M.Sc. GENERAL BIOCHEMISTRY

FULL-TIME COURSE 1991/92

LECTURE TIMETABLES AND COURSE SYNOPSIS

M.Sc. GENERAL BIOCHEMISTRY

The award of the M.Sc. degree is based on performance during the course and in the final examination in June. The degree may be awarded as either a pass (overall marks in the band 50-69%) or pass with distinction (overall marks over 70%).

For part-time students the course lasts for two academic years and continuation into the second year depends on a satisfactory performance during the first year and in the sessional exam at the end of the first year.

For the final exam (held in June of year 2)marks are based on;

a) Coursework during both years 1 & 2	15%
b) Final examination	85%

These marks can be moderated by the viva examination which is held for all candidates following the written examination at the end of year 2.

For full-time students the course lasts for one calendar year and includes a project, which is normally laboratory based running from June -September.

Coursework is $\frac{147-8}{23} \simeq \frac{9.6}{15}$

Final marks are based on;

a) Coursework	15% 9.6
b) Final examination	60%
c) Project	25%

These marks can be moderated by the viva examination which is held for all candidates following the written examination.

For all candidates all elements of the examination (i.e. coursework, written papers and project, where appropriate) must be passed.

N.B. Satisfactory performance during the course means regular attendance at formal lectures, seminars and in-course assessments, and handing in written assignments and practical notebooks on time.

If for any reason you are unable to attend college please inform the appropriate member of staff at the earliest opportunity. For prolonged absence due to illness a medical certificate should be provided.

Monday Tutorial
~~Thursday~~ morning 11.30 → 12.30
Dr. Banner
In my office
on
1.00

TMTBLE1A.WED

MSc GENERAL BIOCHEMISTRY PART 1 (Wednesdays)

TERM 1 1991/92

L = Lecture, P = Practical, S = Seminar

OCT 9	2.00 p.m	Introduction	BANNER
	L 2.15 p.m	Centrifug. & Sub-cell fraction	WRIGGLESWORTH
	T 3.15 p.m	Tutorials	TUTORS
	L 4.00 p.m.	Principles of Spectroscopy	WRIGGLESWORTH
	L 5.30 p.m	Tissue Preparation	PERRY
	L 6.30 p.m	Separation Techniques 1	HALL
OCT 16	L 2.00 p.m	Separation Techniques 2	HALL
	L 3.00 p.m	Separation Techniques 3	HALL
	T 4.00 p.m	Tutorials	TUTORS
	L 5.30 p.m	Enzymology 1	BUTTERWORTH
	L 6.30 p.m	Enzymology 2	BUTTERWORTH
	S 7.30 p.m	Enzyme Assays/Separation Methods	HALL
OCT 23	L 2.00 p.m	Protein Structure 1	WRIGGLESWORTH
	L 3.00 p.m	Protein Structure 2	WRIGGLESWORTH
	P 4.00 p.m	Lab Workshop	HALL
	P	Enzyme assays (Glucose Oxidase + ADH)	
OCT 30	L 2.00 p.m	Separation Techniques 4 (Electrophoresis)	HALL
	T 3.00 p.m	Tutorials	TUTORS
	L 4.10 p.m	Enzymology 3	BUTTERWORTH
	L 5.30 p.m	Separation Techniques 5 (HPLC/GLC)	QUINN
	S 6.30 p.m	Practical Calculations/Test	HALL
NOV 6	L 2.00 p.m	Protein Structure 3	WRIGGLESWORTH
	L 6.00 p.m	Enzymology 4	BUTTERWORTH
	P 3.00 p.m-	Group A - Purification of Lysozyme	
	9.00 p.m	Group B - Gel Filtration (Sephacryl)	
		Group C - SDS PAGE/Western blotting	
NOV 13	L 2.00 p.m	Enzymology 5	BUTTERWORTH
	L 6.00 p.m	Protein Structure 4	WRIGGLESWORTH
	P 3.00 p.m-	Group A - SDS PAGE/Western blotting	
	9.00 p.m	Group B - Purification of lysozyme	
		Group C - Gel filtration (Sephacryl)	
NOV 20	L 2.00 p.m	Enzymology 6	BUTTERWORTH
	L 6.00 p.m	Protein Structure 5	WRIGGLESWORTH
	P 3.00 p.m-	Group A - Gel filtration (Sephacryl)	
	9.00 p.m	Group B - SDS PAGE/Western Blotting	

Mixed types :

Group C - Purification of lysozyme

NOV 27	L 2.00 p.m	Enzymology 7	BUTTERWORTH
	T 3.00 p.m	Tutorials	TUTORS
	L 4.10 p.m	Protein Structure 6	WRIGGLESWORTH
	L 5.30 p.m	Carbohydrate Metabolism 1	PERRY
	L 6.45 p.m	Carbohydrate Metabolism 2	PERRY
DEC 4	L 2.00 p.m	Carbohydrate Metabolism 3	PERRY
	T 3.00 p.m	Tutorials	TUTORS
	L 6.00 p.m	Carbohydrate Metabolism 4	PERRY 11 o'clock
	P 4.00 p.m-	Group A - Enzyme Inhibn (Acid P'ase)	
	9.00 p.m	Group B - MDH	
DEC 11	L 2.00 p.m	Carbohydrate Metabolism 5	PERRY
	T 3.00 p.m	Tutorials	TUTORS
	L 6.00 p.m	Carbohydrate Metabolism 6	PERRY
	P 4.00 p.m-	Group A - MDH	
	9.00 p.m	Group B - Enzyme Inhibn (Acid P'ase)	

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MSc GENERAL BIOCHEMISTRY PART 1 (Wednesdays)

