MEDICAL COURSE BIOCHEMISTRY DETAILED PROGRAM

1st Semester

PROTEINS, BIOENERGETICS

Structure and function of proteins

- groups and structures of amino acids
- 1.,2.,3.,4. structure, supersecondary structure
- definition of domains, examples
- protein conformation, denaturation, precipitation
- role of secondary binding forces (interactions) in the formation of protein structure
- folding of proteins, Anfinsen story, chaperones
- definition of isoelectric point
- ❖ failures in protein folding (misfolding), pathobiochemical background of Alzheimer's disease

Thermodynamics and bioenergetics

- definition of the open system
- definition of chemical (real) equilibrium and steady state
- definition of Gibbs free energy, entropy, ΔG and $\Delta G^{\circ\prime}$
- conditions for spontaneous reactions, exergonic and endergonic reactions
- principle of coupling (endergonic reactions)
- definition of high-energy compounds (e.g. phosphates), examples, formation of high-energy compounds (phosporylation at the substrate and coenzyme level)
- central role of ATP

ENZYMOLOGY

Classification of enzymes and coenzymes
- main classes of enzymes, examples of subclasses, names and examples for all main types
- coenzymes, names of vitamin precursors, functional groups of coenzymes and examples for their involvement in catalyzed reactions

General characterization of enzymes
- general characterization, definition of biocatalysis, decrease of activation energy, which factors are affected and which not in a catalyzed reaction by the enzyme
- characterization of the active site, substrate and reaction specificity
- catalytic site models (lock and key, induced fit, fluctuation)
- ❖ general characterization of enzyme deficiencies (diagnostics, screening, therapeutic approach)

Molecular mechanism of enzyme action
- acid-base catalysis, example for functional group and for the reaction
- covalent catalysis (with charge relay network), example
- strain catalysis
- the roles of metal ions in the mechanism of enzyme action, metalloenzymes and metal-activated enzymes

Optimal conditions of enzymatic reactions
- effect of temperature
- effect of pH
- effect of ionic milieu

Enzyme kinetics
- order of reactions
- Michaelis-Menten kinetics, its preconditions, equation, plot
- definition of K_M és v_{max}, ways to determine them
- Lineweaver-Burk type of linear forms
- direct linear method

Enzyme inhibitions
- competitive and non-competitive inhibitions: changes of K_M and v_{max}, plots, examples from the intermediary metabolism, plots
- uncompetitive inhibition: plot (without example)

Allostery
- definition, models
- kinetic plot of the allosteric enzyme reaction

Multienzyme system
- definition, example, importance

Enzyme activity units
- catal, unit, specific activity
- measurement of enzyme activity in clinical labs

Principles of regulation of metabolic pathways
- committed step
- rate-limiting step, key enzyme
- negative feedback

Regulation of enzymatic reactions (definition and example for each mode)
- compartmentalization
- regulation of enzyme quantity (induction and repression)
- allosteric regulation
- covalent modification
  - phosphorylation
  - limited proteolysis

Isoenzymes
- definition
- examples (e.g. amylase, alkaline phosphatase, LDH)
- diagnostic importance of enzymes and isoenzymes

CARBOHYDRATES

Chemistry and biochemical importance
- monosaccharides (C3-C7); aldoses, ketoses
- stereoisomerism (L and D epimers, α and β anomers)
- furanose, pyranose ring structures
- derivatives of monosaccharides
- disaccharides
- polysaccharides

Digestion and absorption
- carbohydrates in food
- action of digestive enzymes
- absorption and types of transport
- GLUT transporters, types and characterization, insulin dependence
- ❖ deficiency: lactose intolerance

Normal blood glucose level, hypo- and hyperglycemia

Glycolysis
- steps, names of the intermediates, structures!!, names of the enzymes, cofactors, irreversible steps, ATP formation on the substrate level!!, total ATP yield
- comparison of hexo- and glucokinase
- isoenzymes of aldolase
- characterization of the anaerobic pathway (LDH and isoenzymes, Pasteur effect)
- comparison of the aerobic and anaerobic degradation of glucose
- regulation in detail!! (especially PFK1 és PFK2)
  - -allosteric
  - -covalent modification
  - -gene induction and repression
  - -role of hormones!
- ❖ hypoxic heart and skeletal muscle (lactacidosis)

Pyruvate DH complex
- enzymes and coenzymes of the multienzyme complex, intracellular localisation
- catalyzed steps (structures, enzymes, coenzymes involved), regulation
- ❖ reduced action of PDH complex

Gluconeogenesis
- steps, especially the 3 irreversible steps (names and structures! of intermediates, enzyme names, cofactors, intracellular localization)
- role, source / precursors
- regulation (allosteric and hormonal), ATP requirement
- mitochondrial transport of reducing equivalents, malate and glycerophosphate shuttle

Glycogen
- its role, characterization of stores
- synthesis and degradation (structures, enzymes, coenzymes)
- regulation of synthesis and degradation, role of hormones and allosteric regulation

❖ glycogen storage diseases: type 1, 4, 5

Adaptation (regulation of blood glucose level)
- physical exercise (regulation of glycogenolysis in muscle, Cori cycle)
- starvation (glycogenolysis and gluconeogenesis in liver, usage of Ala and glycerol)
- fed state (glycogenesis, effects of insulin)
- role of kidney

Interconversion of hexoses (6-phosphate forms)

Metabolism of fructose
- conversion of glucose to fructose (through sorbitol)
- steps of degradation, names and structures of intermediates, enzyme names

❖ essential fructosuria and fructose intolerance

Metabolism of galactose
- steps, intermediates (names, structures, enzyme names)

❖ galactosemias (defects of kinase and uridyl transferase, screening!)
- synthesis of lactose

Pentose phosphate pathway (HMP-shunt)
- steps with enzymes, coenzymes and intermediates; hexose part with structures
- importance
- regulation of phases depending on NADPH/ribose requirement

❖ G6PDH deficiency (drug/oxidant-induced hemolysis)

Rapoport-Luebering-shunt
- formation of 2,3 DPG (2,3-bisphosphoglycerate) and its importance in RBC

Glucuronate pathway (glucuronate-shunt)
- importance
- steps (names of intermediates, structures till glucuronate)
- connection to HMP-shunt, reason of lack of vitamin C formation in humans

Special fate of glucose in tissues and organs
- RBC, brain, liver, muscle, adipose cells

Glycoproteins

- general characteristics, functions
- components of the oligosaccharide chain, precursors and activated derivatives
- O-linked glycoproteins: properties, main steps of synthesis
- N-linked oligosaccharides: properties, main steps of synthesis
- mucins
- blood group antigens (ABO)

LIPIDS

Chemistry, classification

Fatty acid derivatives

- glycerides
  - triacylglycerols
  - phospholipids
- sphingolipids

Isoprene derivatives

- steroids
- ubiquinone, dolichol

Fatty acids

- common saturated and unsaturated fatty acids, essential fatty acids,
- lipid peroxidation (LPO)

Eicosanoids

- groups, main effects
- their formation from membrane phospholipids (role of PLA_2) and from essential fatty acids
- importance of EPA and DHA
- substances influencing the formation of eicosanoids (especially steroid and non-steroid anti-inflammatory drugs: NSAID, SAID)

