinical Medicine (2012).
3. Biochemistry of Signal Transduction and Regulation; Gerhard Krauss, Wiley-VCH (2003).
4. Elements of Molecular Neurobiology; 3rd Edn. C. U. M. Smith, John Wiley & Sons Ltd, (2002).

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5. Basic Neurochemistry; George Siegel et al., (1999) Wippincott, Williams and Wilkins.
6. Neuroscience; 2nd edn. Purves, Dale; et al., Sinauer Associates, Inc. (2001).
7. G-Proteins coupled Receptors; P. Michael Conn Academic Press (2013).
8. Molecular Biology of the Cell; 6th Edn. Bruce Alberts, Alexander Johnson, Julian Lewis, David Morgan, Martin Raff, Keith Roberts, Peter Walter; Garland Science (2014).
9. Molecular Cell Biology; Lodish et al., 7th Edn. W.H. Freeman and Co. (2012).
10. Cell Signaling; Wendell Lim, Bruce Mayer, Tony Pawson; Garland Science (2014).
11. Cell Biology; A short course; Stephen R. Bolsover et al., John Wiley & Sons, Inc. (2004)
12. Electrochemical methods for neuroscience; Michael AC, Borland LM, editors. Boca Raton (FL): CRC Press (2007).
13. Signal Transduction; Lewis Cantley, CSHL Press (2014).
14. When Cells Die; A Comprehensive Evaluation of Apoptosis And Programmed Cell Death; Richard, A. Lockshin, and Zahra Zakeri, Wiley Liss (2004).
15. Neuroscience; 2nd edn. Purves, Dale; et al., Sinauer Associates, Inc.; (2001).
16. Biochemistry of Signal Transduction and Regulation; 3rd Edn. Gerhard Krauss, Wiley-VCH,
(2003).
17. The Biology of Cancer; Robert A. Weinberg; Garland Science (2013).

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BCOET 303.1 Metabolism I

4 Units (52 hrs)

Carbohydrate metabolism; Introduction, glycolytic pathway and regulation.Rapoport-Leubering cycle –importance.Gluconeogenesis.pathway and regulation..The TCA cycle and its regulation. Alternate pathways: HMP pathway, Enter – Doudoroff, Glucuronate and Glyoxylate pathway, Cori's cycle, Futile cycles and anaplerotic reactions. **8 hrs**

Glycogen and starch metabolism: degradation, synthesis and regulation, glycogen storage disorders. Pasteur effect, fermentative pathways in microorganisms. Warburg effect.Regulation of blood glucose level, hypoglycemiaand hyperglycemia. Diabetes mellitus: introduction, biochemical and clinical changes associated with IDDM and NIDDM, control of hyper glycemia, diagnosis of Diabetes mellitus and GTT. Pentosuria, Hexose interconversion,

fructose and lactose intolerance, Glucose 6 phosphate dehydrogenase deficiency,fructosuria,galactosemia. **12 hrs**

Bioenergetics: Basic concepts of metabolic energy capture and transfer. Biochemical energetic group transfer reactions of ATP, phosphate group transfer potential of ATP and other high energy phosphate donors.Quantification of the Reactivity of Phosphate Functional Groups.Stages in extraction of energy from fuel molecules. **2 hrs**

Biological oxidation: Biological redox couplers, participation in oxidative metabolism. Free energy changes in electron transfer reactions. Mitochondrial electron transfer system Chemical nature, topology and thermodynamic design of electron carriers. Sequence of electron carriers Isolation of mitochondrial complexes, reconstitution experiments and study of specific inhibitors of Electron Transport Chain. Reverse Electron Transport chain. **4 hrs**

Oxidative phosphorylation: Mechanism of proton pumping. Proton motive force and the Mitchell hypothesis.FoF1-ATPase- structure and mechanism, O18 exchange.Coupling of electron transfer to ATP synthesis.Uncouplers, inhibitors and ionophores, partial reactions of OP, P/O ratios and their use in localization of sites of ATP synthesis along the chain.
Mechanism of oxidative phosphorylation . Boyer's Binding change mechanism of Rotational catalysis, mitochondrial specific transport systems and energy charge- Glycerophosphate and Malate-Aspartate shuttle system. Microsomal electron transport.Proton motive force in Halobacteria, ATP synthesis in bacteria.H + pumping by bacteriorhodopsin Photosynthetic electron transport.Structure and function of chloroplast ATP- synthase. **7 hrs**