TERM 2 1991/92

L = Lecture, P = Practical, S = Seminar

JAN 15	L 2.00 p.m	Lipid Metabolism 1	HALL
	3.00 p.m	Tutorials	TUTORS
	L 4.10 p.m	Lipid Metabolism 2	HALL
	L 5.45 p.m	Lipid Metabolism 3	HALL
	S 7.00 p.m	Enzymes	BUTTERWORTH
JAN 22	L 2.00 p.m	Lipid Metabolism 4	HALL
	3.00-6.00 p.m	Data Interpretation	PERRY
	7.00-8.00 p.m.	(Carbohydrate Metabolism)	
	L 6.00 p.m	Lipid Metabolism 5	HALL
JAN 29	L 2.00 p.m	Lipid Metabolism 6	HALL
	T 3.00 p.m	Tutorials	TUTORS
	4.10 p.m	Amino Acid Metabolism 1	PERRY
	5.45 p.m	Amino Acid Metabolism 2	PERRY
	7.00 p.m	Data Exercise	HALL
FEB 5	L 2.00 p.m	Lipid Metabolism 7	HALL
	6.00 p.m	Amino Acid Metabolism 3	PERRY
	P 3.00 p.m - 9.00 p.m	Kinetics of ICD 7	HALL
FEB 12	L 2.00 p.m	Membranes 1	QUINN
	L 6.00 p.m	Membranes 2	QUINN
	P 3.00 p.m - 9.00 p.m	Data Interpn. by Computer	CAMMACK
FEB 19	L 2.00 p.m	Mitochondria 1	BAUM
	T 3.00 p.m	Tutorials	TUTORS
	L 4.00 p.m	Mitochondria 2	BAUM
	L 5.30 p.m	Membranes 3	QUINN
	6.30-9.00 p.m	Data Interpn. 6	HALL
FEB 26	L 2.00 p.m	Mitochondria 3	BAUM
	T 3.00 p.m	Tutorials	TUTORS
	4.00-6.00 p.m	Data Interpretation 6	QUINN
	7.00-9.00 p.m	(Membranes)	
	L 6.00 p.m	Mitchondria 4	BAUM

MAR 4	L 2.00 p.m	Integration of Metabolism 1	PERRY
	L 6.00 p.m	Integration of Metabolism 2	PERRY
	P 4.00 p.m -	Grp. A - Artificial Membranes 7	
	9.00 p.m	Grp. B - Mitochondria -Oxygen electrodes 8	
MAR 11	L 2.00 p.m	Integration of Metabolism 3	PERRY
	L 6.00 p.m	Integration of Metabolism 4	PERRY
	P 3.00 p.m -	Grp. A - Mitochondria -Oxygen electrodes	
	9.00 p.m	Grp. B - Artificial Membranes	
MAR 18	L 2.00 p.m	Integration of Metabolism 5	PERRY
	T 3.00 p.m	Tutorials	TUTORS
	L 4.15 p.m	Integration of Metabolism 6	PERRY
	S 5.45 p.m	Mitochondria/Bioenergetics	WRIGGLESWORTH

At the end got $\frac{146 \cdot 8}{25} = 5 \cdot 87$

Max = 165

Next term $\frac{32}{5}$

TMTBLE1C.WED

MSc GENERAL BIOCHEMISTRY PART 1 (Wednesdays)

TERM 3 1991/92

APR 29	L 2.00 p.m	Isoenzymes	PLUMMER
	L 3.00 p.m	Enzyme Mechanisms 1	BUTTERWORTH
4:45 Drury Lane	L 4.15 p.m	Enzyme Mechanisms 2	BUTTERWORTH
	E 5.45 p.m	Examination (short answer)	HALL
MAY 6	L 2.00 p.m	Enzyme Mechanisms 3	BUTTERWORTH
	L 6.00 p.m	Enzyme Mechanisms 4	BUTTERWORTH
	3.00 - 6.00 p.m	Enzyme Kinetics	BUTTERWORTH
MAY 13	L 2.00 p.m	Enzyme Mechanisms 5	BUTTERWORTH
	T 3.00 p.m	Tutorials	TUTORS
	L 6.00 p.m	Enzyme Mechanisms 6	BUTTERWORTH
	3.00 - 6.00 p.m	Data Interpretation	PERRY
MAY 20	L 2.00 p.m	Lysosomes 1	PLUMMER
	T 3.00 p.m	Tutorials	TUTORS
	L 4.15 p.m	Lysosomes 2	PLUMMER
	5.30 p.m	Data Interpretation	HALL
MAY 27		NO TUITION	
JUN 3	2.00 p.m	Sessional Exam Part 1 - * Calculations & theory of practical work	
JUN 10	2.00 p.m	Sessional Exam Theory Part 2 *	

59 hours of lectures & Data exercise ≈ 60 hours

TUTORIALS: Wednesday tutorials are for part-time students only.

* SESSIONAL EXAMS - for part-time students only.

"MSc Gen Biochem - 1
Lecture Synopsis"

Centrifugation & sub-cellular fractionation

Circular motion and acceleration. "g" forces. The centrifuge, types of rotors. Differential centrifugation, sub-cellular fractionation, marker enzymes, "washing". Density gradient centrifugation, self forming gradients. Sedimentation coefficients and molecular weights.

Principles of Spectroscopy

The electromagnetic spectrum, interaction of radiation with matter, quantization, different regions of the spectrum for different transitions. Instrumentation, basic principles, optical spectroscopy, the spectrophotometer. Beer-Lambert, deviations, stray light, light scattering.

Tissue preparations for the study of metabolism and its control

Tissue preparations used in biochemical experimentation - perfused organs, tissue slices, isolated cells, subcellular organelles, enzymes. The types of experimental questions that can be asked at each level of cellular organisation. Relevance of data obtained using these preparations to the intact organism? How may this be assessed?

Separation Techniques

L1 Problems in protein separation. Advantages and disadvantages of protein purification. Concept of specific activity. Stabilisation of proteins during extraction and separation. Solubilisation of membrane proteins (tissue disruption to be discussed in tutorials). Separation methods of low, intermediate and high resolution. Differential precipitation by ionic strength, pH and organic solvents. Role of surface charges and hydrophobic regions. Protein concentration tech-niques.