Digestion and absorption of lipids

- lipases, colipase, phospholipase; their activation; cholesterol ester hydrolase
- role of bile acids
- absorption

Metabolism of lipoproteins

- classification and characterization of lipoproteins
- functions of apoproteins
- metabolism of lipoproteins, importance of LPL and LCAT; importance of cholesterol transport, LDL receptors, importance of HDL/LDL, HDL cycle (reverse cholesterol transport)

❖ hyperlipoproteinemias

Lipid mobilization

- characterization of the lipid stores (visceral/subcutaneous adipose tissue as an endocrine organ)

❖ obesity, metabolic syndrome (main characteristics)

- phases of lipid mobilization, cases of increased lipid mobilization
- regulation of TG lipase, fate of glycerol

Oxidation of fatty acids

- β-oxidation in detail (localization, phases, carnitine-dependent transport, formulae, enzymes, coenzymes, energy yield)
- oxidation of fatty acids with odd number of carbon atoms (formulae, enzymes, coenzymes)
- ❖ methylmalonic acid uria, proprionic acidemia (possible causes, main symptom)
- oxidation of unsaturated fatty acids
- α-oxidation (main points, function), ω-oxidation (main points, enzyme system), peroxisomal oxidation (main points)

Synthesis of fatty acids

- „de novo" synthesis in detail (localization, formulae, enzymes, coenzymes, function of the fatty acid synthase multienzyme complex, regulation of the key step, adaptive regulation, importance of ATP-citrate lyase and malate-citrate transporter, sources of NADPH)
- elongation (mitochondrial and microsomal)
- formation of unsaturated fatty acids (localization, desaturase complex, localization of the formed double bonds and their cis nature, regulation)

Synthesis of triacylglycerols and phospholipids

- steps of TG and phospholipid synthesis (formulae, coenzymes)
- importance of TG and phospholipid synthesis
- ❖ respiratory distress syndrome (background, occurrence, symptom, diagnosis)

Metabolism of ketone bodies

- ketogenesis (localization, steps, enzymes, coenzymes, formulae)
- utilization of ketone bodies (localization, main route, cofactor, enzyme)
- circumstances and biochemical backgound of increased ketogenesis
- ❖ pathobiochemistry and main symptoms of diabetes mellitus

Metabolism of sphingolipids

- chemistry, occurrence and functions of sphingolipids
- main pathways of synthesis (precursors, coenzymes, main intermediates)
- catabolism of sphingolipids
- ❖ sphingolipidoses (Gaucher's, Tay-Sachs and Niemann-Pick disease: defect, accumulated lipid, symptoms)

Steroid metabolism

- cholesterol synthesis (localization, steps /till active isoprenoids with formulae/, cofactors, energy requirement, regulation of the key enzyme, drugs affecting the cholesterol metabolism, regulation at the cellular/tissue level, esterification in the cell and in the blood, structure of cholesterol)
- metabolism of bile acids (importance, main steps of the synthesis, primary and secondary bile acids, cofactors of the key enzyme and its regulation, enterohepatic circulation, composition of bile)
- other bioactive cholesterol derivatives: pathways of steroid hormone synthesis, localization, elimination of steroid hormones; synthesis of vitamin D_3 vitamin, activation, importance of calcitriol

❖ defect of 21-hydroxylase (background, main symptoms)

Relations between carbohydrate and lipid metabolism

AMINO ACIDS

Chemical and biochemical importance
- structures and chemical classification
- 3 and 1 letter codes of amino acids
- essential, non-essential and semi-essential amino acids (Arg, His)
- amino acids as neurotransmitters
- N turnover in nature

Digestion and absorption of amino acids
- enzymes involved in the digestion of food proteins, their main cleavage sites and their activation (stomach, duodenum-pancreas, ileum-jejunum)
- absorption of amino acids (peptides) – mechanism of transport, carriers
- transport into the cells: gamma-glutamyl cycle (main enzyme, role of glutathione, names of the intermediates (without structures)
- breakdown of endogenous proteins (enzymes, intracellular localization)

General amino acid metabolism
- central role of glutamate and glutamine (synthesis and breakdown of glutamate and glutamine, enzymes, cofactors, importance and tissue localization
- role of amino acids in the synthesis of N-containing compounds
- cleavage and transfer of amino groups of amino acids: deamination and transamination (mechanisms, enzymes, role of cofactor and examples)
- sources of free ammonia and ways of its elimination, most important N-containing compounds in urine (urea/carbamide, uric acid, creatinine, ammonia)
- urea/ornithine cycle (its fundamental importance, organ and intracellular localization, names and structures of the intermediates, enzymes, ATP requirement, regulation)
  - ❖ conditions of hyperammonemia, disorders of urea cycle: citrullinemia (argininosuccinate synthase deficiency), argininosuccinicaciduria II (argininosuccinase deficiency) and their consequences
- decarboxylation of amino acids, and fate of the derivatives (importance, enzyme type, cofactor, examples: histamine, serotonin, catecholamines, production of the amine precursor of acetylcholine, GABA; catabolism of amines)
- fate of the carbon skeleton of amino acids (definition of glucogenic and ketogenic amino acids, further classification of glucogenic amino acids according to their end products: pyruvate, oxaloacetate, fumarate, succinyl-CoA, α-ketoglutarate groups)
- role of amino acids in the production of C1 units (containing one C atom): production of methyl, methylene, formyl, formimino groups, role of SAM and THF; utilization of C1 units in metabolic processes (examples)

Specific amino acid metabolism

A/ Acetyl-CoA group: Ile, Leu; Trp, Lys

- catabolism of branched-chain amino acids (Val, Ile, Leu), (types of enzyme reactions, names and structures of the end products
- ❖ maple syrup urine disease (MSUD) (background: defect of α-keto acid decarboxylase, consequences) /deficiencies of Leu metabolism: isovaleric acidaemia (isovaleryl-CoA dehydrogenase defect) and methyl-crotonyl-CoA carboxylase deficiencies/
- ❖ disorders of the propionyl-CoA – succinyl-CoA pathway /see: fatty acid metabolism/
- catabolism of Trp (production of formyl group, formation of kinurenine, Ala release, structure of end product)
- importance, synthesis and degradation of serotonin (enzymes, structures, cofactors)
- role of tetrahydrobiopterin in amino acid metabolism (metabolism of Trp, Tyr, NO production), precursor for its synthesis (GTP)
- ❖ serotonin-producing tumours (consequences)
- NAD synthesis (without structures, quinolinic acid as the direct precursor)
- importance of melatonin (N-acetyl-5-metoxy-serotonin), its precursor without structure)
- catabolism of Lys (connection to the degradative pathway of Trp, end product of degradation, production of cadaverine, Lys as the precursor of carnitine synthesis)

B/ Succinyl-CoA group: Met, Thr; branched-chain: Ile, Val

- role and formation of SAM, examples for methylation (with structures), remethylation of homocysteine to Met (structures, coenzymes)
- ❖ causes of homocystinuria (enzyme deficiency: cystathionine synthase deficiency, deficiencies of coenzymes: THF, B_{12}, B_6) and consequences (methioninemia /methionine adenosyl-transferase deficiency/)
- degradation of Met, formation of Cys (enzymes, coenzymes, structures)
- Thr metabolism (breakdown with structures), formation of Gly and acetyl-CoA, aminoacetone cycle: formation of pyruvate)

