

M.Sc. (Final) BIOCHEMISTRY

PAPER-VII : BIOCHEMICAL GENETICS AND DNA REPLICATION

UNIT-I HERIDITY AND GENETIC ANALYSIS

Basic concepts of Mendelian and non-Mendelian inheritance. Importance of meiosis in heridity. Sex linked inheritance. Polygenic and maternal inheritance.

Somatic and germinal cell mutations. Different kinds of mutation (Forward and back, point, frameshift, deletion mutations) Conditional mutants, resistance mutants. Suppressor mutations. Chromosomal mutations. Detection, selection and isolation of mutants. Mutation rates. Mechanism of action of mutagens. Polyploidy. Site directed mutagenesis. Photoreactivation and mechanisms for repair of UV damaged DNA (Post replication and SOS repair).

UNIT-II GENOME ORGANIZATION

Genome organization in procaryotes and eucaryotes. Plasmids, transporons, insertion sequences and retroposons. Mitochondrial and chloroplast DNA. Benzer's fine structures of rll loci. Organization of eucaryotic chromosomes. Histones and non-histone type DNA binding proteins. Nucleosomes and higher order structures. C-value pradox and the significance of introns. Single copy genes, repeating sequences, and tandem gene clusters. r-RNA genes, histone genes and immunoglobulin genes. Selfish DNA.

UNIT-III MUTATIONS, RECOMBINATION AND GENE TRANSFER

Mutations. Different kinds of mutations. Isolation of mutants, phage mutants, host range rapidlysis and temperature sensitive mutants. Mechanism of mutants. Gene transfer mechanisms, transformation, trasduction. (generalized, abortive and specialized). Conjugation $F^+ \times F^-$ Hfr strains. Mechanism of recombinant and cross over. Elements of gene mapping. Mapping by recombination analysis, multiple cross over and interference. Circular chromosome and mapping by conjugation. Tetrad and complement analysis Mapping by transformation and transduction. Map units and cytological maps of eukaryotic chromosomes. Somatic cell genetics.

1. Assay of serum transaminase. Determination of bilirubin and calcium. Qualitative tests for normal and abnormal urinary constituents. Determination of urinary creatine and creatinine.
2. Nitrogen estimation by Micro-Kjeldahl Method. Total nitrogen excretion in humans, balance studies.
3. Estimation of Vitamin 'A' in foods. Estimation of Vitamin 'C' in citrus fruits both titrimetric and colorimetric methods. Estimation of tyramine in foods by fluorimetry.

LAB COURSE-II

A. ANALYTICAL METHODS

1. Preparation of buffers.
2. Biochemical preparations. Preparations of egg albumin, casein, glycine, asparagine, cysteine, ATP, glycogen, Preparation of DNP amino acids and separation by TLC and quantitative identification.
3. Determination of calcium as calcium oxalate. Determination of calcium in food stuffs by colorimetry. Use of atomic absorption spectrophotometer to determine Na and K in serum. Determination of Na and K by flame photometry. Determination of Mg in biological samples. Methods of cell disruption. Preparation of tissue homogenates using different homogenizers.
4. Lipid analysis. Determination of lipid content in oil seeds. Triacylglyceride composition by TLC in germinating seeds.

B. CARBOHYDRATE AND LIPID ANALYSIS

1. Isolation of glycogen from liver. End group analysis by periodate oxidation and determination of average chain length of glycogen.
2. Differential analysis of sugars in a mixture. Use of polarimetry for configurational analysis of carbohydrates. Estimation of sucrose.
3. Extraction and adsorption column chromatography of plant pigments. TLC and GC analysis of lipids. Determination of iodine number, saponification and acid value of a fat.
4. Isolation of cholesterol from brain and its estimation. Preparation and analysis of sphingomyelin.