L2 Principles of gel filtration; media, advantages and applications. Hydrophobic interaction chromatography and other substituted support systems.

Ion exchange chromatography; nature and applications. Stepwise and gradient elution. Methods of desalting. Technique of iso-electric focusing and its applications, including 2D gel electrophoresis.

L3 Affinity Chromatography. The preparation of affinity material. Properties of the "ideal" matrix and the importance of a "spacer arm". The attachment of a ligand to the spacer arm illustrated by specific chemical reactions. Water-soluble carbodiimides. Applications. A brief resume of how affinity chromatography can be used to purify receptors, antibodies, glycoconjugates and nucleic acids.
A more detailed consideration of the purification of enzymes illustrated by acetyl-cholinesterase.

L4 Electrophoresis. The principles of zone electrophoresis. The effect of charge, size and shape on electrophoretic mobility. The effect of pH and ionic strength on ionization. Eletroosmosis. A brief review of supporting media. Polyacrylamide-gel electrophoresis (P.A.G.E.). Polymerisation of acrylamide. Gel composition and "pore size". Discontinuous electrophoresis. Molecular weight determination by gradient-gel electrophoresis and SDS-electrophoresis.

L5 HPLC/GLC. Principles of liquid chromatography Column efficiency - theoretical plates. Column capacity ratio. Resolving power of chromatography systems. Factors affecting band spreading. Applications of HPLC.

<u>Protein Structure</u>

L1 Properties of amino acid side chains.
protein Conformation - introduction to general principles, levels of structure. Post-ribosomal modifications including proteolytic cleavage, glycosylation, phosphorylation. Properties of backbone chain - angles, freedom of rotation, properties of peptide bond.

L2 Secondary structures - H-bonds, properties. α-helix, residue arrangement. ß-structures, sheet structures, turns.

L3 Supersecondary Structures. Structure Prediction - The problem, the principles. Secondary structure prediction - Chou-Fasman and other types of analysis.

Helical bundles, beta barrels etc. Secondary structures in membrane proteins, Kyte & Doolittle method for hydropathies.

L4 Tertiary Structure. Stabilizing forces - H-bond, charge group interactions, apolar interactions, Van der Waals interactions. Examples: mutations in haemoglobins. Disulphide bonds. Structural domains - globular structures, evolution of tertiary structures e.g. immunoglobulins. Domains and conformational changes, e.g. citrate synthase.

L5 Denaturation - Definition (does not involve breaking primary structure), temperature effects, use of non-polar solvents, effect of pH, use of strong H-bonding sovents (especially urea). Denaturation and renaturation of RNAase. Quarternary Structure. Definition, forces involved, effect of dilution on quaternary equilibrium.

L6 Quaternary structure and biological activity - modification of activity by substrate binding, cooperative effects e.g. haemoglobin. Regulation - modification of structure and activity by allosteric regulators, e.g. haemoglobin and the Bohr effect, haemoglobin and DPG. Enzyme activity and allosteric regulation.

Properties of Enzymes

L1 Catalytic action and the significance of lowering of the Activation Energy. Enzymes as catalysts: active sites, induced fit, transition state stabilisation, classification, units of activity.

L2 Reaction kinetics: the effect of reactant concentration on rate of reaction, order of reaction; Initial reaction velocity and the influence of substrate concentration on the initial velocity; Michaelis-Menten equation, steady-state hypothesis; Michaelis constant and Vmax. Significance of Vmax/Km.

L3 Determination of Km and Vmax. Comparison of the precision of linear plots of the Michaelis-Menten eqation. Reversible inhibition: competitive, non-competitive, uncompetitive. Identification of type of inhibition and determination of the inhibition constant, Ki.

L4&5 Effect of pH on enzyme activity; application to investigation of mechanism. Bisubstrate reactions: Use of kinetics (primary and secondary plots) and product inhibition to distinguish between ordered, ping-pong and random mechanisms. Pre-steady-state "burst" kinetics.

L6 Binding of ligand to proteins possessing more than one site. Use of Scatchard plot to analyse binding data. Non-equivalence of sites and the Adair Equation.

L7 Cooperativity in binding of ligand to proteins. The models of Hill, Monod-Wyman-Changeux, Koshland. The significance of the Hill coefficient, tests of models.

L8 Isoenzymes. Multimolecular forms of enzymes and isoenzymes. Classification and examples of multiple molecular forms. A more detailed consideration of the isoenzymes of lactate dehyrodgenase and their use in detecting tissue damage caused by disease or toxins.

Mechanisms of enzyme reactions

L1&2 Coenzymes NAD(P)-linked dehydrogenases: hydride ion transfer, A and B sidedness, dinucleo-tide fold. Flavoprotein versatility: dehydrogenases, oxidases, hydroxylases. Thiamine pyrophosphate: decarboxylations, transketolase. Pyridoxal phosphate: Schiff base formation with amino acid. Coenzyme A: properties of thiolesters.

L3 Ribonuclease: Reactivity of histidine residues; pH-kinetics studies of Matthias and Rabin, concerted acid-base catalysis. S-protein and S-peptide. X-ray crystallographic analysis.

L4 Serine proteases: Chymotrypsin, trypsin, elastase, subtilisin. Zymogens. Identification of active site residues by chmical modification and triad (charge relay system); stabilisation of transition state. Structural explanation of specificities and the non-activity of the zymogens.

L5 Lysozyme: X-ray crystallography, substrate distortion hypothesis, stabilisation of transition state. Catalytic perfection: triose phosphate isomerase.

L6 Theories of catalysis: how enzymes work

Carbohydrate metabolism I

L1 Metabolic pathways - relation between the chemistry of the intermediates/flux through the pathway/function of pathway/control of function. The ATP/ADP cycle. Glycolysis - pathway analysis, function and control. Maintenance of glycolytic flux - reoxidation of NADH with formation of lactate, ethanol. Functions of glycolysis in different tissues.