C/ α-ketoglutarate group: Arg, Pro, His, Glu, Gln

- relationship between Arg metabolism and urea cycle (structures, enzymes)
- synthesis and metabolism of creatine (structures, importance of phosphocreatine, diagnostic significance of creatine kinase, creatinine)
- synthesis of NO (NO synthase isotypes, coenzymes), function of NO, glycerol-trinitrate ('nitroglycerin') as a source of NO – therapeutical application, decomposition of NO
- poliamines (types, main functions, enzymes, coenzymes)
- Pro metabolism (synthesis/degradation with structures), importance of OH-Pro (collagen), end products (glyoxalate, pyruvate)
- relationship of Glu metabolism with metabolisms of Arg, Pro and His, formation and importance of N-acetyl-Glu

- potential precursor of His synthesis (PRPP), catabolism (structures, coenzymes, produced C1 unit)
- formation of histamine (structure, enzyme, cellular/tissue localization), its functions and degradation
- ❖ pathological roles of histamine (allergy, gastric hyperacidity), histidinemia (consequences)

D/ Pyruvate group: Ser, Gly, Cys, Ala (Trp), (Thr)

- potential ways of Ser synthesis (connected to glycolysis, from Gly; structures, coenzymes), degradation of Ser (deamination)
- role of Ser in synthesis of Cys (direct and indirect ways, structures), and in phospholipid and sphingolipid synthesis (structures of related phospholipids and sphingosine)
- formation of choline (structures, coenzymes), synthesis and importance of acetyl-choline
- synthesis of Cys (Met-Ser and Ser metabolism, structures), two ways of Cys catabolism (structures), end products excreted by urine (sulfite, mercaptolactate)
- formation of taurine (structures), function of taurine, formation of cystine (structure), formation of PAPS /"active sulfate" (structures and function), role of Cys in the synthesis of gluthatione
- potential ways of Gly synthesis (a/ direct Ser-Gly reaction, b/ breakdown of Thr, c/ CO_2 + NH_3, d/ glyoxalate-Gly); formation of Gly from choline (betaine-dimethyl Gly - sarcosine pathway, possible involvement in the remethylation of homocysteine; breakdown pathways of Gly (a/ cleavage, b/ pyruvate, c/ oxalate formation; structures)
- Gly utilization (formation of creatine, gluthatione; de novo synthesis of purines, porphyrin synthesis, conjugation of biliary acids)
- Ala metabolism (formation from Trp, transamination; structures)

E/ Oxaloacetate group: Asp, Asn

- Asp metabolism (transamination, urea cycle), Asn metabolism (formation, breakdown; structures)
- Asp utilization (nucleotide synthesis)

F/ Fumarate group: Phe, Tyr (Asp)

- formation of Tyr (structures, enzyme, coenzyme)
- ❖ pathobiochemistry of phenylketonuria (PKU), background defects (enzyme or coenzyme deficiency), special type (maternal PKU), consequences, screening (laboratory diagnostic methods), treatment
- Phe-Tyr catabolism (structures, enzymes, coenzymes)
- ❖ tyrosinemia type I and II (fumarylacetoacetate hydrolase and 4-OH-phenylpyruvate dioxygenase deficiency), defect of homogentisate oxidase
- synthesis of catecholamines (structures, enzyme types, coenzymes) and their significance
- ❖ Parkinson's disease (biochemical background, main consequences)
- precursor of melanine (dopaquinone)

- ❖ basics about albinism
- synthesis of thyroid hormones (main steps)

Metabolism of C1 units

- types of C1 units, their production in amino acid metabolism
- transportation of C1 units, synthesis and role of SAM, examples for methylation (with structures), role of THF in Met metabolism
- vitamin precursor of THF (and components of folic acid)
- ❖ causes and consequences of folate deficiency (in adults and in embryo), treatment and prevention
- formation of DHF and THF (and inhibitors of this synthesis), role of THF in nucleotide metabolism
- role of vitamin B_{12} in THF metabolism

Glutathione

- components, synthesis and importance
- role of glutathione in amino acid transportation (gamma-glutamyl cycle)
- antioxidant role of glutathione (cell membrane, eye lens), function of glutathione peroxidase, regeneration of oxidized glutathione (enzyme, coenzyme: NADPH)
- ❖ drug-induced hemolysis (glucose-6-P-DH deficiency)
- ❖ cataract (e.g. galactokinase deficiency)
- role of glutathione in hepatic biotransformation process

<u>PORPHYRIN</u>

- general characteristics of porphyrin structure, heme proteins
- porphyrin synthesis: 1. mitochondrial step (structures of precursors, enzyme, regulation), steps going on in the cytosol (names of intermediates, enzymes), final steps in the mitochondria (names of intermediates, role of ferrochelatase)
- ❖ porphyrias: general features and main symptoms; acute intermittent porphyria, congenital erythropoietic porphyria and porphyria cutanea tarda (enzyme defects); consequences of lead intoxication
- phases of heme catabolism, cell/tissue localizations; formation of biliverdin and bilirubin (enzymes, coenzymes), bilirubin transport in blood, „indirect bilirubin”
- conversion of bilirubin in liver (enzyme and its characteristics), „direct bilirubin”, related transport mechanisms in liver
- conversion of bilirubin in the intestine, enterohepatic cycle, urobilinogen in urine
- ❖ main classes of hiperbilirubinemias: prehepatic /hemolytic/, hepatic, post-hepatic /biliary obstruction/ jaundice/icterus (mechanisms, accumulating or missing bile pigments)
- ❖ neonatal „physiologic jaundice”: mechanism, characteristics and treatment
- ❖ conditions of inherited jaundice: Gilbert syndrome, Crigler-Najjar syndrome type I and II, Dubin-Johnson and Rotor syndrome
- iron metabolism: absorption of iron, characteristics and roles of transferrin and ferritin, mechanism of iron transport into the cells
- ❖ consequence of iron deficiency, its background, toxicity of free iron, disorders of iron storage (hemosiderosis, hemochromatosis)

NUCLEOTIDES

Chemistry and biochemical importance
- structures of purine and pyrimidine bases
- lactim-laktam (enol-oxo) tautomerism
- nucleosides and nucleotides
- nucleotide coenzymes
- nucleotide derivatives (cyclic mononucleotides, activated intermediates, certain coenzymes)
- main structural features of polynucleotides

Digestion, absorption
- nucleotide bases as non-essential nutrients; sources enriched in purines
- digestive enzymes (Rnases, Dnases, nucleotidases, phosphatases)
- forms of absorption (nucleosides, bases)

Synthesis of purine nucleotides
- „de novo" synthesis (general characteristics, energy requirement; first two steps with structures, enzyme names, allosteric regulation; origin of the N és C atoms of the purine structure)
- synthesis of AMP and GMP from IMP (structures, enzymes, coenzymes, regulation)
- salvage reactions (importance, ATP requirement, reactions with enzyme names)
- ❖ HGPRT-defect (Lesch-Nyhan syndrome)
- formation of di- and triphosphate nucleotides