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UNIT-IV DNA REPLICATION-I

Semiconservative replication. Replication forks. Role of DNA gyrase. Continuous and discontinuous synthesis. Evidence for Okazaki model. RNA primers. Enzymes in replication. Single strand DNA binding proteins. Helicases. Topoisomerases. DNA primases, DNA ligases, DNA polymerases. E coli DNA polymerases I and II. Eucaryotic DNA polymerases. Prokaryotic replication mechanisms, Rolling circle replication. Replication of $\phi \times 174$ RF DNA. Bacteriophage M13. Replication of E coli DNA

UNIT-V DNA REPLICATION-II AND REPAIR

Eucaryotic DNA replication, Eucaryotic DNA polymerases. Autonomous replicating Sequences, yeast plasmid replication (Double rolling circle). Mitochondrial DNA replication, Reverse transcriptase, Termination and fidelity of replication, fusion of replicons and termination signals. Telomers, Inhibition of DNA replication. DNA repair : Direct reversal of damage, Excision repair, Recombinant repair, the SOS response, Identification of carcinogens. Inhibitors of DNA replication.

PAPER-VIII : PROTEIN SYNTHESIS AND REGULATION

UNIT-I TRANSCRIPTION

Polynucleotide phosphorylase, RNA polymerase, structure of E.coli RNA polymerase. Interaction between RNA polymerase and template, chain initiation and the () cycle, elongation and termination. Eucaryotic RNA polymerases. Promoter and enhancer sequences. Inhibitors of transcription. Synthesis of different RNA molecules. Synthesis of r-RNA, 5 sRNA and tRNA. Synthesis of eucaryotic mRNA, hnRNA capping. Methylation and polyadenylation.

RNA splicing-introns and split genes. Splicing mechanisms, splicing of nuclear pre-tRNA introns. Group-I & II pre-mRNA introns. Excision of multiple introns. Nuclear cytoplasmic transport. Factors involved in pre m-RNA splicing, RNP's, protein factors, hnRNP proteins. Splicing complexes (Spliceosomes). Transplicing. Catalytic RNA.

UNIT-II TRANSLATION

The genetic code, elucidation, experimental, codon degeneracy, and mutational in vitro translation systems. tRNA structure

and role in protein biosynthesis. Amino acyl t-RNA synthetases. Wobble hypothesis. Mitochondrial genetic code. Nonsense suppression. Ribosomes-structure and composition. Ribosomal proteins and composition. Ribosomal proteins and reconstitution. Mechanism of initiation, elongation and termination of protein biosynthesis. Factors required for translation. Inhibitors of protein synthesis antibiotics and other inhibitors. Nonribosomal biosynthesis of polypeptides. Biosynthesis of gramicidin-S.

UNIT-III REGULATION OF GENE EXPRESSION

Translation feedback. Synthesis of ribosomes and ribosomal RNA. Hemoglobin synthesis. Interferons. Regulation of gene expression at transcriptional level. The lac repressor. Fine structure of lac operon. cAMP and the catabolic activator protein. Gal operon and concept of dual promoters. Dual functions of the repressor the ara operon. Transcriptional control by attenuation. The trp operon.

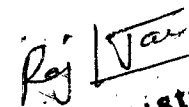
Eucaryotic gene regulation. Positioning chromosomes for transcription. Polytene chromosomes. Gene amplification and gene rearrangements. Transcriptional control by alternative RNA processing and enhancers. Homeotic genes. Regulatory molecules that interact with DNA. Helix-turn-helix. Zinc finger and leucine zipper motifs.

UNIT-IV PROTEIN TARGETTING

Proteins sorting and targeting. Cell organelles and proteins in protein sorting. Post-translational modifications. The signal hypothesis. Signal sequences and signal recognition particle. Molecular chaperones. Protein degradation. Lysosomal degradation. PEST sequences. The ubiquitin pathway. Protein stability and the N-end rule.

UNIT-V SIGNAL TRANSDUCTION

Totipotency and cell signaling. Role of growth factors and cytokines. Signal transduction mediated by cAMP. Role of nitric oxide and cyclic nucleotides. Calcium ions, calmodulin and inositol phosphatides as second messengers. Protein phosphorylation and signal transduction. Glycosylation, acylation and ADP ribosylation of proteins and their role in signal transduction. Programmed cell death and mechanisms involved therein.