L2 Tricarboxylic acid cycle - pathway analysis, function and control. Acetyl CoA - its central position in intermediary metabolism. Acetyl CoA as substrate for the tricarboxylic acid cycle. What happens in one turn of the cycle - function of the cycle in catabolism. Link between the tricarboxylic acid cycle and the electron transport chain. ATP formation and use of oxygen. Pasteur effect.

L3 Pyruvate dehydrogenase as the link between glycolysis and tricarboxylic acid cycle. Multi-enzyme complex nature and function of pyruvate dehydrogenase. Control of PDH in different physiological states. Integration of glycolysis, pyruvate dehydrogenase activity and the tricarboxylic acid cycle.

L4 Distinction between the substrate for, and intermediates of, the tricarboxylic acid cycle. Can intermediates of the cycle be oxidised totally to CO2 and water? Phosphoenolpyruvate carboxykinase.

The anabolic function of the cycle, and its consequences for the bioenergetic function of the cycle. Anaplerotic pathways. Pyruvate carboxylase -function and control. Glyoxylate cycle - occurence, physiological role in prokaryotes and plants, control. Regulation of isocitrate dehydrogenase activity and therefore flux through glyoxylate cycle in E. coli.

L5 Gluconeogenesis - physiological role. Mechanisms for reversal of irreversible steps in glycolysis. Mitochondrial/cytosolic interrelationships in gluconeogenesis. Nature of gluconeogenic substrate and consequences for exit of oxaloacetate and reducing equivalents from mitochondria. Introduction to fructose-2, 6-bisphosphate. Substrate cycles and control of flux. Pentose phosphate pathway - production of NADPH for reductive biosyntheses.

L6 Glycogen metabolism and its control. Pathways for glycogen synthesis and glycogenolysis. Difference in function of glycogen stored in liver and muscle - control of glycogen metabolism in relation to this difference in function. Hormonal control of phosphorylase - interactions between two signal transduction systems involving Ca^{2+} and cyclic AMP. Control of glycogen synthase. Protein phosphatases. Role of inhibitor-1 and its control by cyclic AMP.

Lipid Metabolism

L1 Revision lecture. The nature of lipids. Saturated and unsaturated fatty acids. Essential fatty acids. Role of PUFA (membranes, epidermal water barrier, prostaglandins, etc), triglycerides and phospholipids. Phospholipases. Behaviour of lipids in an aqueous environment. Role of bile and pancreatic enzymes in lipid digestion.

L2 Fate of digested lipids. Re-esterification of monoglycerides. Chylomicron formation. Structure and role of plasma lipoproteins. Characteristics of white adipose tissue. Location and directional role of lipoprotein lipase. Importance of apo-proteins. Fate of chylomicrons.

L3 Receptor mediated endocytosis. Nature and inter-relationships of VLDL, IDL, LDL, and HDL. Apo B48 and post-transcriptional modification. Hyperlipoproteinemia and abetalipoproteinemia. Role of LCAT. LDL receptors and receptor-mediated endocytosis. Genetic defects. Fate of cholesterol esters. Regulation of ACAT and HMGCOA reductase by cholesterol and by phosphorylation. Relevance to atherosclerosis (very brief).

L4 Fat mobilisation. Products of mobilisation. Hormone-sensitive lipase and its regulation by 5'AMP-and cAMP-dependent kinases. Cholesterol-esterase activity.

Control of lipolysis by hormones and glucose levels. Uptake of FA by cells and mitochondria. Role of carnitine. Inhibition by malonyl CoA.

L5 ß-oxidation and ketone bodies. Key features and regulatory aspects of ß-oxidation. Oxidation of unsaturated and odd chain length FA (brief). Nature, formation, utilisation and role of ketone bodies. Ketosis. Regulatory aspects of ketone bodies.

L6 Fatty acid synthesis. Sources of Acetyl CoA. The citrate shuttle. Supply of NADPH by malic enzyme and pentose phosphate pathway. Subunit structure of Acetyl CoA carboxylase. Polymer formation. Acyl carrier protein. Reactions of FA synthesis. End products. Multifunctional enzyme complexes in yeast and mammals.

L7 Lipid synthesis. Elongation and desaturation of FA (brief). Regulation of FA synthesis. Effect of hormones, citrate and Facyl CoA on Acetyl CoA carboxylase. Relation to polymerisation and phosphorylation (both 5'AMP- and cyclic AMP dependent).

Synthesis of triglycerides and phosphoglycerides. Interconversions of phosphogylcerides. Regulatory aspects.

Amino acid metabolism

L1 Protein digestion and assimilation. Enterokinase and the protease activation cascade. Amino acid and peptide absorption. Catabolism of amino acids and urea synthesis. Transaminases and glutamate dehydrogenase. Transdeamination. Control of urea synthesis. Relation between urea synthesis and gluconeogenesis.

L2 Essential and nonessential amino acids - definition and methods of assessment. Glucogenic and ketogenic amino acids. Metabolism of selected amino acids. (i) Phenylalanine and tyrosine - synthesis of catecholamines. Catabolism of phenylalanine and tyrosine. Phenylketonuria. (ii) Methionine and cysteine. S-adenosylmethionine and methylation reactions. Catabolism of methionine and cysteine.

L3 Tissue-tissue relationships in amino acid metabolism. Glucose-alanine cycle. Synthesis of alanine and glutamine by muscle particularly during starvation. Physiological roles of alanine and glutamine released by muscle. Synthesis of urea or glutamine from ammonia in the liver - relation to pH homeostasis and excretion of nitrogen as ammonium ions or urea through the kidney. Activation of glutamine metabolism by acidosis in the kidney.

Intergration of metabolism

L1 Importance of the control and integration of metabolic pathways in vivo. General considerations of control of metabolic pathway flux. Types of control mechanisms. Non-equilibrium reactions as control sites. Flux-generating steps - definition, examples and importance in vivo.

