Detailed syllabus of lectures of the subject „Biochemistry“

Lecturer: prof. MUDr. Radim Černý, CSc.

1. Introduction to biochemistry
Biochemistry within the system of biological, chemical and medical sciences. The role of biochemistry in contemporary medicine and its perspectives.

2. Properties of peptides and proteins
Amino acids, their properties, and their classification according to the polarity of their side chains and according to the acid-base properties. Essential and non-essential amino acids. Structure of peptides and proteins, their primary structure. Structures of higher order and their meaning for the function of peptides and proteins.

3. Enzymes
Basic characteristics of enzymes and basic enzyme properties. Substrate and reaction specificity of enzymes. Enzymes as proteins. Apoenzymes and coenzymes. Active sites of enzymes. Mechanisms of catalytic activity, examples. Thermodynamic and catalytic aspects of enzymatic catalysis. Velocity of enzymatic reaction, Michaelis´ constant and its practical meaning. Inhibition of enzymatic reactions. Competitive and non-competitive inhibitors. Allosteric enzymes and allosteric inhibiton and activation and their importance in metabolism. Isoenzymes – relationship to the organization of genes, their importance for the metabolic regulations and for the diagnosis in medicine. Classification and nomenclature of enzymes.

4. Bioenergetics and the role of high energy compounds
Chemical reactions as thermodynamic processes. First and second low of thermodynamics. Enthalpy, free energy, entropy. Endothermic and exothermic reactions. Entropy and living systems. Conservation and transfer of energy in living systems, high energy compounds, ATP.

5. Redox reactions as a source of energy
Oxidation and reduction. Redox potential and standard redox potential. Thermodynamics of electron transfer. Redox reactions as a main source of energy in aerobic organisms.

6. The respiratory chain and oxidative phosphorylation
Mitochondria and its structure. Dehydrogenases and their function. Coenzymes NAD, NADP, FMN, and FAD. NADH dehydrogenase, coenzyme Q, cytochrome system. Cytochrome c reductase and cytochrome c oxidase. Organisation of the respiratory chain, transfer of electrons and transfer of protons. Formation of proton gradient and its usage. ATP synthesis as a function of proton gradient. Stoichiometry of dehydrogenation and formation of ATP. Proton gradient and formation of heat. Inhibitors of the respiratory chain, their experimental and medical importance.

7. The citrate cycle
Structure of CoA, its formation and function. Acetyl-CoA. Description of individual reactions of the citrate cycle. Dehydrogenation steps in citrate cycle and their connection with the respiratory chain. Energy output of the citrate cycle. Regulation of the citrate cycle at the individual enzyme level. Amphibolic character of the citrate cycle – the need for the supplementing (anaplerotic) reactions – pyruvate carboxylase. Mechanism of carboxylation, the role of biotin.

8. Glycolysis
Description of individual glycolytic reactions and glycolytic enzymes. Glycolysis in aerobic and in anaerobic conditions. Formation of lactate. Relationship between glyceraldehydephosphate dehydrogenase and lactate dehydrogenase and its importance. Energy production in glycolysis under aerobic and anaerobic conditions. Regulation of glycolysis – importance of phosphofructokinase and fructose-2,6-bisphosphate. Activation of *feed-forward* type.

9. Metabolism of pyruvate
Anaerobic conversion into lactate and its importance. Alcoholic fermentation. Carboxylation of pyruvate into oxaloacetate. Oxidative decarboxylation of pyruvate into acetyl-CoA. Total energy production of glucose metabolism under different conditions. Compartmentation of individual processes in the cell, connection of extra- and intramitochondrial redox processes, malate shunt and glycerolphosphate shunt.

10. Gluconeogenesis
Description of individual steps in gluconeogenesis, relation to the glycolysis. Non-reversible reactions of glycolysis and their specific solution in gluconeogenesis. Cycling of some reactions and its meaning. Regulation of gluconeogenesis in connection with regulation of glycolysis.

11. Synthesis and degradation of glycogen
Structure of glycogen, reducing end non-reducing ends of the molecule. The role of glycogen in the energy producing metabolism. Degradation of glycogen. Phosphorylase, mechanism of activity, its regulation. cAMP cascade.

Debranching enzyme, its transferase and glucosidase activity. UDP-glucose. Gycogen synthase and branching enzyme. The overall regulation of glycogen metabolism. Control of blood glucose.