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PAPER-IX MICROBIAL BIOCHEMISTRY AND VIROLOGY Syllabus : M.Sc. Biochemistry

UNIT-I MICROBIOLOGY INCLUDING PARASITOLOGY

Isolation, cultivation and identification of bacteria. The bacterial cell wall structure. Gram positive and gram negative bacteria. Microbial nutrition and growth. Bacterial growth and kinetics. Diauxic growth. Synchronous growth. Chemostatic culture. Continuous cultivation of microbes, Bacterial and viral diseases. enteric diseases, tuberculosis typhoid, tetanus, malaria, Kalazar prion diseases.

UNIT-II FERMENTATION

Introduction to fermentation. Fermentative production of ethanol, penicillin, riboflavin, glutamic acid, lysine, amylases and proteases. Solid state fermentation. Antibiotics : chemistry and mode of action of penicillin, streptomycin, chloramphenicol, tetracyclines and rifampicin.

Basic design of fermentors. Production of enzymes (amylases, proteases, lipases and cellulases) and high fructose syrup. Microbial transformations of sterols and steroids. Environmental applications of microorganisms in sewage and effluent treatment (aerobic and anaerobic digestors). Downstream processing of valuable products.

UNIT-III VIROLOGY-I

Nature of virusoids, prions and viruses. Composition and structure of viruses. Virus-host interactions. Isolation and assay of viruses. General methods of virus isolation with examples of TMV and T₂ phages. Assay of TMV. Plaque assay for bacteriophages. Assay of animal viruses with special reference to oncogenic viruses. Pock assays. Cytopathic effects. Bacteriophages-structure, regulatory mechanisms and development of T even phages. OX 174, QB M13. Bacteriophage life cycles. Lytic growth of bacteriophages, initial events, one step growth, single burst. Eclipse.

UNIT-IV VIROLOGY-II

Eucaryotic viruses, SV 40 virus system, cell transformation interactions in permissive and non-permissive hosts. Retroviruses RSV as prototype virus. Animal viruses. General features and outlines of adenovirus, poliovirus 40, retrovirus and HIV/AIDS. Oncogenic viruses and carcinogenesis. Oncogens and mechanisms of cell transformation.

UNIT-V PLANT AND ANIMAL VIRUSES

General features : Host-virus interactions, permissive/nonpermissive hosts, structure of naked and enveloped viruses, cytopathic effects, assay methods (Pock assay, haemagglutination assay, transformation assay) and purification methods (ultrafiltration, ultracentrifugation and affinity methods).

PAPER-X : IMMUNOLOGY

UNIT-I BASIC IMMUNOLOGY

Elements of immunity. Natural and acquired immunity. Cells and tissues of immune system. Elements of cellular and humoral immunity. Immunogens, antigens, haptans, adjuvants. Immunoglobulin nature, structure, classification and biological properties. Generation of antibody diversity. Genes involved in antibody production. Theories of antibody production. Effector mechanisms of humoral immunity. Activation of B-lymphocytes. T-cell receptors. Triggering the immune response. Cellular cooperation immune response. Complement and its role in immune response.

UNIT-II APPLIED IMMUNOLOGY-I

Hybridoma technique and monoclonal antibodies. Antigen-antibody interactions. Immuno-analytical methods based on Ag-Ab interactions (Gel diffusion techniques, immunoelectrophoresis, immunofluorescence, RIA, ELISA and western blotting). Vaccines. Methods of vaccine production. DNA vaccines, synthetic vaccines.

UNIT-III APPLIED IMMUNOLOGY-II

Hypersensitivity. Basic concept and types of hypersensitivity. Autoimmune diseases. Theories of breakdown in self-tolerance. Selected autoimmune diseases (Organs specific and systemic diseases). Immune deficiency disorders-AIDS. Immunosuppressive agents in clinical practice.

UNIT-IV IMMUNO ANALYTICAL METHODS

Production and immuno technology and purification of polyclonal antibodies. Antigen-antibody interactions-gel diffusion, immunoelectrophoresis, immunofluorescence, RIA, ELISA Western blotting and FACS techniques. Vaccines-types and their applications. (DNA, recombinant DNA, peptide and antiotypic vaccines).