L2 Integration of metabolism with contraction in muscle. Fibre types and metabolism. Creatine phosphate and sources of ATP in contracting muscle. Activation of glycogenolysis, glycolysis and tricarboxylic acid cycle in an integrated way during contraction. Control of phosphorylase and phosphofructokinase. Adenylate kinase, AMP deaminase and the purine nucleotide cycle. Adenylate energy charge.

L3 Integration of metabolism between tissues in vivo. Glucose-fatty acid cycle. Experimental evidence for relationship between glucose and fatty acids.

Control of fatty acid release from white adipose tissue - hormone-sensitive lipase. Fate of glycerol and the triglyceride/fatty acid cycle. Regulation of fatty acid re-esterification. Preferential oxidation of fatty acids by red skeletal muscle spares glucose oxidation. Metabolic consequences of enhanced fatty acid oxidation in muscle - inhibition of glycolysis by inhibition of phosphofructokinase. Implications for muscle glycogen metabolism.

L4 Integration in response to starvation (i). Short-term and long-term starvation. What metabolic problems are posed by starvation? Conservation of glucose reserves by fatty acid oxidation in muscle. Increased rates of gluconeogenesis and ketogenesis in liver. Control of gluconeogenesis by hormones and substrate supply. Phospho/dephospho regulation of pyruvate kinase. Fructose-2, 6-bisphosphate - effect on phosphofructokinase-1 and fructose-1, 6-bisphosphatase to control glycolysis/gluconeogenesis. Regulation of fructose-2, 6-bisphosphate levels in liver. Cyclic AMP and fructose-2, 6-bisphosphate as indicators of the starved and fed states respectively.

L5 Fatty acid metabolism in liver during starvation. Why does oxidation take precedence over esterification? Role of malonyl CoA and control of the carnitine shuttle. Central role of acetyl CoA carboxylase in response of liver to hormonal and metabolite signals reflecting the physiological state of the animal. Production of ketone bodies from increased supply of acetyl CoA from fatty acids. Decreased oxaloacetate favours ketogenesis. Fate of ketone bodies - oxidation in muscle and brain conserving glucose. Effects on adipose tissue regulating lipolysis.

L6 Storage of excess carbohydrate-carbon as triglyceride in the fed animal. Integration of pathway from glucose metabolism in liver to triglyceride stored in adipose tissue. Central role of insulin.

Role of key enzymes - especially acetyl CoA carboxylase. Integration of fatty acid synthesis and cholesterol synthesis. Review of the metabolic effects of insulin, glucagon and catecholamines. Review of phospho/dephospho control mechanisms.

Cell Membranes

L1 Morphology of cell membranes. Membrane functions. Methods of isolation and purification. Enzyme markers. Membrane composition: lipid, protein and carbohydrate constituents. Structure of membrane substituents.

L2 Properties of polar lipids in aqueous systems. Monomolecular films. Critical Micelle Concentration. Aggregation and polymorphic phase behaviour. Integration of protein into the lipid matrix. Singer and Nicolson fluid mosaic model. Dynamic behaviour, motion of lipids and proteins.

L3 Membrane molecular biology. Signal hypothesis of membrane protein synthesis. Secretion. Transmembrane signal processes. Poly-phosphoinositide system. Adenylate cyclase. Surface receptors.

Mitochondria

L1 General morphology of mitochondria in situ and after isolation. Topology of sub-mitochondrial particles. Chemical composition and ultrastructure, especially of inner membrane. Brief review of biogenesis. Permeability characteristics of outer and inner membranes. Electroneutral and electrogenic permeases of inner membrane. Outline of "ancillary" and "fundamental" functions of mitochondria and their localisation with respect to mitochondrial compartments.

L2 Food and energy needs in terms of ATP turnover. Light and dark reactions of photosynthesis. Intermediary metabolism as reversal of dark phase of photosynthesis. ATP requirement of dark reaction in relation to substrate level phosphorylation. Oxidative phosphorylation as reversal of light phase. Substrates for oxidative phosphorylation. Transfer of cytoplasmically-generated reducing equivalents. The respiratory chain: chemistry and ultrastructural organisation. Phenomenology of the common pool of conserved energy; respiratory control, P/O ratios, uncouplers, Ca^{2+} uptake, reversed electron transfer etc.

L3 The chemiosmotic theory and how it accounted for phenomenology and successfully predicted other phenomena. Chemiosmotic explanation of photo-synthetic phosphorylation. Mechanistic problems of generation by respiratory chain and utilisaion by ATP synthetase. Proton stiochiometries. Q-cycle. Cytochrome oxidase as proton pump. Ligand conduction versus trans-membrane Bohr effect.

Lysosomes

L1 Lysosomes in Biology. Discovery, acid hydrolases, a family of particles, stability, isolation, and purification. Physiological function. The function of lysosomes in normal physiology considered. More details about their role in fertilisation and production of thyroid hormone.

L2 Lysosomes in Pathology. Damage to lysosomes. Lipid peroxidation, role of lysosomes in tissue injury, the effect of radiation and adverse physical conditions. Lysosomes in disease. The uptake of exogenous material by lysosomes. Lysosomes and arthritis and cancer. Lysosomal storage diseases.

TMTBLE2A.TUE

M.Sc. GENERAL BIOCHEMISTRY PART 2 (Tuesdays)
TERM 1 1991/92

(L = lecture; P = practical; T = tutorial; S = seminar)