Synthesis of pyrimidine nucleotides
- „de novo" synthesis to the formation of UTP and CTP (general characteristics, energy requirement; the whole pathway with structures, enzyme/enzyme system names, allosteric regulation)
- salvage reaction (importance, ATP requirement, enzyme)

Formation of deoxyribonucleotides
- main reaction of dNDP formation (structures, enzyme)
- role and components of the thioredoxin and glutaredoxin systems
- mode of allosteric regulation
- salvage reactions
- formation of dTMP via „de novo" and salvage reactions (structures, enzymes, coenzymes, regulation)

Importance of folic acid/THF and vitamin B_{12} in the nucleotide synthesis
- general importance of THF and its role in nucleotide synthesis
- ❖ folic acid hypovitaminosis and its consequences in adult
- ❖ folic acid hypovitaminosis and its consequences during pregnancy, importance of prevention
- role of vitamin B_{12} in the metabolism of THF and in the intermedier metabolism

- storage and sources of vitamin B_{12}
- ❖ B_{12} hypovitaminosis and its consequences

Inhibitors of nucleotide synthesis

- ❖ general characteristics and side-effects
- ❖ fields of application (as cytostatics, immunosuppressors; certain compounds are used in antibacterial or in antiviral therapy)
- ❖ DHF- and folic acid antagonists (sulfonamides), purine and pyrimidine analogs, glutamine analogs, nucleoside analogs

Degradation of nucleotides

- degradation of purine nucleotides to the formation of uric acid (structures, enzymes)
- importance of the salvage pathway
- ❖ hyperuricemia: primary and secondary causes
- ❖ gout (pathomechanism, symptoms, therapy)
- degradation of pyrimidine nucleotides (structures, enzymes)

CITRIC ACID CYCLE (Szent-Györgyi-Krebs cycle)

General significance and intracellular localization of the citric acid cycle, connection with the PDH complex

- general funtion of the cycle in the catabolic and anabolic processes, in aerob condition, intracellular localization
- oxidative decarboxylation of pyruvate, activity of the pyruvate dehydrogenase enzyme complex (components, intermediers, coenzymes/ prostetic groups, emphasized function of vitamin B1, regulation)

Process and energy balance of the citric acid cycle

- reaction of the cycle: individual steps and intermediers (structures), enzymes, irreversible reactions, decarboxylation reactions
- function of the á-ketoglutarate-dehydrogenase enzyme complex
- localization of NADH+H and $FADH_2$ formation, phosphorylation at substrate level (GTP formation)
- total energy balance (during one cycle, and during the terminal oxidation and oxidative phosphorylation)

Regulation of the citric acid cycle

- allosteric activators and inhibitors of enzymes of the irreversible reactions; velocity determining step
- anaplerotic reactions (guarantee of adequate oxalacetate concentration)
- effect of hypoxia

Relationship between the cycle and other metabolic pathways

- catabolic pathways (glycolysis - PDH complex, metabolism of amino acids and fatty acids)
- anabolic pathways (gluconeogenesis, synthesis of amino acids and porphyrins)

TERMINAL OXIDATION, OXIDATIVE PHOSPHORYLATION

Structure of the mitochondria, transporters

- property of outer and inner membrane, typical lipid and protein molecules
- transporters of the inner membrane, their functions
- the matrix and its metabolic functions
- transport of the reducing equivalents in mitochondria (malate and glycerophosphate shuttle), significance and relationship with the cytoplasmic metabolism

Terminal oxidation

- steps, essence and function of the respiratory chain, close contact with the citric acid cycle
- redox potential, concept of redox pairs and redox capacity; significance of the relationship between redox pairs
- Relationship between the changing of redox potential and the free energy; energetics of the respiratory chain
- 4 complexes of the respiratory chain : members (enzymes, coenzymes, cytochromes, FeS proteins), parameters, functions and localizations)
- the last oxidative step, opportunities of water and formation of superoxide/reactive oxygen species
- components of the respiratory chain (except complexes), functions, characterization of ubiquinone
- general parameters of cytochromes, types and functions
- regulation and inhibitors of the respiratory chain

The oxidative phosphorylation

- conformation and function of ATP synthase
- chemiosmotic theory, function of proton pumps; formation and function of proton gradients
- emphasized function of ADP in the regulation (acceptor control)
- efficiency of oxidative phosphorylation (P/O quotient, energetic efficiency)
- aerob and anaerob catabolism of the glucose, comparison of the energy production
- close contact with terminal oxidation, uncoupling agents (UCP) and effects in terminal oxidation; function of the brown adipose tissue (thermogenin)

❖ mitochondrial diseases, their characterization (defects of PDH/respiratory chain/oxidative phosphorylation, special defects of fatty acid-amino acid metabolism)

MEDICAL COURSE BIOCHEMISTRY DETAILED PROGRAM
2nd Semester

BIOCHEMISTRY OF BLOOD - LEUKOCYTES

General characteristics of neutrophil granulocytes

- main types of granulocytes
- neutrophil granulocytes as microphages (essence and importance of phagocytosis, activation and degranulation)
- general metabolism of neutrophils

Oxidative stress

- ROI: reactive oxygen intermediates (types and reactivity)
- role of Fe and Cu ions (Fenton and Haber-Weiss reaction)
- ROI-producing physiological processes/reactions and their localizations
- endogenous and exogenous antioxidant compounds (uric acid, bilirubin; glutathione, vitamins and vitamin-like substances)
- antioxidant enzymes and their functions (SOD, catalase, GSH-peroxidase)
- essence of oxidative stress and states associated with it
- consequences of oxidative stress (lipid peroxidation, protein-, carbohydrate- and nucleic acid damages)

Special pathogen-killing mechanisms in neutrophils

Oxidative mechanisms

- respiratory burst (basics, localization during phagocytosis, properties and function of NADPH-oxidase)
- ❖ chronic granulomatous disease (CGD)
- myeloperoxidase (localization, function)

Non-oxidative mechanisms

- breakdown /digestive/ enzymes (proteinases, glycosidases); antiproteinases and their importance
- ❖ α-1 antiproteinase deficiency
- bakteriostatic and baktericid proteins: lactoferrin and defensins;(lysozyme)

Chemotaxis

- steps of chemotaxis and receptors involved in adhesion
- importance of chemotaxins (examples)
- ❖ leukocyte adhesion defect (LAD1) /see: also at the material on adhesion/

BIOCHEMISTRY OF BLOOD - RED BLOOD CELLS

Characterization of red blood cells (erythrocytes)
- shape, changes in shape, size, cell count, life-time
- formation of erythrocytes, importance of enucleation
- factors required for production of red blood cells (role of folic acid, vitamin B_{12}, erythropoietin)
- ❖ megaloblastic anemia
- general functions
- response to hypo- and hyperosmolarity

Structural composition of red blood cells
- membrane structure
- components of cytoskeletal system, and characterization
- ❖ inherited spherocytosis, elliptocytosis (see: actin-cytoskeleton)
- membrane transporters (Na-K ATPase, Band 3 protein)
- specific antigens (ABO and Rh blood groups)