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UNIT-V CYTOKINES

Effector molecules, cytokine receptors. Complement, classical and alternate pathways of complement activation, regulation of complement activation pathways. Immunological tolerance, hypersensitivity, Autoimmunity, immunology in cancer and AIDS, Vaccines.

PAPER-XI BIOTECHNOLOGY**UNIT-I PROTEIN ENGINEERING**

Immobilized enzymes and cells. Methods of immobilization and applications. Resolution of amino acid racemates. Synthesis of improved penicillin's increased protein stability and enhanced specific activity. Altering the kinetic properties and pH.

UNIT-II MICROBIAL BIOTECHNOLOGY

Introduction to microbial biotechnology. Large-scale cultivation of microbes, problem of oxygen supply, basic fermenter design, current design of stirred tank reactor, aseptic operation, control systems batch versus continuous operation, down-stream processing. Production of biomass (microbial insecticides, starter cultures, single cell proteins production). Production of low molecular weight compounds—primary and secondary metabolites. Metabolic end products. Bioconversions. Microbial polysaccharides and production of microbial enzymes. Microbiological mining. Introduction to drug design and delivery.

UNIT-III ANIMAL BIOTECHNOLOGY

Introduction to animal biotechnology. Cells and cell lines, media for cell structure and equipment. Production of viral vaccines. Production of high value therapeutics interferon and plasminogen activator, urokinase. Monoclonal antibodies. Immunotoxins as therapeutic agents. Chimeric antibodies. Introduction to transgenic animals. Human gene therapy. Animal cloning techniques. Gene knockouts.

UNIT-IV PLANT BIOTECHNOLOGY

Introduction to plant biotechnology. Plant cell culture, plant protoplast and protoplast fusion, plant viruses as vectors. Ti plasmid as vector and transgenic plants. Transgenic technology. Sense RNA and DNA.

UNIT-V MICROBIAL PATHOGENS AND ANTIMICROBIAL AGENTS-II

Antibiotics : Assay of antibiotics, chemistry and biosynthesis of important antibiotic compounds. First, second, third and fourth generation antibiotics with reference to modified penicillins. Antibiotic resistance. Biochemical modes of action of antibiotics acting as inhibitors of ribosomal function (e.g., aminoglycosides, tetracyclines, puromycin, chloramphenicol etc.) inhibitors of nucleic acid metabolism, actinomycin D, mitomycin C etc.) inhibitors of cell wall biosynthesis (penicillins, bacitracins etc.) and inhibitory of membrane function (polyenes, peptide antibiotics etc.)

PAPER-XII GENETIC ENGINEERING**UNIT-I GENETIC ENGINEERING-I**

Introduction and overview of methodology for cloning. Homologous and heterologous expression of genes. Methods of ligation. DNA ligases, ligation of fragments with cohesive ends. Adapters and linkers. Blunt and ligation. Homopolymer tailing. Use of restriction nucleases in cloning. Use of viral and plasmids YAC, shuttle vectors. Eucaryotic vectors. Copy number subcloning strategies.

UNIT-II GENETIC ENGINEERING-II

Identification of clones of interest. The use of genomic DNA library and DNA library in gene cloning. Chromosome walking and mapping techniques. Use of expression vectors to over produce proteins. Baculoviral expression. Reporters genes and identification of upstream control elements. Secretion of recombinant proteins. Fusion proteins. Yeast expression. Site directed mutagenesis. Subtractive cDNA cloning. Phage display of proteins and peptides. 2-hybrid system.

UNIT-III DNA CLONING, TOOLS AND TECHNIQUES

Production of recombinant proteins with examples of insulin, somatostatin and interferon. PCR and its applications. RFLP and its applications. DNA finger printing, trans genics and cloning techniques.

DNA Sequencing methods. Maxam and Gilbert's method. Dideoxy chain termination method of Sanger. Gene probes in detection prenatal and antenatal detection of disease. Human genome project.