12. The pentose phosphate pathway
Description of individual steps of pentose cycle. Importance of glucose-6-phoshpate dehydrogenase for production of NADPH, reduced glutathion and degradation of peroxides. Formation of pentoses and their significance. Transaldolase and transketolase. Relationship between the pentose cycle and glycolysis. The role of pentose cycle in photosynthesis. Brief description of photosynthesis as a basic process in autotrophic organisms.

13. Metabolism of other saccharides
Carbohydrates as nutrients. Digestion of starch and glucose resorption. Digestion of sucrose, utilisation of fructose and its problems. Conversion of glucose to galactose, biosynthesis of lactose. Digestion of lactose, utilisation of galactose and its disorders.

14. Metabolism of fatty acids
Fatty acids in energy-producing metabolism. Activation of fatty acids – acyladenylates and acyl-CoA. Transport of activated fatty acids into mitochondrial matrix, acyl-carnitine. Beta-oxidation of fatty acids, production of acetyl-CoA. Energy output of fatty acid oxidation. Comparison with carbohydrates with respect to the oxygen consumption and energy production. Degradation of fatty acids in mitochondria and in peroxisomes. Biosynthesis of fatty acids. Transfer of substance and reducing potential for this synthesis from mitochondria into cytosol. Formation of malonyl-CoA and its regulation. Importance of ACP (acyl-carrying protein) and its role in fatty acid synthesis. The regulation of fatty acid synthesis and degradation.

15. Ketone bodies. Relations between the carbohydrate and fatty acid metabolism
Conversion of acetyl-CoA into 3-hydroxy-3-methyl-glutaryl-CoA (HMG). Formation of acetoacetate and other ketone bodies. Ketone bodies as water-soluble equivalent of fatty acids. Utilisation of ketone bodies in different tissues. Ketone bodies in starvation. Equilibrium of ketone bodies formation and utilisation. Conversion of carbohydrates into fatty acids and triacylglycerols, impossibility to convert fatty acids into carbohydrates. Explanation of this phenomenon. Metabolic disorders related to the preferential utilization of fatty acids, starvation, diabetes mellitus. Ketone bodies as indicators of metabolic disorder, ketoacidosis.

16. Triacylglycerols in metabolism
Triacylglycerols (TAG) as energy storage molecules. Their biosynthesis and degradation. TAG in nutrition. Digestion and resorption of TAG. Resynthesis of TAG in enterocytes and formation of chylomicrons. (VLDL). Lipoprotein lipase. Utilisation pf fatty acids in different tissues, utilisation in adipocytes. The dynamic equilibrium (steady state) of stored TAG in adipocytes. The absence of glycerol kinase in adipose tissue and its significance. Blood glucose level and TAG in adipocytes. Hormone-sensitive lipase and its regulation. Mobilisation of fat in adipose tissue. Free fatty acids (FFA) in blood and their transport on albumin.

17. Metabolism of cholesterol
Biosynthesis of cholesterol. HMG reductase as the checkpoint of cholesterol synthesis and target of therapeutic action. The role of cholesterol in organism and the role of the liver in cholesterol metabolism. Degradation and excretion of cholesterol. Primary and secondary bile acids and their importance, their enterohepatic circulation. The excretion of bile acids, the influence of dietary fiber and therapeutic agents.

18. Lipoproteins of blood plasma
Digestion and resorption of lipids. Formation of chylomicrons, their circulation and degradation with lipoprotein lipase. Fianl degradation of chylomicrons remnant in the liver. Formation of VLDL in the liver, circulation, degradation and conversion to LDL. The role of LDL and LDL receptors. Reverse transport of cholesterol, HDL. The role of individual apoproteins, their biological significance. The risk factors of atherosclerosis. The examination of lipoproteins, hyperlipoproteinemias.

19. Steroid hormones and vitamins D
Vitamin D and its conversion to calcitriol. Biosynthesis of individual groups of steroid hormones, their significance in organism. Degradation of steroid hormones and the estimation of degradation products in body fluids.

20. Metabolism of acylglycerols and sphingolipids
Biosynthesis of diacylglycerol phosphate (phosphatidate), activation to CDP-derivative, formation of phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol-4,5-bisphosphate. Cardiolipins, ether phospholipids, plasmalogens, PAF. Phospholipases, their role in degradation and in production of regulatory molecules – diacylglycerol and inositol trisphosphate. Biosynthesis of sphingosine, ceramide and sphingolipids – sphingomyelins, cerebrosides, sulfatides, and gangliosides. Degradation of sphingolipids, sphingolipidoses.