Special metabolism of red blood cells
- GLUT-transporter
- anaerobic glycolysis, Cori-cycle
- ❖ glycolytic enzyme deficiencies: hemolytic anemias
- synthesis and role of 2,3 DPG (Rapoport-Luebering shunt)
- HMP-shunt, NADPH production
- ❖ glucose-6-phosphate dehydrogenase enzyme deficiency and its consequences
- components of the antioxidant system and their action; production and regeneration of gluthatione, antioxidant role of glutathione

Metabolism of iron
- iron content of the human body and its tissue distribution, daily iron requirement
- characterization of iron absorption and its regulation
- storage: ferritin
- transport: transferrin
- cellular uptake: receptor-mediated endocytosis
- ❖ iron deficiency anemia

Hemoglobin and myoglobin
- structure and function in details
- position of the hem functional group and its structural changes during oxygenation and deoxygenation
- R and T conformation, kinetics of conversion of two conformation into each other, factors influencing the stability
- factors influencing the oxygen binding affinity of hemoglobin (shift of sigmoid curve to right and left)
- binding of 2,3-DPG, its effect on oxygen affinity

- molecular background of Bohr-effect, its role in the oxygen supply of tissues
- changes in oxidation state of iron ion, formation and regeneration of methemoglobin
- ❖ methemoglobinemia
- ❖ carbon monoxide poisoning
- shift in expression of globin genes during human development
- differences in between fetal and adult hemoglobin
- ❖ thalassemia
- ❖ sickle cell anemia

BIOCHEMSTRY OF BLOOD - FUNCTIONS OF PLATELETS, HEMOSTASIS, FIBRINOLYSIS

Steps of hemostasis (vasoconstriction, platelet aggregation and formation of the thrombus, retraction procedure)

Functions of platelets

- formation, structure of platelets, important organelles, special metabolism
- α-granules, lysosomes (lambda granules) and dense bodies (delta granules): composition, platelet specific proteins
- molecular mechanism of platelet activation, important cell-surface receptors, signaling pathways, importance of Ca^{2+}, role of prostaglandin derivatives, role and action of TxA_2 and PGI_2
- inhibition of platelet activation
- primary adhesion: definition and its regulation, direct and indirect collagen binding (β1 integrin/ GpIa-IIa and von Willebrand factor and its receptor (GpIb-IXa)
- ❖ Bernard-Soulier syndrome
- platelet aggregation: definition and its regulation (GPIIb/IIIa-receptor ($\alpha_{IIb}\beta_3$ integrin) receptor)
- ❖ Glanzmann-type trombasthenia
- ❖ In vivo inhibition of platelet aggregation (therapy/ prophylaxis): 1. GPIIb/IIIa-receptor ($\alpha_{IIb}\beta_3$ integrin)-inhibitors 2. ADP-receptor antagonists 3. acetyl-salicylic acid, mechanism of action
- role of platelets in activation of coagulation cascade and formation of thrombus

Coagulation system

- coagulation factors: synthesis, biochemical characterization, their role in the cascade
- types and processes of coagulation cascade (intrinsic, extrinsic), way of activation of coagulation factors (contact activation, limited proteolysis, serin proteases, activation complexes, role of Ca^{2+}
- role of vitamin K, synthesis of vitamin K dependent factors (II, VII, IX, X, protein S and C), formation of gamma-carboxy –glutamate
- ❖ anticoagulants: vitamin K antagonists (kumarin-derivatives)

- sructure and function of thrombin
- structure of fibrinogen, formation of fibrin net, stabilization of fibrin, mechanism of transglutaminase (Laki-Lóránd factor) reaction
- laboratory testing of coagulation system: definition and importance of Prothrombin-time PT (Quick-probe), Activated partial thromboplastin time APTI, Thrombin time TT), INR (International Normalized Ratio)

❖ coagulation factor deficiencies:
  ❖ inherited lack of factor VIII, IX, XI: hemophilia A,B és C; characterization, symptoms
  ❖ inherited lack of factor Von Willebrand
  ❖ secondary lack of coagulation factors (for ex.: liver disorders)
  ❖ disseminated intravascular coagulation (DIC): biochemical background, symptoms

- biochemical basics of anticoagulation: mechanism of antithrombin III, role of vitamin K

❖ in vivo anticoagulation and prophylaxis: 1. Heparin; LMWH: low molecular weight heparin 2. vitamin K antagonists (kumarin derivatives), mode of action, importance, advantages/disadvantages
❖ in vitro anticoagulation: heparin, citrate, oxalate, EDTA; mechanism of action

Fibrinolysis

- mechanism of fibrinolysis, biochemical importance
- plasminogen, activation of plasmin system
- role of plasma kinins, complement, strepto-and urokinase system in activation of fibrinolysis
- fibrin degradation procedure, (FDP: fibrin degradation products), importance

❖ in vivo fibrinolytic therapy and its importance (acut dissolving of thrombi)
❖ DIC

BIOCHEMISTRY OF BLOOD - PLASMA PROTEINS

Composition of blood
- corpuscular elements
- main components of the plasma
- differences between blood serum and plasma

General characterization of plasma proteins
- synthesis (location, general characteristics)
- role of oligosaccharide side chains
- reference range of plasma total protein, pathological increase and decrease
- ❖ malnutrition, kwashiorkor

Separation of plasma proteins
- separation methods
- characterization of native electrophoresis, its application in clinical practice
- plasma protein fractions, pattern, serum levels
- ❖ pathological electrophoretic patterns: nephrosis, liver cirrhosis (ß-γ bridge formation), hypogammaglobulinemia, monoclonal gammopathy, acut and chronic inflammation (iron deficiency anemia, hemolytic anemia)

Characterization of plasma proteins
- names of proteins in albumin, alpha1, alpha2, beta, gamma fractions
- detailed characterization (synthesis, main characteristics, physiological-biochemical functions, disorders):
- albumin
- ❖ analbuminemia
- alpha-1-acidic glycoprotein
- alpha-1-lipoprotein (HDL)
- alpha-1-antitrypsin
- alfa-fetoprotein (AFP)
- ❖ inherited alpha-1-antitrypsin deficiency (emphysema)
- ceruloplasmin (+ copper metabolism)
- ❖ Wilson and Menkes disease
- alpha-2-macroglobulin
- haptoglobin and hemopexin
- ❖ hemolytic anemias
- beta-lipoprotein (LDL)
- prebeta-lipoprotein (VLDL)
- transferrin, ferritin (+iron metabolism)
- ❖ iron deficiency anemia
- fibrinogen
- immunoglobulins

Acut phase proteins

- characterisation and importance of acut phase response
- positive and negative acut phase proteins

BIOMEMBRANES

Main functions of biomembranes
- the essence of compartmentalization, its advantages (eukaryotes – prokaryotes)
- barrier function (semipermeability), regulated flow of materials and information
- chief functions of membrane proteins (enzyme, receptor, transporter, antigen)
- special functions of biomembranes (endo- and exocytosis, phagocytosis; receptor-mediated endocytosis /LDL, transferrin/; cell polarity, cell shape, cell movements; cell division; cell fusion /skeletal muscle/)

Composition of biomembanes
- „unit membrane": uniform thickness and similar composition
- characteristic differences between the main biomembrane types
- chief types of membrane lipids, types of membrane phospho- and sphingolipids
- importance of membrane carbohydrates, glycoproteins, glycolipids
- antigenitás (ABO vércsoport)