Lipid metabolism: Oxidation of fatty acids: even and odd numbered, unsaturated and branched chain fatty acids. Catabolism of triacylglycerols and phospholipids. Glycerol metabolism Energetic of β-oxidation. Brief mention of alpha and omega oxidation.Metabolism of ketone bodies; their formation, oxidation and clinical significance.Biosynthesis of triacylglycerols, phospholipids and sphingolipids.Sphingolipidodystrophies.Biosynthesis of steroids from isoprenoid units.Metabolism of prostaglandins.Cholesterol biosynthesis, catabolism and regulation.Transport of cholesterol -LDL receptor pathway. Reverse Cholesterol Transport. Lipoproteinemias, fatty liver, hypercholesterolemia.Chemical composition, biological functions and metabolic fate of VLDL, LDL and HDL. Arachidonic acid metabolism- Leukotrienes **11 hrs**

Photosynthesis: Introduction, chloroplast/thylakoid structure. Ultra structure and organization and lipid composition of thylakoid membranes. photosynthetic apparatus - PS I and PS II.Hill reaction, light reaction, cyclic- and non-cyclic photophosphorylation. Quantum yield of Photosynthesis. Dark reactions, CO2 fixation : C3and C4-pathways. Crassulacean acid metabolism. Photorespiration.Bacterial photosynthesis.**8 hrs**

References

1. Biochemistry; Voet , D. and Voet, J.G. [Eds.] (1999) 3 Ed. Jhon Wiley and sons.
2. Biochemistry; David Rawn, J. (1989) Neil Patterson Publishers.
3. Principles of Biochemistry; Smith et al., [Ed.] (1986) McGarw Hill.
4. Bioenergetics; A Practical Approach, G.C. Brown and C.E. Cooper (1995) IRL- Oxford

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University Press.
5. Photosynthesis, D.O. Hall and K. K. Rao, (1999), 6th Edn. Cambridge University Press.
6. Hawk's Physiological Chemistry, Oser (1976) 14th Edn Tata-McGraHill.
7. Advances in Carbohydrate Chemistry and Biochemistry; Horton, Elseveir (1994).
8. Biochemistry of Foods, Eskin Elseveir (2012).
9. Text Book of Biochemistry with Clinical correlations; 6th Edn. Thomas M. Devlin, Wiley-Liss (2012).
10. Lehninger- Principles of Biochemistry; D. L. Nelson and M.M. Cox 6th Edn. Macmillan Publications (2012).
11. Biochemistry and Molecular Biology; 5th Edn. D. Papachristodoulou, A. Snape, W.H. Elliott, and D. C. Elliott, Oxford University Press (2014).
12. Biochemistry; David Rawn, Panima Publishers (2012).

BTS 307- Research Methodology

Total hours -42

Unit – I Introduction to Research:

Meaning of Research, Types of Research, Research and Scientific Method, Research Process, Criteria of Good Research, Research Problem - Identification and defining a research problem, **10 hrs**

Unit- II ResearchDesign: Collection and review of research literature, Sources of literature and their evaluation. Designing research methodologies, Framing the objectives, Formulation of hypothesis, execution of designed experiments. **10 hrs**

Unit III Research Process & Report: Analysis of data, Presentation of research findings.General strategies for report writing. Organization of the research report, Contents of report, Bibliography, Appendices,Stylemanuals, Criteria for the evaluation of the research report.**8 hrs**

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Unit IV Research Ethics: Preparation andPresentation of proposal to the ethical committee for approval. Causes of unethical acts. **2 hrs**

Unit V Intellectual property rights: Patenting and the procedures involved in the application for patents and granting of a patent, Compulsory licenses, Patent search, Patent Cooperation Treaty (PCT), Examples of patents, Legal implications, Patent exploitation and protection. **12 hrs**

Reference

1. Research Methodology: Methods and Techniques - C. R. Kothari, 2nd Edition, New Age International Publishers.
2. Research Methodology: An Introduction" by Stuart Melville and Wayne Goddard,2nd edition, Juta and Company Ltd
3. RESEARCH METHODOLOGY a step-by-step guide for beginners by Ranjit Kumar
4. "Research methodology"second edition, by Paneerselvam, PHI publishers.