21. Metabolism of unsaturated fatty acids and eicosanoids
The most important unsaturated fatty acids and their significance. Desaturases, their significance and limitations. Elongases, combinatory effects with desaturases. Essential fatty acids. Formation of eicosanoids – prostaglandins, prostacyclins, thromboxanes, and leukotriens. Cyclic and linear pathways of their synthesis. Effect of aspirin and non-steroid analgetics, antiphlogistics, and antipyretics. Polyunsaturated fatty acids, EPA, their significance.

22. Metabolism of amino acids – overview
Nitrogen-containing compounds in metabolism. Autotrophs and heterotrophs in amino acid metabolism, essential amino acids. Digestion of proteins, resorption of amino acids. Degradation of amino acids. Ammonotelic, ureotelic, and uricotelic organisms. Degradation of amino acids in humans, central role of glutamate and aspartate. Oxidative deamination, transamination. Detoxification of ammonia, glutamine. Urea cycle. Relationship of urea cycle and acid-base balance.

23. Metabolism of individual amino acids
Glycogenic and ketogenic character of amino acid products. Glutamate – degradation, formation of glutamine, proline, ornithine, arginine, and GABA. Formation of NO. Aspartate – degradation, donation of amino-group, formation of asparagine, role in the synthesis of pyrimidines. Alanine – degradation. Methionine – transmethylation, degradation, formation of cysteine. Cysteine – degradation, cysteic acid, taurine, PAPS (active sulphate). Selenocysteine and selenomethionine. Valine, leucine, isoleucine – degradation and significance. Phenylalanine and tyrosine – phenylalanine hydroxylase, PKU. Formation of catecholamines, their degradation, melanines. Metabolic disorders of tyrosine. Threonine – degradation. Tetrahydrofolate and its role in metabolism. Metabolism of glycine and serine, choline cycle, degradation. Histidine – biosynthesis and degradation, histamine. Tryptophan – formation of tryptamine, serotonine, melatonine, degradation.

24. Biosynthesis of porphyrins and heme, their degradation
Biosynthetic pathway of porphyrins, formation of heme, its significance. Heme degradation –biliverin, bilirubin, conjugation of bilirubin, urobilinogens, urobilins. Relationship of bile pigments to the liver function, diagnosis of jaundice.

25. Biosynthesis and degradation of nucleotides
Biosynthesis of pyrimidine nucleotides, its regulation. Biosynthesis of purine nucleotides, its regulation. APRT and HGPRT and their significance for the regulation of purine synthesis. The role of nusleotides in metabolism. Formation of deoxynucleotides, ribunucleotide reductase, thioredoxin. Mechanism of methylation of dUMP to dTMP. Degradation of nucleotides, nucleosides, and bases. The sigificance of AMP deaminase and adenosine deaminase. Formation of uric acid and its importance.

26. Nucleic acids – structure and function
The common principles and differences in structure of DNA and RNA. The significance of structure for the function. The principle of complementarity and its significance for the maintainance and usage of genetic information. Primary, secondary and structure of higher orders in DNA. Estimation of DNA sequence. Chromatin, its structure.

27. Biosynthesis of nucleic acids
Biosynthesis of DNA. DNA polymerases, DNA ligases. Mechanism of DNA replication. Telomers and their significance. Postsynthetic control of replicated DNA, mismatch repair. DNA repair. RNA polymerases, biosynthesis of the different types of RNA. Regulation of transcription, posttrancriptional modifications of rRNA, tRNA, mRNA. Mechanism of splicing and its significance. Structure and organisation of the gene, gene families, relationship towards isoenzymes.

28. Proteosynthesis
The role of individual RNA types in proteosynthesis. The structure of ribosome. Codon and anticodon. Genetic code, its principles. Mutations, their significance. Proteosynthetic mechanism. Postsynthetic modifications of proteins. The role of signal peptide and endoplasmic reticulum. Glycosylation, phosphorylation, proteolysis, and other modifications of proteins.

29. Replication cycles of viruses
Viruses as models of nucleic acid replications. Mechanism of replication of DNA viruses. Lysogenic viruses. Mechanism of replication of RNA viruses. Retroviruses, mechanism of their replication. HIV. Viruses in oncogenesis. Oncogenes, protooncogenes.