OCT 8	L 2.00 p.m	Introduction	BANNER
	L 2.30 p.m	Nucleic Acid Structure - 1	BANNER
	L 4.00 p.m	Gene Arrangement - 1	HALL
	* 5.00 p.m	Welcome to Students	
OCT 15	L 2.00 p.m	Nucleic Acid Structure - 2	BANNER
	L 3.15 p.m	Gene Arrangement - 2	HALL
	L 4.15 p.m	RNA Synthesis	BANNER
	T 5.30 p.m	Tutorial - Data Intepretation	BANNER
OCT 22	L 2.00 p.m	Data Interpretation Exercise	BANNER
	L 3.15 p.m	Gene Cloning - 1	DUDLEY
	L 4.15 p.m	Gene Cloning - 2	DUDLEY
	T 5.30 p.m	Gene Arrangement	HALL
OCT 29	L 2.00 p.m	Gene Cloning - 3	DUDLEY
	P 3.00 p.m-9.00 p.m	PRACTICAL 1	BANNER
	L 5.30 p.m	DNA Replication 1	BANNER
NOV 5	L 2.00 p.m	Gene Cloning - 4	DUDLEY
	P 3.00 p.m 9.00 p.m	PRACTICAL 2	BANNER
	L 5.30 p.m	DNA Replication 2	BANNER
NOV 12	L 2.00 p.m	Gene Cloning - 5	DUDLEY
	P 3.00 p.m-9.00 p.m	PRACTICAL 3	BANNER
	L 5.30 p.m	DNA Sequencing	DUDLEY
NOV 19	L 2.00 p.m	Gene Structure & Expression 1	HALL
	P 3.00 p.m - 9.00 p.m	PRACTICAL 4	BANNER
	L 4.30 p.m	Polymerase Chain Reaction	STIRLING
NOV 26	L 2.00 p.m	Gene Structure & Expression - 2	HALL
	P 3.00 p.m-9.00 p.m	PRACTICAL 5	BANNER
	L 5.00 p.m	Protein Biosynthesis - 1	BANNER
DEC 3	L 2.00 p.m	Protein Biosynthesis - 2	BANNER
	L 3.15 p.m	Gene Structure & Expression - 3	HALL
	S 4.30 p.m	Gene Cloning - Seminar	DUDLEY
DEC 10	L 2.00 p.m	E X A M (Multiple Choice)	
	S 4.00 p.m	Gene Structure & Expression - Seminar	HALL

M.Sc. GENERAL BIOCHEMISTRY PART - 2

TERM 2 1991/92

(L = Lecture; P = Practical; T = Tutorial; S = Seminar)

JAN 14	L 2.00 p.m	Control Prok. Gene Expression - 1	HALL
	S 3.15 p.m	Data Interpretation Exercise	BANNER
	L 4.30 p.m	Control Prok. Gene Expression - 2	HALL
	L 5.30 p.m	Signal Transduction - 1	PERRY
JAN 21	L 2.00 p.m	Control Euk. Gene Expression - 1	HALL
	T 3.15 p.m	Data Interpretation Review	BANNER
	L 3.45 p.m	Signal Transduction - 2	PERRY
	L 4.45 p.m	Basis of Immunology - 1	STAINES
JAN 28	L 2.10 p.m	Control Euk. Gene Expression - 2	HALL
	L 3.15 p.m	Signal Transduction - 3	PERRY
	S 4.30 p.m	Seminar : Gene Expression	HALL
FEB 4	L 2.00 p.m	Basis of Immunology - 2	STAINES
	L 3.15 p.m	Immunology;antibody based methods for Biochem.	STAINES
	L 4.15 p.m	Oncogenes - 1	HALL
FEB 11	P 2.00 p.m	Practical Test	BANNER
	L 4.30 p.m	Oncogenes - 2	HALL
	L 5.30 p.m	Oncogenes - 3	HALL
FEB 18	L 2.00 p.m	Cytoskeleton - 1	EAGLES
	L 3.15 p.m	Cytoskeleton - 2	EAGLES
	D 4.00 p.m	Demonstration	EAGLES
	L 5.15 p.m	Cytoskeleton - 3	EAGLES
	L 6.00 p.m	Cytoskeleton - 4	EAGLES
FEB 25	L 2.00 p.m	Membrane Transport - 1	QUINN
	P 3.00 - 9.00 p.m	Membranes Practical	BANNER
	L 6.00 p.m	Membrane Transport - 2	QUINN
MAR 3	L 2.00 p.m	Membrane Transport - 3	QUINN
	L 3.15 p.m	Membrane Transport - 4	BAUM
	L 4.30 p.m	Membrane Transport - 5	BAUM
	L 6.00 p.m	Connective Tissue Macromols. - 1	PRICE
MAR 10	L 2.00 p.m	Connective Tissue Macromols. - 2	PRICE
	T 3.00 p.m	Tutorial	TUTOR
	L 4.15 p.m	Connective Tissue Macromols. - 3	PRICE
	S 5.30 p.m	Seminar - Membrane Transport	QUINN

MAR 17 2.00 p.m - 5.30 p.m SESSIONAL EXAMINATION

TMTBLE2B.TUE

TMTBLE2C.TUE

M.SC. GENERAL BIOCHEMISTRY PART - 2

TERM 3 1991/92

The main teaching for this term will take place as seminars on topics chosen to cover a wide range of biochemical interests. The seminars will take the form of one or two introductory lectures by a member of staff with the object of providing both a brief revision of the background material and an introduction to the specific topic areas to be covered during the rest of the seminar. This will be followed by presentations by students of the specific topic areas. These areas will be selected by the lecturer and advised to students before the Easter vacation. After each presentation there will be an opportunity for class discussion.

The objectives of this approach are to provide a stimulus for revision of topics from both Part I and Part II of the course and to raise topics of current interest in biochemistry.

A full programme of the seminars, including references, will be available a few weeks before the Easter vacation.

N.B. Although it will be necessary for each student making a presentation to thoroughly prepare their allotted topic, all students will be expected to have made sufficient preparation of every topic to be able to make a contribution to the discussion.

Topics covered will be :

1.	Apr 28th	Mol. Biol. Techniques	DUDLEY
2.	May 5th	Bioenergetics	WRIGGLESWORTH
3.	May 12th	Diabetes	PERRY
4.	May 20th	Multienzyme Complexes	BUTTERWORTH

A suggested programme for the seminars (which may vary according to the lecturer) is:

3.00 p.m	Lecture 1
4.00-4.15 p.m	Tea
4.15 p.m	Lecture 2 (followed by discussion?)
5.15 p.m	Student presentation 1 followed by discussion
5.45 p.m	Student presentation 2 followed by discussion

The above seminars begin at 3.00 p.m following tutorials etc. from 2.00 p.m-3.00 p.m as detailed below:

Apr 28th	Revision Seminar	BANNER
May 5th	Data Interpretation	BANNER
May 12th	Numerical Problems	BANNER
May 20th	Revision Seminar	

44 hours of lectures.