BCP – 305: Clinical Biochemistry (4 Credits)

Analysis of Blood and Urine for diagnostic investigations

1. Estimation of glucose by Folin Wu method.
2. Estimation of glucose by Dubosky's method.
3. Estimation of cholesterol by Zack's method.
4. Estimation of haemoglobin by Wong's method
5. Estimation of urea in blood by Diacetylmonoxime method.
6. Estimation of serum calcium by Clark and Collips method.
7. Determination of A/G ratio by Biuret method.
8. Analysis of SGOT-SGPT (AST, ALT) / creatine kinase / acid or alkaline phosphatase.
9. Qualitative analysis of Urine sample for normal and abnormal constituents.
10. Determination of titrable acidity of urine.
11. Estimation of uric acid in serum and urine by Caraway's method
12. Estimation of creatinine and creatine in serum and urine by Zaffe's method.
13. Estimation of urea in urine by Nesslerization method (Urease method).
14. Determination of urine Chloride by Volhard-Arnold method.

15. Estimation of 17-ketosteroid by Zimmerman's method.
16. Estimation of urine Bilirubin.
17. Lipid profile

BCP – 306: Molecular Biology (4 credits)

1. Isolation, quantification and characterization (Spectrophotometric and agarose gel electrophoresis) of genomic DNA from bacteria (E. *coli*).
2. Isolation, quantification and characterization (Spectrophotometric and agarose gel electrophoresis) of genomic DNA from plant by CTAB method.
3. Isolation, quantification and characterization (Spectrophotometric and agarose gel electrophoresis) of plasmid DNA from animal source. (Not by TCA method)
4. Isolation, quantification and characterization (Spectrophotometric and agarose gel electrophoresis) of total RNA from bacteria, mRNA from plant and microbial sources.
5. Restriction digestion and ligation of DNA.
6. Spectroscopic determination of melting temperature(Tm) of calf thymus DNA.
7. Amplification of desirable gene by Polymerase chain reaction.
8. Rapid amplification of polymorphic DNA.
9. Reverse transcriptase- Polymerase chain reaction RT-PCR
10. Southern blotting
11. Phage Titration.

BCT 401 Molecular Biology II

4 UNITS (52 hrs)

Ribosomes: Prokaryotic ribosomes; molecular components, in vivo assembly, dissociation of subunits, and polysomes.Eukaryotic components and their assembly, organelle ribosomes **3 hrs**

Genetic code; breaking the code, experimental results leading to deciphering genetic code, coding properties of mRNA, Co-linearity of genes and proteins, **decoding** properties of tRNA, triplet binding assay, use of synthetic oligo nucleotides (works of Khorana and Neirenberg), base pairing between codon and anti-codon, Wobble **hypothesis** Properties of genetic code, deviation from universal genetic code. **Contextual reading 4 hrs**

Translation: Initiation of protein synthesis in prokaryotes, Shine-Dalgarno sequence, formation of initiation complexes; effect of GTP hydrolysis by IF2. exchange of ribosomal subunits. Eukaryotic translation initiation-scanning model, eukaryotic initiation factors, role of eIF4E, F, and G. Formation of stable 48S initiation complex, role of eIF1 and eIF1A, toeprint assay, direction of polypeptide synthesis and mRNA translation. Control of translation in bacteria and eukaryotes. Elongation ; Amino acyl-tRNA synthetases, formation of ternary

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complex among amino-acyl tRNA, EF-T, and GTP, three site model of ribosome, peptide bond formation, ,Termination of translation :stop codon suppression-**supressor tRNA**, release factors, aberrant termination, non-stop mRNAs, , no-go-decay of mRNA. Inhibitors of prokaryotic and eukaryotic translation. Mechanism of translational control **(trasferrin/ferritin)** .Post-translational modifications of proteins. **7 hrs**

