APPENDIX-V

SRI KRISHNA DEVARAYA UNIVERSITY: ANANTAPUR
M.Phil/Ph.D DEGREE COURSE IN BIOCHEMISTRY
(With effect from 2001-2002)

PAPER I - RESEARCH METHODOLOGY

1. **DATA PRESENTATION AND STATISTICAL APPLICATIONS:**

Principles of experimental design, collection, assembly, analysis and interpretation of experimental data.

Data presentation- Tabular, graphical and diagrammatic representation of data. Use of simple, semi-log & double graph paper in data representation.

Statistical applications in research-averages, standard deviation, standard error, analysis of variance, regression, coefficient of variation. Chi-square test, students test (t) and Duncan's new multiple range test.

2.SEPARATION METHODS:

Classification: Zonal & Frontal methods.

Chromatography: adsorption, partition, paper,thin layer, paper, cellulose derivatives affinity.

Electrophoresis: Moving boundary, zone, starch gel, paper, cellulose derivatives Isotachophoresis, Isoelectro focusing, high voltage electrophoresis.

PAGE: Preparation of native & denaturing polyacrylamide gels and separation of proteins. Preparation of PAGE gels for DNA sequencing. Preparation of DNA fragments. Maxam and Gilbert and Sangers DNA sequencing methods. Generation of DNA sequence.

3. SPECTROSCOPIC TECHNIQUES:

Spectra Law of colorimetry, Visible and UV Spectrophotometry Florimetry; Atomic absorption spectrophotometry; NMR spectrophotometry; (Basic principles only).

4. RADIOSOTOPE TECHNIQUES:

Nature and type of Radio activity; Decay units; Detection measurement of Radio activity (GM, Scintillation and Auto radiography) Preparation of labeled compounds; Radioactive precursors used in labeling of Nucleic Acids; Nick translation; End labeling; polynucleotide kinase and its use in labeling of the DNA, RNA fragments purification and measurement of DNA radioactivity in detection of specific proteins in plant and animal cells.

5. IMMUNO-CHEMICAL TECHNIQUES:

Production of Antisera; Adjuvant; Haptane; Precipitation reaction; Precipitation in solutions; Immunodiffusion and immunoelectrophoresis; Radio immunoassay; Elisa; Complement fixation; immunofluoresecence.

6. CENTRIFUGATION TECHNIQUES:

RCF; Svedgerg coeffient, preparative centrifugation, Differential rate-zonal; Isopycnic and sedimentation equilibrium; centrifugation; Analytical ultracentrifugation; Analysis of cellular fractions.

7. ELECTRODE AND INDICATOR DYE TECHNIQUES:

P^H calculation; Henderson-Hasselbaclch equation; standard hydrogen electrode; Reference electrode- Calomel electrode and silver, Silve chloride electrode; P^H measurement using organic indicator and potentiometry. Ion specific electrode (Oxygen electrode).

8. ISOLATION METHODS:

Approaches to Biochemical investigation- whole organisms. Perfusion, Slices, culture and cell fraction; enzyme isolation; methods of solubilization; isolation and concentration and assay.

9. MANOMETRY:

Types of manometry; wargerg constant manometer; Gilson differential respirometer and practical aspects and applications.

10. RECOMBINANT DNA TECHNOLOGY:

Generation of DNA fragment; restriction and modification enzymes and their in generation DNA fragments. Restriction fragment polymorphism (RFLP); DNA finger printing; PCR Amplication; chemical synthesis of DNA and c DNA synthesis.

11. VECTROS:

Plasmids and Cosmid vectors. Promotor probe vectors; expression vector and vectors for cloning plants and Eukaryotes.

12. CLONING STRATEGIES:

Cloning of blunted and DNA fragments, use of DNA linkers; Homopolymer tailing. Cloning DNA fragments with cohesive end; use of alkaline phosphatase. Introduction of cloned genes. Transformation, Transduction, Electroporation. Detection of recombination. Hybridization, Immuno-chemical techniques.

SRI KRISHNADEVARAYA UNIVERSITY, ANANTAPUR

DEPARTMENT OF BIOCHEMISTRY

M.Phil / Ph.D written examination syllabus for paper –II (Special paper)

Broad areas of research:

1. **Metabolic disorders-Oxidative stress-Diabetes-Treatment**
2. **Recombinant DNA Technology**
3. **Cardiovascular diseases and treatment**
4. **Membrane biochemistry**
5. **Modelling, Docking and Dynamics of novel protein in micro organisms**

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Metabolic disorders-Oxidative stress-Diabetes-Treatment

1. CARBOHYDRATE AND LIPID METABOLISM:

The Glycolytic pathway, Fermentation, Anaerobic fate of pyruvate, Metabolism of hexoses other than glucose (Fructose, Galactose, mannose). Regulation of Glycolysis.