30. Molecular biology as a tool for biomedical research
Restriction endonucleases and their role in manipulation with DNA. DNA cloning. Genomic and cDNA libraries, their usage. Production of recombinant proteins in bacterial cells, diagnostic and therapeutic significance. PCR, its

methodological and diagnostic significance. Use of DNA and RNA analysis in diagnosis. siRNA as a tool in regulation of gene expression in research and in therapy. Principles of gene and cell therapy.

31. Hemoglobin, its structure and function
Hemoglobin structure, gene families for alpha- and beta- globins, their significance. Hemoglobin function as transport molecule and as a buffer, the connection of both functions. Hemoglobin as allosteric molecular complex. The role of BPG, CO_2 and protons in regulation of hemoglobin functions. Molecular mechanism of Bohr´s effect.

32. Immunoglobulins - structure and function
Structure of immunoglobulins, heavy and light chains, constant and variable parts. Immunoglobulin classes, their structural basis. The structure of immunoglobulin genes, their reconstruction and gene expression. Mechanism of production of individual immunoglobulin classes. Stracture and meaning of T-receptors, their analogy with immunoglobulins. Other proteins of immunoglobulin superfamily, biological role of MHC I and MHC II.

33. Biochemistry of the liver and basic xenobiochemistry
The role of liver parenchyme in carbohydrate, lipid, and amino acid metabolism. The role of the liver in biotransformation (xenobiochemistry). Main mechanisms of biotransformation, two-step system. Examples of biotransformation. Possible toxic and cancerogenic effect of biotransformation.

34. Biochemistry of the muscle
The main muscle proteins and their role in muscle contraction. Mechanism of muscle contraction, its regulation. The role of calcium ions, trponin, tropomyosin, actin and myosin, role of ATP. Contraction in smooth and striated muscle. Metabolism in muscle, role of myoglobin, creatine, glycogen, oxidative processes, and anaerobic processes. The cytosceleton.

35. Neurobiochemistry
Specific aspects of neural tissue metabolism. Oxygen consumption and glucose consumption, the reasons. Metabolism of ketone bodies in brain. Metabolism of amino acids in brain. Some properties of hematoecephalic barrier and its significance. Neural synapses – cholinergic, adrenergic, GABA. The overview of neurotransmitters.

36. Structure and function of membranes
The structure of different membranes and reasons for their composition. Transfer of molecules across the membrane. The need for transporting mechanisms: passive transport, cotransport, antiport, active transport, secondary active transport. The principle of pump and channel, the significance.

37. Biochemistry of extracellular matrix
Extracellular matrix, its composition and function. Collagens, their role in tissues. Biosynthesis of collagen, posttranslation processing. Other matrix proteins – elastin, fibronectin, connection with cells, integrins. Proteoglycan complexes in connective tissue, their significance. Bone and tooth mineral, composition, formation, resorption.

38. Biochemistry of nutrition and starvation
Composition of human body and the need for permanent turnover and energy formation. Food components and the requirements for the correct food composition. Essential components of the diet. Starvation, 4 phases according to the type of metabolism. Characterisation of metabolism in prolonged starvation.

39. Antimetabolites and their significance
Antimetabolites. the use of competitive inhibitors as antimetabolites for treatment purposes. Examples of successful antimetabolites: analogues of THFA, analogues of bases, nucleosides, nucleotides, and coenzymes. Importance for chemotherapy and transplantations.

40. Oxygen derived free radicals, antioxidation protection in organism
Properties of molecular oxygen, tendency to form free radicals: superoxide ion, hydroperoxyl radical, hydrogen peroxide, hydroxyl radical. Radical reactions, peroxidation of lipids. Protection: superoxide dismutase, peroxidases, catalase, glutathione peroxidase, glutathione reductase. Relationship to the pentose cycle, possible disorders. Other free radical (not oxygen derived), negative effect of biotransformation. Free radical scavangers, antioxidants.

41. Regulation of biochemical processes
Regulation in time – regulation of gene expression, regulation of enzymes, allosteric enzymes. regulation in space – compartmentalization and regulated transport. Metabolites forming levels and metabolites passing through the tunnels.

Note: The subject **Biochemistry** is extended in 4th year as **Pathobiochemistry** and in 5th year as **Clinical Biochemistry**.