Gene Expression in Prokaryotes: Definition of regulon, operon, *cis* and *trans* acting elements. Bacterial trascription control; induction and diauxy.Discovery and structure of lac operon.Utility of merodiploids in understanding regulation of operon.Molecular basis of repression.Isolation of repressor, assay of binding of lac operator and repressor.Effect of repressor on dissociation of RNA pol. Positive control of lac operon; mechanism of action of CRP/CAP, transcription activation by recruitment, characterization of binding of cAMP-CAP-DNA.Activation of lac **PI** transcription by CAP-cAMP.Catabolite repression, inducer exclusion and prevention mechanism.Anatomy and regulation of arabinose and tryptophan operons.Riboswitches; discovery and models of riboswitch action.Phage strategies; Regulatory cascade controlling lytic development.Functional clustering of phage genomes.Antitermination in lambda phage, maintenance of lysogeny by lambda phage.Characterization of λ-repressor-DNA binding, molecular properties of λ-repressor, establishment of lysogeny.Sigma switching in phage infection. **7 hrs**

Gene Expression in Eukaryotes: Stages of regulation of gene expression in eukaryotes; Chromatin structure and its effect on transcription .Nucleosome positioning; SV 40 mini chromosome, experimental location of nucleosomal positions; DNase hypersensitive sites and mapping.Locus control regions.Histone modifications; Acetylation of histone tails.Identification of histone acetyl transferases (HATs).Properties and roles of P55 and Gcn-5 HATs.Histone deacetylases; experimental demonstration of HDACs in repressor complexes.**5 hrs**

Chromatin remodeling; Major classes of remodeling complexes; assay of remodeling; ChIP.Composition of SWI2/SNF2 and ISWI complexes.Model of SWI2/SNF2 mechanism.Remodeling in yeast HO gene and human IFN-β promoter.Histone code.Heterochromatin silencing; chromo and bromo domains, histone methylation, HMTases, SFR and RAP-proteins.Transcription elongation through nucleosomes; FACT and PARP. **5 hrs**

Mapping and quantifying transcripts; Northern blots; S1 mapping of 5' and 3' ends of transcripts. Primer extension, and G-less cassette transcription, measuring in-vivo transcription rate- nuclear run on transcription.Quantification of gene expression by measuring protein product. **4 hrs**

Transcriptional activators; classification, structure and function, domains of activators.DNA binding motifs; Zn fingers- Gal 4 activator of yeast.Nuclear receptor- structure and function of glucocorticoid, thyroid and orphan receptors.Domains of nuclear receptors; homeo, bZIP and bHLH domains.Modularity of domains of activators; chimeric transcription factors- Gal4-LexA, two hybrid assay.Dimerization of activators, modular arrangement of enhanceosomes.Recruitment of TFIID and holoenzyme; evidence, role of enhancers, interaction between enhancer and promoter-control region of human metallothionine gene.Insulators-working, insulator bodies, Co-activators and mediators; discovery of mediators- mediators factors; activation of CRElinked gene model for nuclear receptor activation.Regulation of **activity of** transcription factors- phosphorylation, ubiquitination, sumoylation and acetylation. **6 hrs**

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Regulation of gene expression via stability of mRNA; Casein mRNA and transferring-receptor mRNA, gel mobility shift assay for IRE binding protein, model for TFR mRNA destabilization by iron. RNA interference; post transcriptional gene silencing (PTGS) and quelling. Definition, mechanism of RNAi. Classical experiments with petunia and C. elegans. Simplified model, composition and function of Dicer and RISC.Role of Argonaute.siRNAs, role of RNAi machinery in heterochromatin formation and gene silencing- EF1A gene. miRNAs; control of gene expression by miRNAs example and experimental proofs, pathways of gene silencing by miRNA.. **5 hrs**

Molecular biology of Drosophila development; Overview of D .Melanogaster development Differential development by Morphogenic gradient, dorso-ventral patterning of embryo.Regulatory DNAs, role of snail and twist proteins in patterning. Localization of biocoid and nono (oskar) mRNAs in embryo, Regulation of segmentation genes expression by bicoid. Regulation of hunchback expression, and gap genes, production of segmentation stripes, Expression of eve gene, and eve stripe-2. **6 hrs**