Structure & Organisation of Nucleic Acids

L1 Types of nucleic acid; structure and nomenclature of nucleotides and polynucleotides; base pairing; helical forms; structural features of types of RNA.

L2 Size and distribution of DNA. Supercoiling of DNA. The bacterial nucleoid. Packaging of DNA in eukaryotes; histone proteins; the nucleosome as the basic structural unit of chromatin

Gene Arrangement

L1 Actual and theoretical gene numbers. Anomalies in eukaryotes. C-value paradox. The non-coding component of eukaryotic genomes. Construction and interpretation of Cot curves. Highly repetitive DNA (satellite DNA); nature, location and possible function. Mini-satellites and genetic finger-printing.

L2 Unique genes and genes of limited duplication. Gene families. α- and ß-globin gene clusters. Evolutionary origin. Homologies and common intron pattern. Significance of gene families with examples. The nature of psuedogenes. Processed pseudogenes. Mechanisms of gene duplication.

L3 Moderately repetitive DNA. Non coding sequences (SINES e.g. Alu, and LINES) r-RNA gene organisation in E.coli and eukaryotes. The nucleolus and nucleolar organiser regions. Processing of r-RNA transcripts. E/M visualisation. 5S r-RNA genes. Gene amplification (r-DNA, DHFR, chorion proteins) and its significance Duplication of histone and Ig genes. Supergene families.

Gene Structure and Expression

L1 Nature, distribution and evidence for gene introns. Details of splicing including enzyme independent mechanisms. The spliceosome and roles of small nuclear RNA. Alternative splicing systems. Enzymic properties of RNA. Significance of introns.

L2 Eukaryotic transcription by RNA polymerases I, II and III. Modifications to primary transcripts. 5' capping and its significance for T/L. Comparison with bacterial T/L. 3' polyandenylation; addition sites, terminal processing, poly A polymerase. Significance. Transport of mRNA from the nucleus. Role of nuclear matrix and nuclear envelope.

L3 Methods for studying gene regulatory regions. Promoters. Characteristics of TATA, CAAT and GC boxes. Enhancers and hormone response elements. Cis and Trans regulatory elements. Transcription factors and structural motifs (zinc fingers, etc). Downstream promoters.

DNA Replication

L1 Evidence for the semiconservative nature of replication. Principle features of the replication process. Role of DNA polymerases. Requirement for many other proteins in replication.

L2 Roles for topo-isomerases, helicase and single strand binding proteins. Priming of RNA polymerase and primase. Semi-discontinuous replication. Models for replication. Accuracy of DNA replication.

RNA Synthesis

Enzymology of transcription. RNA polymerases. Structure of E.coli RNA polymerases; role of subunits; recognition of promotor sequences; elongation and termination.

Mechanism of Protein Synthesis

L1 & 2 Amino acid 'activation' by amino-acyl synthetases. Polypeptide chain synthesis; 2-site model for ribosomes; codon-anticodon interaction; formation of initiation complexes; protein factors involved in initiation, elongation and termination. Comparison of protein synthesis in prokaryotes and eukaryotes. Brief outline of post-translational modification of proteins.

"MSc Gen Biochem - 2
Lecture Synopsis"

Control of Prokaryotic Gene Expression

L1 The bacterial chromosome. Constitutive, inducible and repressible genes. Details of genes and regulatory sites of the Lac operon. Promoter consensus sequences. Polycistronic mRNA. Induction by lactose and allolactose. Gratuitous inducers. Negative transcriptional control mechanism. Identification of regulatory mutants and construction of partial diploids.

L2 Repressor-operator interactions-methods of study. Catabolite repression. Effect of glucose on cAMP levels. Catabolite gene activator protein and its role. Repressible operons.

Control of Eukaryotic Gene Expression

L1 General comparison with gene control in prokaryotes. Possible levels at which gene expression may be regulated. Major controls appear to be transcriptional. Evidence that gene activation is associated with changes in chromosome conformation. DNA'ase I sensitivity and hypersensitivity. A role for nucleosomes in gene expression?

L2 The chromatin domain as a unit of gene regulation. Nature of domains. Scaffold associated regions (or MAR) and their components. Topoisomerase II and supercoiling. Location of promoters and enhancers. Current ideas on the significance of DNA methylation.

Gene Cloning

L1 Isolation of RNA from tissues using chaotropic agents. Assessment of quality of RNA by analysis on agarose gels and by *in vitro* translation. Preparation of mRNA using oligo T cellulose chromatography.

Characteristics of mRNA and relative percentages of RNA molecules found in a typical eukaryotic cell.

L2 Manufacture of double stranded cDNA. Concept of a library and sequence abundance. Use of reverse transcriptase to make the first strand. Alternative second strand methods; Klenow using the hairpin, or RNase H/DNA polymerase. Methylation of internal restriction sites. Concept of linkers and addition of linkers using T4-DNA ligase. Use of S1 nuclease and T4 DNA polymerase to polish ends.

L3 Concept of plasmids as vectors using pUC as an example. Drug resistance, origin of replication, growth and preparation of plasmids. Introduction of plasmids into E. coli. Plating out bacteria and selecting for transformants. Use of blue/white inactivation to select for recombinants. Introduc-tion to other plasmids commonly used e.g. p Blue script and pBK322.

L4 Introduction of cDNA into plasmid vectors and preparation of the library. Concept of genomic cloning. Introduction to bacteriophage lamda as vector for cloning large DNA fragments (EMBL 4), as well as use in cDNA cloning (lamda gt 10). Preparation of genomic DNA fragments for cloning. In vitro packaging and plating out lamda phages. Differences between cDNA clones and genomic clones.