Structure of biomembranes
- formation and characteristics of phospholipid bilayer; micelles, liposomes
- the essence of „fluid mosaic model"
- integral and peripheral membrane proteins (main features, examples)
- lipid-anchored membrane proteins
- membrane asymmetry (from the aspect of lipids and proteins), its alteration
- factors affecting the fluidity of the membrane

Dynamism of biomembranes
- mobility of lipids in the membrane (rotation, lateral movement, flip-flop); investigations on flip-flop
- mobility of proteins in the membrane (rotation, lateral movement); the basics of FRAP method (fluorescence recovery after photobleaching)
- mikrodomains in the membrane, lipid rafts

MEMBRANE TRANSPORT

Passive transport- diffusion
- characterization
- materials transported by passive transport (examples)
- importance of free diffusion in uptake of drugs and organic solvents

Passive transport- facilitated diffusion

- characterization, kinetics
- classification: uniport, symport, antiport types with examples
- Band 3 and GLUT transporters and their action, some mitochondrial transports

Active transport

- characterization, kinetics
- classification: uniport, symport, antiport types with examples
- classification, detailed characterization:

  1/ F-type-ATPases

  inner mitochondrial membrane ATP synthase

  2/ P-type-ATPase

  Na^+ K^+ ATPase - characterization, reaction cycle

  Ca^{2+} ATPases (SERCA): types, characterization, reaction cycle

  H^+ / K^+ ATPase (stomach mucosa)

  3/ V-type-ATPase

- ABC transporters: characterization, types, examples

❖ multidrog resistance: definition, importance

❖ cystic fibrosis

- secondary active transport, examples

Ion channels

- channel-forming proteins, their classification based on conductivity, selectivity, gating
- experimental approach for channel-forming proteins (electrical observations, inhibitors, antibodies, patch clamp)
- voltage-gated, ligand-gated, stretch-gated channels, examples, their role in physiological processes
- selectivity of ion channels
- definition and action of ionophores, ionophore antibiotics: valinomycin, gramicidin
- pore-forming proteins; structure, function and regulation of connexin and porin channels

LIVER

Characterization of hepatic tissue

- structural units of hepatic tissue (lobulus, acinus)
- microcirculatory system (position of portal triad, central vein)
- metabolic consequences of the microcirculatory system (metabolic characterization of periportal, intermedier and pericentral zones)
- risk regions within hepatic tissue according to absorption of different compounds and hypoxia
- main cell types and their characterization
- structure of hepatocytes, importance of cell organelles

Central role of liver in the intermediate metabolism

Carbohydrate metabolism

- adaptation of carbohydrate metabolism (resorption and postresorption phases, starvation)
- glucose control function (regulation of blood glucose level), storage of glycogen, role of GLUT 2 and glucokinase as glucose sensor
- Cori-cycle, glucose-alanine cycle
- importance of pentose-phosphate cycle in liver
- specific metabolic pathways (metabolism of fructose and galactose); uronic acid cycle

Lipid metabolism

- adaptation of lipid metabolism (resorption and posztresorption phases)
- storage of triacylglycerols and phospholipids
- specific metabolic pathways: synthesis and importance of ketone bodies
- metabolism of VLDL and HDL
- metabolism of cholesterol: complex regulation of cholesterol biosynthesis in the liver; (synthesis of bile acids, see below)

Amino acid metabolism

- special issues of amino acid acid metabolism
- urea cycle, hyperammonemia
- glutamine cycle
- synthesis of plasma proteins

Porphyrine and iron metabolism in the liver

- formation and circulation of bilirubin
  - ❖ types and laboratory background (differential diagnosis) of jaundice
  - ❖ inherited bilirubin disorders

Bile production

- synthesis, circulation and function of bile acids
- composition of bile
- transport mechanism in bile canaliculi
- ❖ molecular basics of bile stone formation

Other pathobiochemical aspects:

- ❖ biochemical signs of liver functional problems; liver function laboratory tests
- ❖ mechanism of fatty liver formation

Biotransformation

- definition of biotransformation, its biolological importance and main phases
- reactions of first phase, formation of reactive side chains (hydrolysis, reduction oxidation)
- cytochrome P_{450} system and its action
- classification of CYP enzyme family members with examples
- ❖ adduct-formation (DNA, protein) and its consequences (mutation, carcinogenesis)
- formation of reactive oxygen radicals, its role and ways of elimination
- reactions of second phase, conjugation reactions (conjugation with glucuronic acid, sulphate, glycine, glutathione; acetylation, methylation)
- transport/elimination of biotransformation products
- induction and inhibition of enzymes of biotransformation, addiction, interactions, importance of therapeutic drug monitoring

Biochemical effects of alcohol

- fate of ethanol in the human body (first pass mechanism)
- role of ADH and MEOS (+ catalase) system
- ❖ metabolic effects of alcohol: imbalance of reduced coenzymes, fatty liver, lactate acidosis, ketosis
- ❖ toxic effects of acetaldehyde
- ❖ harmful effects of reactive oxygen radicals
- ❖ lack of proteins, amino acids, vitamins and minerals in case of enhanced alcohol intake
- ❖ metabolic and circulatory consequences of liver cirrhosis, their interaction, definition of hepatic coma
- ❖ fate of methanol, toxicity of converted products
- ❖ competitive inhibition of methanol degradation

CONNECTIVE TISSUE – CELL ADHESION – CYTOSKELETON

CONNECTIVE TISSUE

Introduction

- functions of the connective tissue (structural role and functional importance/ regulatory role of cell adhesion – effects on metabolism, cell migration, cell differentiation and cell proliferation)
- main types of the connective tissue
- cell types occuring in the connective tissue, cell types involved in synthesis of fibers and matrix

Fibers

Collagen

- functions, general properties
- repeating unit of amino acids, importance of this in the formation of the molecular structure, characteristic amino acids
- molecular structure of the collagen fiber
- steps of biosynthesis (in detail: hydroxylation, glycosylation, formation of triple helix, importance and cleavage of register peptides), maturation of collagen (oxidation)
- characteristics of "young" and "matured" collagen
- main collagen classes, characteristics and distributions of the important types (fibrillar: I, II, III; network forming: IV; anchoring fibril: VII; non-collagen proteins with collagen domain: eg. acetylcholinesterase)

❖ disorders of biosynthesis: mutations of collagen genes (I: osteogenesis imperfecta, II: chondrodysplasias, III: Ehlers-Danlos /IV/, IV: Alport syndrome, VII: epidermolysis bullosa); vitamin C defect (scurvy)

- degradation (breakdown enzymes), cases/conditions of fast collagen turnover and

❖ scarring-fibrosis (cases)

Elastin

- function, distribution, properties
- characteristic amino acids, molecular structure
- biosynthesis (in detail: Lys oxidation, desmosine, isodesmosine)
- degradation

Fibrillin

- function, distribution, main feature

❖ Marfan syndrome

Extracellular matrix (ECM)