References

1. Biochemistry and Molecular Biology of Plant; Buchanan, Gruissum and Jones, (2000), ASPP, USA.
2. Biochemistry; David Rawn, Panima Publishers (2012).
3. The Bacteriophages; Richard Calendar, 2nd Edition, Oxford University Press (2005).
4. Basic Virology; Wagner and Hewlett; Blackwell Science, (2004)
5. LEWINS Gene XI; J.E. Krebs, E.S. Goldstein, and S.T. Kilpatrick, Jones and Barttlett Publishers (2012).
6. Molecular Biology of the Cell, Alberts et al., Garland Publications, (2012).
7. Molecular Biology, David Freifelder, Narosa Publishers, (1997).
8. Molecular Biology Robert F. Weaver, McGraw Hill (2012).
9. Microbial Genetics; Maloy et al., Jones and Bartlett Publishers, (1994).
10. Modern Microbial Genetics; Uldies N. Streips and Ronals E. Yasbin, Wiley Leis Inc. New York, (2002).
11. Principles of Developmental Genetics; S.A. Moody, Academic Press (2007).
12. Developmental Biology; S. P. Gilbert, 8th Edn, Sinauer Associates Inc.,(2006)
13. Molecular biology and Biotechnology; 4th Edn., J.M. Walker and R. Rapley, RSC (2000).
14. Molecular Biology of Gene; Watson, J.D. et al., 5th Edn. Pearson Education; (2004).
15. Principles of Virology; S.J. Flint et al., ASM Press (2000).
16. Biochemistry and Molecular Biology; 5th Edn. D.Papachristodoulou, A. Snape, W.H. Elliott, and D. C. Elliott Oxford University Press (2014)
17. Chromatin structure and Gene Expression; 2nd Edn. Sarah Elgin, Jerry Workman, Oxford University Press (2000)
18. Molecular Cell Biology; Harvey Lodish 5th Edn. (2010)
19. Biochemistry 5th Edn. Jeremy M. Berg, John L. Tymoczko, Lubert Stryer (2011).
20. Genome Stability: DNA Repair and Recombination; James Haber, Garland Science (2013)
21. Retroviruses; Coffin JM, Hughes SH, Varmus HE, editors; CSH Press, (1997)
22. Viruses: Biology, Applications, and Control; David Harper, Garland Science (2011).

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BCT -402 Biochemical Genetics

4 Units (52 hrs)

Introduction: Nature of genetic material. Chromosomes and genes.**Structure of chromatin;** nucleosomes and higher orders of organization. Mutation: types of mutation, mutagens, mechanism of mutation, induction and isolation of mutants and their role in genetic studies. **Complementation**. **7 hrs**

Classical Genetics: Review of classical genetics; work on Pisum sativum, Drosophila Melanogaster, Neurospora Crassa etc. inheritance (sex-linked and others). Population genetics,extranuclear inheritance. Sex determination, Morgan's discovery of sex linked inheritance of sex linked genes, X;linked traits in humans. Identification of sex chromosomes, XX,XY, mechanism of sex determination. X inactivation Structure of Y chromosome.
8 hrs

Quantitative Genetics:

Human quantitative traits, discontinuous traits and continuous traits, Breeding analysis, genetics basis of quantitative variation, Multiple factor hypothesis and analysis of polygenes.Genotype-Environment Interaction and models for their measurement, estimation of Heritability Index. **8 hrs**

Human genetics: **Biochemical methods for genetic studies-** Chromosome banding, Chromosome mapping based on recombination frequency data. Transposons.Transposition in human chromosomes. Overview of human genome project, mapping of human genes; techniques used(give appropriate ex), annotation of important genes. Chromosomal abnormalities. (give appropriate example). Use of SNPs for detection of diseases**13 hrs**

Bacterial Genetics: Bacterial chromosomes, plasmids; fertility, resistance, colicinogenic and others. Recombination in bacteria.Mechanism of recombination, transposable genetic elements, transformation and conjugation in bacteria.Linkage map of bacterial chromosomes.
8 hrs

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Viral Genetics: Life cycles of bacteriophages, lytic cycle; replication of T- phages. Lysogeny and its regulation.Transduction; specialized, generalized and abortive.Fine structure analysis of T-phages; Benzers work, concept of cistrons. **8 hrs**