Glycogen metabolism: Glycogen breakdown, glycogen synthesis, control of glycogen metabolism, Direct allosteric control of glycogen phosphorylase and glycogen synthetase, covalent modification, glycogen phosphorylase bicyclic cascade, glycogen synthetase bicyclic cascade. Integration of glycogen metabolites, control mechanism. Maintenance of blood glucose level. **Citric acid cycle:** Metabolic sources of acetyl Co-A, Amphibolic nature of citric acid cycle, Regulation of citric acid cycle. **Gluconeogenesis:** Pathway regulation and control of blood glucose, metabolic and hormonal regulation, cori cycle. **Pentose phosphate pathway:** Control of the pathway, Glucose-6-phosphate dehydrogenase deficiency.

Lipid metabolism: Biosynthesis, Regulation and Catabolism of fatty acids, Triacyl glycerols, Phospholipids and Cholesterol. Lipoprotein metabolism: chylomicrons, VLDL, LDL and HDL.

Integration of Carbohydrate and Lipid metabolism. Role of liver and adipose tissue in lipid metabolism during fed, fasting, IDDM, NIDDM and obese conditions.

2. THE ENDOCRINE PANCREAS AND HORMONES OF ADRENAL MEDULLA:

Insulin: synthesis, secretion, physiological regulation and alternation in diabetics. Biochemistry of insulin action: insulin receptors, second messengers, protein tyrosine phosphatases and regulation of insulin action. Insulin action on glucose transport. Insulin gene regulation, GTP binding proteins, regulation of pancreatic islet function and β-cell function. **Glucagon:** synthesis, secretion, factors regulating glucagon release. Biochemical effects of glucagon. **Hormones of Adrenal medulla:** chemistry, synthesis, secretion, regulation, physiology and functions of epinephrine and nor-epinephrine.

3. DIABETES MELLITUS:

Primary, secondary diabetes, classification of diabetes on clinical grounds: IDDM, NIDDM, Gestational diabetes mellitus. Clinical syndromes associated with insulin resistance. Vital importance of plasma glucose homeostasis, glucose transporters and pathophysiologic states. Major signs and symptoms of diabetes. Clinical and laboratory features of primary diabetes mellitus, diagnosis, glucose tolerance test. Consequences of disturbed carbohydrate, lipid and protein metabolism in chronic insulin deficiency and excess. Animal models of auto immune diabetes mellitus and NIDDM.

4. DIABETIC COMPLICATIONS AND MECHANISMS:

Micro and macro vascular complications: diabetic retinopathy, nephropathy, neuropathy, atherosclerosis, hypertension. **Oxidative stress:** Free radicals, reactive oxygen species, nitric oxide, oxidative damage:

lipid peroxidation, protein oxidation and oxidative damage to nucleic acids. Protection against oxidative damage: chemistry, metabolism of glutathione, role of glutathione in defense mechanism, regulation of glutathione levels, enzymes of defense mechanism. The Sorbitol-Osmotic and Sorbitol-Redox hypothesis, advanced glycation end products and the pathogenesis of diabetic complications. The role of growth factors in the pathogenesis of diabetic vascular complications. Pathogenesis of platelet dysfunction.

5. DIABETES-THERAPEUTICS:

Type-I diabetes: diet therapy, exercise, immune intervension, islet cell transplantation, pancreas transplantation, gene therapy for metabolic disease. Engineering the pancreatic β-cell. **Type-II diabetes:** prevention strategies, diet therapy, exercise, α-glucosidase inhibitors, intensive insulin therapy, oral hypoglycemic drugs (sulphonyl ureas, biguanides, thiozolidinediones), fatty acid oxidation inhibitors. **Medicinal plants:** role of medicinal plants in protecting human health. Antidiabetic plants and their acive constituents.