Proteoglycans

- general properties
- 6 main types of glycosaminoglycans (repeating disaccharide units, main characteristics, tissue/cell distribution)
- functions of hyaluronic acid, heparin and heparan sulfate
- 3 main classes of proteoglycans, main types, characteristics, localization (large, aggregating PGs: aggrecan, perlecan; small, Leu-rich PGs: biglycan, decorin; cell surface PGs: syndecan, appican)
- molecular structure of aggrecan, properties

❖ Alzheimer disease

- main steps of proteoglycan biosynthesis, degradation of GAG

❖ mucopolysaccharidoses (Hurler, Hunter)

- inorganic and organic components of bone; degradation/remodelling of bone (role of osteoclasts and osteoblasts)

❖ osteoporosis

- components and types of cartilage

❖ FGF-receptor3 defect: dwarfism (achondroplasia)

Adhesion glycoproteins

- roles, general characteristics, classification according to function
- „pathway forming": fibronectin, tenascin (mol. structure, binding domains, functions)
- basement membrane components: laminin, entactin (mol. structure, binding domains)
- structure and components of the basement membrane
- involved in hemostasis: thrombospondin, vWf (mol. structure, binding domains, functions)
- involved in mineralization and remodelling of the bone: osteonectin, -pontin

CELL ADHESION

- importance of cell-matrix and cell-cell adhesion, possible effects on cell function (eg. anchorage-dependent cell proliferation, contact inhibition)
- formation of focal adhesional complex, „adhesosome" as a signalling complex
- main classes of adhesion receptors
- integrins: general characteristics, classification (β1-β4), distribution, functions

❖ gene defects of integrins (β2: leukocyte adhesion defect (LAD), β3: Glanzmann disease)

- selectins: general characteristics, types (L, P, E), distribution, function
- IgCAM superfamily: main characteristics, types (ICAM-1,-2, NCAM), distribution, functions
- cadherins: main characteristics, functions, types (E; N, P, R, M)

❖ E-cadherin as a negative carcinoma marker

- role of adhesion receptors in extravasation of neutrophil granulocytes (selectins, β2, β1 integrins, ICAM-1,2; see: chemotaxis)

CYTOSKELETON

- general characteristics, roles; cytoskeletal classes, properties, functions
- ***actin-cytoskeleton*** (microfilaments): aktin types, polymerization; importance and functions of actin-binding proteins
- motor protein of microfilaments: myosin II (importance in cell migration)

❖ hereditary spherocytosis (spectrin defect)

❖ progresive muscular dystrophies (defect of dystrophin: Duchenne disease, laminin defect)

- ***intermediate filaments***: general characteristics, main types and distributions

❖ importance in cell typing (tumour diagnostics)

- ***mikrotubules:*** molecular structure, formation and dynamics
- mikrotubular motor proteins (kinesin, dynein) and their function

❖ MT toxins (colchicine, Vinca alkaloids, taxol) as cytostatic drugs

BIOCHEMISTRY OF NUTRITION

Basics of „food science"
- essence and goal of optimal, balanced diet
- components of our food (nutrients, alimentary fibers, bioactive compounds, additives)
- classification of nutrients

Proteins
- importance of food proteins
- average daily protein demand, its caloric ratio in the diet/food, states with increased protein requirement, „protein sparing"
- significance of essential amino acids in the diet; limiting amino acid
- quality of food protein (foods with high or low biological value)
- full-value vegetarian and vegan diet (completing – complementation)
- ❖ protein-poor nutrition (main features of marasmus senilis and symptoms of kwashiorkor)
- ❖ protein-enriched nutrition
- advised protein sources, vitamin intake (B6)

Carbohydrates
- importance of food carbohydrates
- minimal carbohydrate demand, its caloric ratio in the diet/food
- ❖ carbohydrate-poor nutrition (glucagon predominance, metabolic changes, ketosis)
- ❖ carbohydrate-enriched nutrition (consequences of insulin predominance, fast rise in blood glucose - hyperinsulinemia - obesity)
- recommended carbohydrate intake (complex carbohydrates); disadvantages of simple sugar intake; vitamin intake (B1)

Lipids
- importance of food lipids
- caloric ratio of lipids in the diet/food; intake of essential fatty acids
- ❖ lipid-poor nutrition (in the background: generally digestive problems) and its consequence (absence of fat-soluble vitamins; absence of EPA and DHA)
- ❖ lipid-enriched nutrition and its consequences; fatty acids increasing or decreasing the blood cholesterol level
- features of advised lipid intake, appropriate lipid sources; vitamin intake (E), importance of carnitine

Micronutrients
- general importance, conditions affecting vitamin requirement
- functional classification of vitamins and vitamin-like substances (coenzymes, antioxidants, ones with other functions) and examples for natural sources
- importance of vitamins with coenzyme functions in the metabolism (vitamin – coenzyme form – example for a reaction)

- classification of minerals according to requirement; examples for natural sources
- examples for functions for microminerals and for trace elements

Alimentary fibers

- general daily requirement and average daily intake
- classification of dietary fibers, main types and their chemical nature
- characteristic effects of alimentary fibers and their significance in the prevention of certain diseases

❖ consequences of fiber-poor nutrition – increased risk for certain diseases
❖ disadvantages of fiber-enriched nutrition

MUSCLE

General points

- main muscle types, chief features and their importance
- characteristics of the macro- and microstructure in skeletal muscle, cell organelles of the skeletal myofiber
- characteristics of the macro- and microstructure in cardiac muscle
- characteristics of the macro- and microstructure in smooth muscle
- ultrastructure of striated muscle myofibrils: the sarcomeric structure (bands, lines), filaments
- the principle of „sliding" filament" model

Composition and main components of the striated muscle

- general composition (organic and inorganic components)
- water-soluble proteins (enzymes - examples, myoglobin), membrane proteins (examples)
- structural proteins (groups: contractile and regulatory proteins; main cytoskeletal proteins)
- molecular features of myosin, ATPase activity, binding sites; thick filaments
- α-actin; G-actin, F-actin; connections of F-actin
- characteristics of tropomyosin and its function
- members of the troponin complex, functions
- structure of the thin filament, its components and formation

Contraction of the striated muscle

- basic mechanism of muscle contraction, formation of crossbridges, „power stroke"
- steps of striated muscle contraction (contraction-relaxation cycle)
- importance of Ca^{2+} ion concentration in the sarcoplasm, its value in resting muscle, and before contraction
- importance of troponin complex and tropomyosin in the initiation of contraction

Excitation – contraction coupling („EC coupling")

- basics of direct/mechanical coupling and its steps ***in skeletal muscle***
- importance and functioning of nicotinic acetylcholine receptor
- localization and functioning of dihydropiridine receptor (DHP-R) and ryanodine receptor (RyR)
- the sarcotubular system: structure and importance of T-tubules and the sarcoplasmic reticulum; localization and function of triads
- Ca^{2+} -induced Ca^{2+} release: essence and steps ***in cardiac muscle***
- excitation – contraction coupling in ***smooth muscle,*** role of PLC signaling
- basics of smooth muscle contraction: myosin phosphorylation and its regulation

Mechanism of relaxation in striated muscle
- steps of relaxation
- importance and functioning of acetyl cholinesterase
- ❖ inhibitors of cholinestherases
- importance and functioning of SR - Ca^{2+} ATPase (SERCA)
- storage of Ca^{2} in the sarcoplasmic reticulum