References

1. Genetics, Strick Berger, M.W. (1990) 3rd edn. McMillan.
2. Human Molecular Genetics; Peter Sudbery, (2002) Printice Hall.
3. Introduction to Modern Virology, Primrose and Dimmock (1988), Blackwell Sc.
4. Genetics and Molecular Biology; Robert Schleif, The Johns Hopkins University Press Baltimore, (1993).
5. Discovering Genomics, Proteomics and Bioinformatics, Campbell A M & Heyer L J, 2nd Edn. Benjamin Cummings, (2007).
6. Introduction to Genetics: A Molecular Approach; T A Brown, Garland Science (2011).
7. Molecular Cell Biology; Lodish et al., 7th Edn. W.H. Freeman and Co. (2012).
8. Molecular Biology of the Cell; 7th Edn. Bruce Alberts et al., Garland Publications (2008).
9. Nuclear Organization; Chromatin Structure and Gene- Expression, Roen Van Driel and Arie P. Otte Oxford University Press (1997).
10. Principles of Developmental Genetics; S.A. Moody, Academic Press (2007).
11. The Cell; Geoffrey Cooper, and Robert E.; 5th edn. Hausman Sinauer Associates (2009).
12. The Science of Genetics, George W. Burns and Paul J. Bottino (1989), Maxwell-McMillan.
13. Human Genetics; Lewis, 7th Edn. WCB & McGraw Hill (2007).
14. Essential Genetics: A Genomics Perspective; Daniel L. Hartl, 6th Edition, Jones and Barlett Learning (2012).
15. Bacterial and Bacteriophage Genetics; Edward A. Birge, 5th Edition, Springer (2006).
16. Nucleosome Histone, and Chromatin; Part-A; Carl Wu and C. Allis, Academic Press (2012).

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BCT – 403: Biotechnology and Bioinformatics

4 Units (52 hrs)

Introduction

Introduction to cloning procedures. Isolation of nucleic acids and their characterization – vectors and genomic DNA. **2 hrs**

Restriction endonucleases and DNA modifying enzymes:

Restriction enzymes Discovery, classification, properties, and applications. Reactions, application of the following modifying enzymes employed in rDNA technology; DNA- and RNA ligase, Alkaline Phosphatases and Poly nucleotide kinases(PNK)DNase (DNase-I) and RNases (RNase A, H), S1- and Micrococcal nuclease, double and single stranded exonucleases. DNA and RNA polymerases (Klenow fragment), template independent RNA polymerases. **7 hrs**

Cloning Vectors and Cloning :

Desirable properties of vectors,basic properties of plasmids, plasmids as vectors. Directional cloning in plasmid vectors (pUC), blunt end cloning in plasmids, use of linkers and adapters in cloning. Preparation of competent cells and transformation of cloning host , electroporation, Screening colonies using X-gal and IPTG (α-complementation), screening by hybridization,restriction digestion.

Bacteriophage lambda vectors- Insertional and replacement lambda vectors, transfection, *in vitro* packaging, screening recombinant phages. Cloning in M13 vector and COSMID vectors and their applications.

Expression vectors: Characteristics of expression vectors, bacterial expression vectors. Super vectors-characteristic features and utility of BAC and YAC **10 hrs**

Genomic and cDNA libraries:

Outline of methodology for genomic library construction, creation of genomic libraries using lambda and cosmid vectors. Growth, evaluation, storage and amplification of genomic libraries.cDNA libraries; methodology, screening cDNA libraries- hybridization, screening with antibodies. **5 hrs**

PCR:

Discovery, principle and procedure, variants of PCR- Reverse transcriptase-PCR, long PCR, differential display RT PCR, inverse PCR, multiplex PCR, real-time PCR, Cloning PCR products, TA-cloning, Application of PCR- PCR in screening clones, colony PCR, PCR-based methods for mutagenesis (overlap-extension, megaprimer) , PCR in diagnostics **5 hrs**

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Sequencing: Principle of DNA sequencing**,** automated sequencing, extending the sequence, shot gun sequencing.Analysis of sequence data; annotation, ORF, exon-intron boundaries, identification of genes and their products. **4 hrs**