L5 Analysis of gene expression and structure using Northern and Southern blotting. Method of setting up each type of blot. Concept of radioactive probes. How to make probes; oligolabelling and nick translation. Labelling oligonucleotides and reverse transcribed probes. Hybridisation and stringency; effect of temperature and ionic strength on stability of DNA/RNA or DNA/DNA hybrids.

Transgenic animals. What methods are available to generate transgenic animals? Analysis of different uses for this technology including;

identification of promotor sequences from tissue specific genes; analysis of the mode of action of oncogenes; analysis of development using lineage ablation. Anti-sense RNA as a possible therapy for inherited diseases.

DNA Sequencing

Reasons for sequencing DNA. Format of DNA for sequencing. M13? Double stranded plasmid DNA now the normal choice. Principles of the dideoxy method/chain termination. Use of 35S ATP (thioester). Manufacture of sequencing gels and principles of sample separation at high voltage. Analysis of data, how to read a sequencing gel. Maxam & Gilbert chemical method: when is it used? Brief description of the method.

Oncogenes

L1 Background. Nature and cause of cancer. Action of carcinogens. Evidence for cancer as a mutational event. Predisposing factors. Immunological aspects. Properties of transformed cells. Growth factors.

L2 Brief survey of DNA T.V. Genome structure and replication of RNA T.V. Main features of acute RNA T.V. including types, origin and locations of v-oncogenes. The scr oncogene. Chronic RNA T.V.: Promoter insertion and enhancer insertion. Evidence for enhanced expression. Use of 3T3 cells and transgenic mice. Use of primary cultures to show simultaneous requirement for two or more oncogenes.

L3 Methods of activation of c-oncogenes with particular regerence to point mutations, gene translocation and gene amplification. Roles of oncogene proteins in detail (src, erb A, erb B, fms, sis, ras, myc, fos, ros, etc) and effects on cell growth. (2.29)

Signal transduction

L1 General principles of signal transduction systems. Receptors - general properties. Second messengers - general properties. The insulin receptor in detail - structure, location and properties. Tyrosine kinase activity - possible role in insulin action? Use of anti-receptor antibodies to probe receptor functioning.

L2 The adenylate cyclase-cyclic AMP-phosphodiesterase signal transduction system. Receptors for glucagon and catecholamines. Location and properties of adenylate cyclase. G-proteins - use of cholera and pertussis toxins to probe G-protein functioning. cAMP dependent protein kinase A. Phosphodiesterases.

L3 The polyphosphoinositide/inositol-1,4,5-trisphosphate/Ca^{2+} signal transduction system. Ca^{2+} as a second messenger in hormone action. Measurement of intracellular Ca^{2+} - photoproteins and fluorescent dyes. Link between Ca^{2+} - dependent hormone action and phospholipid metabolism. Polyphosphoinositides and inositol phosphates. Calmodulin. Protein kinase C.

Basis of Immunology

L1&2 The role of the immune system in protection against disease. Basic mechanisms of natural immunity. Lymphocytes - diversity, origins, functions and the nature of their receptors. Antibodies. Structural and functional aspects in combination with antigen, genetic origins and organisation of the genes. The T-cell receptor - genetics and structure.

Immunological Techniques

This concentrates on antibodies and deals with production and characterisation of antisera. Methods for making monoclonal antibodies and the properties of the antibodies. Basic principles of immunoassays.

"MSc Gen Biochem - 2
Lecture Synopsis"

Cytoskeleton

L1 Introduction to the cytoskeleton. Composition and function. Structure of acton myosin, microtubules, intermediate filaments and their associated proteins.

L2 Assembly and disassembly of cytoskeletal components and control of the process. How muscles work and the detailed structure of muscle. Control of muscle contraction. In vitro assays for actin/myosin based movements. Actin/myosin interaction during cell movements. Cytoplasmic streaming and microvilli movements.

L3&4 Motility-based around microtubules. Bending of cilia and flagella and role of digrein. Detailed structure of cilia. Arrangements of microtubules in cells. Role of microtubules in organelle mobility and during mitosis. Detailed analysis of mitosis and current views on how it occurs.

Membrane Transport

L1 Categories of membrane transport. Passive diffusion. Fick's first Law of diffusion. Effect of solute size, chemical structure and charge. The role of surface charges oF membranes on passive diffusion of electrolytes.

L2 Facilitated diffusion. Kinetics of diffusion. Mechanisms of facilitated diffusion. Characteristics of membrane channels. Mobile carrier hypothesis. Examples of facilitated diffusion in signal transduction, nerve conduction, muscle contraction, vision.

L3 Active transport. Mechanisms of energy coupling. Group translocation of sugars in bacteria. UIon-linked translocations in linked transport systems. Exchange transport, Na^+-K^+ATPase.

"MSc Gen Biochem-2
Lecture Synopsis"

Transport and Metabolic Control

L1 General characteristics of passive permeation, facilitated transport and active transport. ATP-, redox- and light-driven primary pumps and their reversibility. Natural and artificial secondary transport. Ionophores, electrogenic and electroneutral. Properties of mitochondrial anion exchangers.

L2 Secondary transport networks, artificial and natural. Transport networks in control of mitochondrial metabolism and its relation to meta-bolic pathways in the cytoplasm. Ornithine: citrulline exchange and urea synthesis. ADP:ATP exchange and control and stoichiometry of ox-phos. Import and export of reducing equivalents in relation to anabolic and catabolic pathways of carbohydrate and lipid metabolism. Control of carrier function.

Connective tissue macromolecules

L1 Collagen genes, synthesis and structure of type I, II, and III collagens. Relationship of structure to function.

L2 Basement membranes - type IV collagen laminin, heparan sulphate Proteoglycones. Function of basement membranes in development and specialisation in kidney - glomerular basement membrane.

L3 Proteoglycans, structure, membrane associated types in extracellular matrix, viscoelastic gels, Function. Elastin - a stretchable protein. It's sequence. How does stretching occur?