REFERENCES

1. Principles of biochemistry - A. Lehninger.
2. Harpers biochemistry – Robert K Murry, Peter Myes, Dryl K. Grmmer, Victor W. Rod well.
3. Text book of Biochemistry with clinical correlations 4th edition. Thomas M. Devlin.
4. Human physiology- Chaterjee.
5. Biochemistry by Lubert Stryer.
6. Biochemistry by Voet and Voet.
7. Diabetes Mellitus-A Fundamental and Clinical Text - Derek LeRoith, Simeon I. Taylor and Jerrold M. Olefsky

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Recombinant DNA Technology

UNIT – I

Nucleic acids: Composition and structure of nucleic acids.Chromosome of E-Coli and nature of human genome. Enzymes and proteins in DNA replication. Replication of *E. coli* chromosome and M13 genome. Eukaryotic DNA replication.Regulation of ColE1 plasmid DNA replication. Inhibitors of DNA replication.DNA damage and repair: Photoreactivation, direct reversal of damage, excision repair, recombination repair and SOS response. Initiation, elongation and termination of RNA synthesis. Monocistronic and polycistronic RNAs. Post-transcriptional modifications of eukaryotic hnRNA – capping, methylation and polyadenylation. RNA splicing and splicing mechanisms. introns. Role of catalytic RNA. Mechanism of initiation, elongation and termination of protein synthesis. Translational factors. . Post-translational modifications.Inhibitors of protein synthesis - antibiotics and other inhibitors.

UNIT- II

House-keeping genes, constitutive genes, and regulatory genes. Induction and repression. Regulatory proteins – DNA-binding motif of regulatory proteins. Role of zinc fingers, leucine zippers, helix-turn-helix. Fine structure of *lac* operon. Negative regulation and positive regulation of *lac* operon. Dual functions of the repressor in *ara* operon. Transcriptional control by attenuation in *trp* operon. Regulation of gene expression in λ and *nif* operon. Regulation of gene expression in eukaryotes – *gal* operon in yeast. Eukaryotic translation control - translation control by heme, interferon, mRNA masking, antisence RNA. Harmones and environmental factors affecting gene expression. Homeotic genes and their regulation.

UNIT – III

Genetic engineering - Introduction and outlines of gene cloning**.** Restriction and modification enzymes. Restriction mapping, DNA ligases, polynucleotide kinase, alkaline phosphotases, S1 nuclease, terminal transferase, Bal 31 nuclease. Polymerase chain reaction - principle, types, primer design, and applications

of PCR. Cloning vectors – Characteristics of a vector. Natural plasmids used as vectors – advantages and disadvantages. Construction of P^{BR322} for its use as a vector.Vectors used for cloning in *E.coli.* (plasmids, bacteriophage derivatives, cosmids, BACs), yeast (YACs, shuttle vectors), higher plants(Ti plasmid derivatives, Caulimoviruses) and animal cells (Construction of SV40retroviruses), characterization of expression vectors. Generation of DNA fragments containing a Gene (Shotgun Method, Southern analysis and cDNA Synthesis) preparation of DNA probes, Construction of DNA libraries- Genomic cDNA libraries.

UNIT- IV

Introduction of recombinant DNA molecules into appropriate hosts – Competent cells preparation, **e**lectroporation, microinjection, transfection and particle bombardment method. Screening of recombinants for a positive clone – Genetic, biochemical and hybridization methods and expression of cloned genes – characterization of expression vectors: Vectors having inducible *lac, taq* promoters. Use of IPTG and its role in induction of a cloned gene. Expression of proteins with His tag and its significance in simultaneous expression and purification of recombinant proteins. technologies – synthesis of plantibodies. Problems associated with expression of cloned genes – inclusion bodies, solubilisation and reconstruction of expressed proteins.

UNIT - V

Plant tissue culture: Major types of media, basic techniques in plant tissue culture, plant transformation in molecular – aided breeding, RFLP markers, linkage analysis-AFLP, QTL, map- based cloning and molecular assisted selection. Development of recombinant E-Coli that experesses human insulin. Developoment of transgenic plants for glyphosate resistance. Develop ment of transgenic sheep for wool,milk and meat. General account on the application of genetic engineering in agriculture, medicine and industry.

References

1. Molecular Biology of the Cell- B. Alberts, D. Barly, J. Lewis, M. Raff, K. Roberts and J.D Watson.
2. Molecular Biology- A Comprehensive Introduction to Prokaryotes and Eukaryotes- D. Freifelder
3. Molecular cloning: Laboratory Manual – Maniatis, E.F. Fritsch and J. Sambrook
4. Genes V(1994)- Benjamin Lewin
5. Molecular Biology of the Gene 4^{th} by- Watson, Hopkins, Roberts, Steitz and Weiner
6. Biochemistry- Donald Voet and Judith Voet
7. Animal Biotechnology- Ranga
8. Molecular Biotechnology – Glick and Pasteurnak
9. Plant Biotechnology – J. Hammond, P. McGarvey and V. Yusibov
10. Plant Biotechnology – Purohit 2000.

Cardiovascular diseases and treatment

UNIT-I :

Classification, brief account of chemistry and biological role of fatty acids, phospholipids , fats , lipoproteins. Physico-chemical properties and analysis of neutral fats and oils .Extraction and analysis of lipids. Metabolism of triacylglycerols, phospholipids, fatty acids, cholesterol and their regulation. Lipoproteins–Function,metabolism and disorders associated with lipoprotein metabolism.