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UNIT-IV GENE REGULATION AND HUMAN DISEASE

Oncogenesis with reference to protooncogenes. Transcription factors as oncogenes (Fos, Jun, AP, 1, V erb A and thyroid hormone receptor). Antioncogenesis. P53, Retinoblastoma protein.

UNIT-V REGULATION OF GENE EXPRESSION IN PROKARYOTES AND EUKARYOTES

Negative and positive control of gene expression (Lac operon). Gal promoters (Gal operon) Dual function of repressor (ara operon). Transcriptional control by attenuation (trp operon). Phase variation (Salmonella flagellar protein synthesis). Translational feedback.

COURSE-I

A. ANALYSIS OF BIOMOLECULES

Absorption spectra of nucleic acids and base derivatives. Separation and quantitation of nucleic acids. Analysis of nucleic acids for base composition and GC content.

Chromophores. Correlation of ϵ_m and base composition. Incorporation of 3H thymidine into DNA. Plasmid mini-preparations

Large scale isolation of a plasmid DNA. Use of restriction endonucleases and ligase. Agarose gel electrophoresis.

Insertion of foreign DNA into a vector and transformation. Blot analysis for RNA and DNA. DNA sequencing by Sanger's method (demonstration).

B. ENZYME KINETICS AND IMMUNOLOGY

1. Determination of blood groups. Ouchterlony double immunodiffusion.

2. Immuno electrophoresis. RIA and ELISA methods (demonstrations).

3. Cell-fractionation. Preparation of cell free homogenate. Isolation of mitochondria. Intracellular localization of dehydrogenases and respiratory enzymes. Preparation of chloroplasts and nuclei. Isolation and purification of enzymes (lysozyme from egg white, amylase from jack bean meal, arginase from liver, pyrophosphatase from yeast)

4. Kinetic studies including determination of K_m and K_i -Metal ion activation of enzymes. Determination of activation energy of an enzyme. Turnover number of catalase or trypsin. Enzyme inhibition.

COURSE-II

A. ANALYTICAL METHODS AND ENZYMOLOGY

1. Qualitative tests for salivary amylase. Determination Of enzyme activities (V_{max} and specific activity) of the following enzymes, Sweet potato amylase, horse gram urease, liver catalase, arginase. yeast acid and alkaline phosphatases, yeast invertase. Proteolytic activity of pancreatin.

2. Qualitative tests for inhibition of enzyme activity with above enzymes. Determination of order of a Chemical reaction.

3. Saponification of esters, Identification of organic functional groups by qualitative tests. Formol titration of amino acids.

4. Determination of pK of amino acids. Polarimetric experiments. Respirometry, study of tissue respiration by tissue slices and effect of inhibitors on oxygen consumption.

B. PROTEIN ANALYSIS

1. Absorption spectra of proteins and methods of protein estimation. Determination of aromatic amino acid content in proteins.

2. Isolation of a protein by salt or solvent or isoelectric precipitation.

3. Purification of protein and determination of molecular weight by SDS-PAGE. End group analysis by DABITC method.

4. Incorporation of labeled amino acids into proteins (demonstration). Protein phosphorylation (demonstration). Western transfer. Identification of proteins on membranes using avidin-biotin and/or antibodies.

M.Sc. BIOCHEMISTRY

(Previous and Final)

Instructions to examiners to all theory papers.

Max. Marks of each theory paper is : 100

Time : 3hrs.

Note:

1. Ten questions will be set in all selecting two questions from each unit.
2. Candidates have to attend five questions, one from each unit.

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M.Sc. BIOCHEMISTRY
(Previous and Final)

Max. Marks : 200

Duration of Exam : 12 hrs.
(Spread in 2 days)

4 Exercises to be performed selecting one exercise from each section.

Two quantitative exercises	= 50×2	= 100
Two qualitative exercises	= 25×2	= 50
Viva		= 30
Record		= 20
		—
		= 200

Note— The practical examination will be conducted by the board of two external and one internal examiners who will conduct practical on both days.

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