Genetic engineering in animals cells: Over view of strategies, transfection methods-phospholipids as delivery vehicles, electroporation and direct transfer, transient and stable transformation, Cotransformation and selection of stable transformants, selectable markers for animal cells. Mammalian plasmid expression vectors, reporter genes. Gene transfer by viral vectors- adeno and baculo virues, retroviral vectors. **5 hrs**

Genetic engineering in plants:
Plant cell, protoplast, and callus culture and their manipulations. *Agrobacterium*-mediated transformation, properties of Ti plasmid, Ti-plasmid derivatives as plant vectors (disarmed T-DNA), cointegrate and binary vectors, selectable markers for plants, analysis of transgene expression in plants. Plant expression vectors; CaMV and TMV vectors.Gene transfer techniques in plants-Direct gene transfer, protoplast transformation, particle bombardment. **5 hrs**

Bioinformatics: Definition and overview, sequence data, nucleotide and protein sequence, Databases: Nucleotide and Protein database, EST tag databases and SNP database. Sequence Alignment. Pairwise and multiple sequence alignment.Methods of local and global alignment. Dynamic programming, Scoring matix, PAM, searching sequence databases by sequence similarity-BLAST and FASTA. Tools for secondary structure analysis.Tertiary structure prediction. Structural databases: PDB, MMDB. Visualization of protein structure: RasMol, Swiss-prot Pdb viewer, Homology modeling and application in drug discovery.
9 Hrs

References
1. Molecular Cloning; A laboratory manual; Michael R. Green, CSHL Press (2012).
2. Molecular Cell Biology; Lodish et al., 7th Edn. W.H. Freeman and Co (2012).
3. Molecular Biology of the Cell; 7th Edn. Bruce Alberts et al., (2008), Garland Publications
4. Molecular Biology; Robert F. Weaver, McGraw Hill (2012).
5. Principles and Techniques of Biochemistry and Molecular Biology; 7th Edn. Keith Wilson and John Walker (2010).
6. Principles of Gene Manipulations; 6th Edn. S.B. Primrose, R.M. Twyman, and R.W. Old, Blackwell Science (2012).
7. Gene Cloning and DNA analysis- An Introduction; T. A. Brown, 5th Edition, Wiley-Blackwell Publishing (2006).
8. Molecular biology and Biotechnology; 4th Edn., J.M. Walker and R. Rapley, RSC (2000).
9. Plant Biotechnology and Agriculture; Arie Altman and Paul Hasegawa Academic Press (2011).
10. Bioinformatics, Andreas D Baxevanis Wiley Interscience, 1998.
11. Bioinformatics,- David W Mount Cold spring harbor, 2001.
12. Essentials Bioinformatics,- Jin Xiong ,Cambridge University Press
13. Bioinformatics, Methods and Applications- Genomics, Proteomics and Drug, Discovery–Phi, 2006 S C Rastogi, N Mendiratta and P Rastogi
14. Bioinformatics-Stuart M Brown ,NYU Medical Center, NY USA. 2000
15. Computational methods for macromolecular sequence analysis, R F Doolittle,Academic Press, 1996

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16. Introduction to Bioinformatics, -Arthur Lesk Oxford, 2006.

BCT 404 – Metabolism II

4 Units (52 hrs)

Nitrogen Cycle: Introduction, biological and non-biological nitrogen fixation, nif genesand their regulation , regulation of nitrate and nitrite reductase , Assimilation of ammonia, formation of amino acid amides by glutamine synthetase and its regulation. Nitrogen homeostasis . **6 hrs**

Nucleotide Metabolism: Biosynthesis of purine and pyrimidine nucleotides and their inter conversion, regulation of biosynthesis. Other pathways of purine nucleotide formation.Role of Thymidylate synthase.Biosynthesis of deoxyribonucleotides and coenzymes nucleotides.Chemical inhibition of the biosynthesis of nucleic acid precursors. Degradation of purine and pyrimidines, and disorders associated with their metabolism; gout, Lesch-Nyhan syndrome, oroticaciduria and xanthinuria. **8 hrs**