UNIT-II:

Heart – basic anatomy and physiology (Cardiac cycle and ECG)

Normal myocardial function-cellular basis of cardiac contraction (the contraction process , cardiac activation , the role of muscle strength.)Abnormal myocardial function- Heart failure: causes of heart failure – Age , Sex, Diet, Hypertension, Diabetes, Smoking , Physical exercise. Clinical manifestations of heart failure - dyspnea, orthopnea, paroxysmal dyspnea, cheyne-stokes respiration , fatigue ,weakness and abdominal symptoms and cerebral symptoms . Biochemical abnormalities in heart failure - altrations energy metabolism , altrations regulatory proteins, abnormalities of excitation-contraction coupling.

UNIT III

Metabolic complications of cardiovascular diseases-Atherosclerosis, ischemic heart disease: myocardial infarction , angina pectoris. Diagnosis of cardiovascular disease -ECG findings and cardiac markers .Biochemistry and tissue distribution of cardiac markers - creatine kinase isoenzymes, Lactatedehydrogenase isoenzymes, Myoglobin, Cardiac troponin I and T . Clinical utility of cardiac markers in detection of acute myocardial infarctions

UNIT-IV

Chemistry and generation of free radicals,reactive oxygen species
and nitric oxide.Antioxidants–tocopherols , carotenes,oxidized and reduced
acorbic acid,reduced glutathione. Cytoprotective enzymes-superoxide dismutase, catalase , glutathione peroxidase . Assessment of reactive oxygen species including free radicals. Role of reactive oxygen species in pathology.

UNIT –V

Collection and preservation of specimens. Biopharmaceutical properties of drug substances,β-adrenergic receptors . Receptors and drug action, factors affecting drug metabolism. Phytochemical investigation. Medicinal plants used in cardiovascular disease. Extraction and isolation methods of plant material.

References :

1. Harpers biochemistry-Robert K Murry , Peter Mayes, Daryl KGrammer, Victor W. Rod Well.
2. Text book of biochemistry with clinical correlations- (4^{th-} edition)-Thomas M.Devlin.
3. Principle of biochemistry- A.Lehninger.
4. Medicinal chemistry- 4^{th} edition,William O.Foye et al.
5. Clinical biochemistry, William J.Marshall et al.
6. Medicinal biochemistry –Chaterjee.
7. Text book of biochemistry- Voet and voet.
8. Clinical biochemistry –Teitz.
9. Pathology- Robbins.
10. Principle of internal medicine- Harrisons.

Membrane biochemistry

1. Biomembranes:

Chemical composition and molecular structure of biomembranes, lipids, carbohydrates and proteins of biomembranes & membranes asymmetry, Fluid mosaic model of membranes, structure of RBC and mitochondrial membranes, Isolation of ;membrane biogenesis; Donann membrane equilibrium; Membrane transport processes-pumps, gates, channels, active transport, passive transport; ionophores; liposomes.

2. Enzymes:

Purification and assay; enzyme kinetics, effects of pH, temperature, substrate concentration- Michalis-Menten equation, Vmax, Km; Inhibition- irreversible, reversible, competitive, noncompetitive and uncompetitive, Mechanism of enzyme action. Antioxidant machinery, Vitamins as co-enzymes.

3. Metabolism

Blood and its importance in integration of metabolism,Composition of blood, plasma, RBC, WBC, Platelets,

Haemopoesis, functions of blood plasma lipoproteins, transport of gases, biochemistry & mechanism of blood clotting, and fibrinolysis. Nitric oxide and its role in physiological processes. Biochemistry of digestion-Gastro
intestinal tract, liver, pancreas, Kidney and its role in excretion.

Glucose homeostasis; Glycoslysis, HMP shunt, TCA cycle, gluconeogenesis, cholesterol biosynthesis, oxidation of fatty acids & synthesis, urea cycle, basic biochemical reactions of amino acids.

4. Cell communication:

General principles: Cell surface receptors (Ion channel linked G-Protein linked and enzyme linked receptors) and intracellular receptors, forms of intracellular signaling- autocrine, paracrine, contact dependent, synaptic and endocrine signaling. Response of cell to signals. Intracellular signaling proteins: different types and their role. Second messengers- cAMP pathway and role of calcium.

5. Alcohol, Drugs & Xenobiotics:

Biochemical aspects related to alcohol and alcoholism: drug addiction, anesthetics, membrane tolerance, psychoactive substances; cigarette smoking; drug-membrane interactions. Alcohol induced changes biochemical changes in alcoholics. Basic components and biochemical aspects of human disease. Diabetes mellitus. Liver disease, xenobiotics and metabolism.

References

1. Principles of biochemistry by A. Lehninger.
2. Harpers biochemistry – Robert K Murry, Peter Myes, Dryl K. Grmmer, Victor W. Rod well.
3. Text book of Biochemistry with clinical correlations 4th edition. Thomas M. Devlin.
4. Human physiology- Chaterjee.
5. Biochemical aspects of human diseases by R.S. Elkeles/A.S. Tavall, Blackwell scientific publication.
6. Biochemistry by Lubert Stryer.
7. Dynamic of biological membranes by M.D. Houslay and K.K. Stanley.
8. Biochemistry by cell membranes by S. Papa and J.M. Tagar.
9. Molecular biology of membrane structure and functions by H.R. Petty, Plenum press.
10. Biochemistry by Voet and Voet.

Modelling, Docking and Dynamics of novel protein in micro organisms

1. Introduction to Computers:

Components of computers, storage devices, graphic devices, concepts of Hardware and Software; concepts, methods and types of Networks- Intranet and Internet.

2. Operating Systems and programming:

Basics of operating systems and types; UNIX (Basic commands and concepts), Introduction to LINUX operating systems.

3. Computer Networking:

Introduction. Types-Intranet and Internet. History of internet and LAN. Components of internet setup, utilities of internet-web(WWW), Telnet, search engines, e-mail, and web interface searching.

4. Introduction to Bioinformatics: Various disciplines and applications of Bioinformatics. Genome projects: General introduction to genome projects-Human Genome Project (HGP).

5. Biological Databases:

General introduction, database searching options-sequence alignment, gapped sequences, substitution matrices, filters. An overview of Public molecular DATABASES (EMBL, GenBank, DDBJ, GSDB)-Protein Database Introduction, main databases (PIR international protein sequence database), SWISS-PROT database, TrEMBL, Brookhaven protein databank (PDB). Database querying (NCBI, EXPACY & EMB) using keywords and search engines.

6. Proteomics:
Introduction, principle, technique, MALDI analysis. Application of proteomics in biology.

7. Sequence Analysis:
Concepts of sequence alignments and their importance. Sequence alignment methods and programes: BLAST, BLAST reading output, matrix option (BLOSSUM, PAM), e-value and fetching of sequences. Understanding of FASTA, reading the FASTA output, Pair wise and multiple sequence alignment.

8. Molecular Modeling:
Model building tools (Modeler) – Preparation of files for model building, building of protein models.

9. Visualization packages:
Rasmol-basic utilities and commands for protein visualization in 3-D.

10. Structure of proteins:
Classification and biological functions of proteins. Forces stabilizing protein structure. Primary structure and its determination. Secondary structure – helical, beta pleated structure, non-repetitive-Ramachandran's plot. Super secondary structure. Tertiary and quaternary structures. Three-Dimensional structures of globular proteins- structure and functional relationship.

References:

1. Introduction to computers by -Peter Norton's.
2. *Inside the Machine: An Illustrated Introduction to Microprocessors and Computer Architecture*. Stokes, Jon. San Francisco: No Starch Press. ISBN 978-1-59327-104-6.
3. Operating System Concepts (7th Edition) by Abraham Silberschatz, Peter Baer Galvin, and Greg Gagne.
4. Principles of Operating Systems: Design and Applications by Brian Stuart.
5. Computer Networks (Hardcover)by Andrew S. Tanenbaum
6. Bioinformaics: Sequence, Structure and Databanks: A Practical Approach By Des Higgins (ED), Wilie Tylor (Ed)
7. Fundamental Concept of Bioinformatics By: Dan E. Krane, Michael L. Raymer
8. Structural Bioinformatics by Phil Bourne and Helge Wessing.
9. Bioinformatics: Sequence and Genome Analysis by David W. Mount
10. Proteins and Proteomics: A Laboratory Manual by Richard J. Simpson.
11. Molecular Modeling. Basic Principles and Applications by Hans-Dieter Höltje, Wolfgang Sippl, Didier Rognan, Gerd Folkers
12. Principles of Biochemistry, 2006. A.L.Lehninger, Nelson & Cox (CBS, India)
13. Biochemistry - Donald Voet and Judith Voet (John Wiles and sons)

14. Question Paper Model

Max marks: 100 Time: 3 hours

The question paper shall consists of **Eight** questions with **Six essay** type and **Two short notes** type carrying equal marks, of which the candidate shall answer any **Five questions**.