Amino acid Metabolism: General metabolic reaction of amino acids– transamination, pseudotransamnation, glucose – alanine cycle, oxidative deamination (glutamate dehydrogenase), minor pathways of amino acid degradation – transdeamination, amino acid oxidase, and non – oxidative deamination (α–deaminase, dehydrase, asparaginase and glutaminase). Urea cycle– regulation and metabolic disorders.Biosynthesis of creatine and creatine phosphate, polyamines– putrescine, spermidine and spermine, glutathione (γ-glutamyl cycle), physiologically active amines (γ–amino butyric acid, serotonin, α – histamine and catecholamines – dopamine, epinephrine and epinephrine). 9**hrs**

Degradation of the individual amino acids: Pathways in animal, plant and microbial systems; Amino acids forming from pyruvate (alanine, glycine, threonine, serine, cystine and cysteine), oxaloacetate (aspartic acid and asparagine), α- ketoglutarate (glutamic acid, glutamine, arginine, histidine and proline), succinyl CoA (valine, isoleucine and methionine), acetoacetate and/or acetyl CoA (leucine and lysine), pyruvate, formaldehyde, acetoacetate and/or acetyl CoA (tryptophan), and fumarate, acetoaetate and/or acetyl CoA (phenylalanine and tyrosine). Inherited disorders associated with glycine, aromatic, branched chain, basic and sulfur containing amino acid metabolism. **12 hrs**

Biosynthesis of the individual amino acids: Pathways in animal, plant and microbial systems– biosynthesis of non – essential amino acids from pyruvate (alanine), intermediates of glycolysis (serine) and TCA cycle (aspartic acid, asparagine, glutamic acid and glutamine),

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essential amino acid (tyrosine), non – essential amino acid (glycine, proline and arginine), and essential & non – essential amino acid (cysteine). Biosynthesis of essential amino acids from aspartate family of amino acids (threonine, lysine and methionine), pyruvate family of amino acids (valine and leucine), pyruvate and α−ketobutyrate family of amino acid (isoleucine), aromatic family of amino acids (phenylalanine, tyrosine and tryptophan) and histidine. Regulation of amino acid biosynthesis by sequential & concerted feedback inhibition.

12 hrs

Heme Metabolism: Biosynthesis and degradation of porphyrin and their regulation, porphyrias, jaundice and Hemoglobinopathies. **5 hrs**

References

1. Biochemistry- R. Garret, Charles M Grisham, Belmont (2013)
2. Biochemistry; Geoffrey Zubey, (1998), WCB Publishers.
3. Biochemistry; David Rawn, Panima Publishers, (1989).
4. Text Book of Biochemistry with Clinical correlations; 6th Edn. Thomas M. Devlin (2012), Wiley-Liss.
5. Lehninger- Principles of Biochemistry; D. L. Nelson and M.M. Cox 6th Edn. Macmillan Publications (2012).
6. Principles of Biochemistry; Smith et al., [Ed.] (1986) McGarw Hill.
7. Bioenergetics; A Practical Approach, G.C. Brown and C.E. Cooper (1995) IRL- Oxford University Press.
8. Biochemistry Ed. Donald Voet & Judith G. Voet, John Wiley & Sons, Inc. (2010).
9. Bioenergetics; David Nicholls and Stuart Ferguson, Elsevier (2013).

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BCP – 405: Genetic Engineering and Protein Chemistry (4 Credits)

Genetic Engineering

1. Isolation and characterization of DNA fragments for cloning
2. Restriction digestion of plasmid DNA.
3. Preparation of Competent cells.
4. Transformation of DNA by $CaCl_2$ method (recombinant vectors – plasmids / phages).
5. Characterization of clones by restriction digestion.
6. Northern Hybridization (using non-radioactive probes)
7. Cloning, Expression, Isolation and purification of recombinant proteins .

Protein Chemistry

1. Extraction and isolation of enzymes (phosphatases / esterases / amylases) from Insect / Microbial / Plant sources.
2. Fractionation by Ammonium sulfate, acetone and pH precipitation
3. Ion exchange chromatography.
4. Gel filtration.
5. Kinetic characterization of the enzyme

6. Analysis of purity of isolated proteins by NATIVE and SDS PAGE.

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