Muscle differentiation and regeneration
- main steps of skeletal muscle development
- basis of skeletal muscle regeneration: satellite cells

Mechanics of muscle action
- dynamics of muscle stretching (stimuli – latent period – contraction –peak - relaxation)
- factors influencing muscle stretching (number of motor units, Ca^{2+} concentration, temperature, pH, basic stretched state: role sarcomer length)
- muscle contraction in case of repeated stimuli, according to frequency of stimuli (separate contractions, complete and incomplete tetanus)
- isotonic and isometric contractions

Energy need for muscle contraction
- ATP sources and their usage in muscle
- immediate energy supply: creatine phosphate, myokinase, myoglobin
- short-term energy supply: role of glycogen
- long-term energy supply: role of aerobic glycolysis
- adaptation of respiratory and cardiovascular system during exercise for improved energy supply

Types of muscle fibers
- characterization of fast, glycolytic fibers
- characterization of slow, oxidative fibers
- characterization of fast, oxidative fibers
- muscle plasticity
- special metabolism of heart muscle

Adaptation of muscle metabolism
- metabolic basis of muscle fatique
- comparison of sprinter and marathon runner
- metabolic consequences of short-term, intensive training
- metabolic consequences of long-term, endurance training
- muscle fever
- endurance
- oxygen debt

Muscle disorders

Ion channel diseases (channelopathies)

- malignant hyperthermia (detailed characterization)
- myotonic states (e.g.: hypo-/hyperkalemic periodic paralysis, congenital myotonia)
- muscular dysgenesis (deficiency of dihydropiridin-receptor)
- Brody-disease (SERCA1-deficiency)

Diseases affecting the neuromuscular junction

- myasthenia gravis (detailed characterization)

Diseases affecting the cytoskeletal system

- progressive muscle dystrophies, Duchenne dystrophy (detailed characterization)

BIOCHEMISTRY OF THE NERVOUS SYSTEM

Brain: general characteristics of the metabolism

- intensive metabolism (high glucose and oxygen demand; hypoxia as a great risk)
- background of the high energy demand (active transports, axonal transport, synthetic processes)
- characteristics of carbohydrate metabolism (GLUT-1,-3; intensive aerobic /+anaerobic/ glycolysis; basics of the classic PET diagnostics)
- ❖ acute hypoglycemic coma (essence, main causes)
- ❖ hyperglycemic coma and chronic hypoglycemic coma (main causes, characteristic metabolic disorder, risky consequences of ketosis)
- characteristics of lipid metabolism (brain as an organ enriched in lipids – main lipid types, characteristics of fatty acid metabolism, utilization of ketone bodies)
- characteristics of amino acid – protein metabolism (elimination of ammonia, neurotransmitters formed from amino acids, importance of the formation of cytoskeletal proteins)
- characteristics of nucleotide metabolism (main pathways)
- ❖ hereditary defects affecting the general metabolism of the central nervous system: sphingolipidoses (Nieman-Pick and Tay-Sachs disease), mucopolysaccharidoses, Lesch-Nyhan disease

The blood-brain barrier (BBB) and the cerebrospinal fluid

- structure and main transports via BBB (passive diffusion, facilitated transport)
- ❖ background of „kern-icterus" (pathological icterus/jaundice of neonates)
- main characteristics of the cerebrospinal fluid

General features of neurotransmission

- main pre- and postsynaptic processes (pre: synthesis of neurotransmitters, formation of synaptic vesicles, axonal transport, transmitter release)
- main characteristics of neurotransmitters and classification according to their chemical character (major groups: amino acids, amines, amine derivatives; special types: peptides, purines, NO /gas/, lipid derivatives /endogenous cannabinoides/
- elimination of neurotransmitters (generally: breakdown, presynaptic reuptake)
- general characteristics of ionotropic receptors (structure, function, cation/anion flow and its consequence)
- main examples for ionotropic receptors (Glu, nicotinic cholinergic, GABA and Gly receptors)
- general characteristics of metabotropic receptors (structure, linkage to G protein), and the two main ways of action (signaling pathways, opening ion channels)
- main examples for metabotropic receptors (Glu, GABA, catecholamines, serotonin and muscarinic cholinergic receptors)

Cholinergic neurotransmission

- presynaptic synthesis of acetylcholine (enzyme, reaction with structures), sources for choline
- transport into the synaptic vesicles
- ❖ agents influencing the acetylcholine release (botulinum toxin, 4-aminopyridine, latrotoxin), their effects
- characteristics of the nicotinic receptor, the two types and their localization, function
- ❖ inhibitors of the nicotinic receptor (curare, succinyl-choline), their effects
- ❖ myasthenia gravis
- function of the muscarinic receptor, main types (characteristic localization, signaling, effect)
- ❖ inhibitors of the muscarinic receptor (atropine, scopolamine), their effects
- importance of cholinergic neurotransmission in the brain (learning, memory)
- breakdown of acetylcholine (involved enzyme and its characteristics)
- ❖ low-activity acetylcholine esterase: possible consequences
- ❖ inhibitors of acetylcholine degradation and their characteristic effects, reversible inhibitors (physostigmine, neostigmine), irreversible inhibitors (alkyl phosphates)

Catecholamines

- synthesis of catecholamines (reactions with structures, enzymes, names cofactor)
- main types of adrenergic receptors (characteristic localization, signalization, effect)
- dopaminergic neurotransmission (characteristic localization), function of the receptors
- transport of dopamine into the synaptic vesicles, presynaptic reuptake
- ❖ inhibition of presynaptic reuptake and its effect (cocain)
- ❖ Parkinson disease (background, main symptoms, treatment)
- ❖ the effect of excessive dopamine release (dopamine theory of schizophrenia, biochemistry of being in love)
- degradation of catecholamines (main breakdown enzymes, names of the end products)

Glutamate neurotransmission

- main characteristics, localization, synthesis (Glu-Gln cycle, enzymes, structures)
- main types of ionotropic glutamate receptors, mechanism of action of the NMDA-receptor and its significance
- ❖ excessive functioning of the NMDA receptor in hypoxia
- metabotropic glutamate receptors: characteristics of their function
- elimination of glutamate

GABA and Gly neurotransmission

- synthesis of GABA (enzyme, coenzyme, structures)
- ionotropic and metabotropic GABA receptors and their function
- inactivation and degradation of GABA (enzymes, GABA-shunt)

- characteristics of Gly neurotransmission

Serotonin neurotransmission

- general characteristics: localization, effects
- synthesis (enzymes, coenzymes, reactions with structure); presynaptic reuptake
- ❖ inhibitors of presynaptic reuptake (SSRI) and their effect
- general function of serotonin receptors
- breakdown of serotonin (enzymes, endproduct)
- ❖ serotonin-analog drug (LSD)

Other neurotransmitters

- histamine: formation (reaction with structures, enzyme, coenzyme), localization and effects
- ATP: formation and breakdown, receptors (purinergic)
- neuropeptides (e.g. endorphines/endogenous opiates: formation and main effect)
- endocannabinoids (membrane lipid derivatives), their function (modulation of inhibitory neurotransmission)
- NO / nitric monoxide (action of NO synthase, coenzymes, structures), degradation (formation of peroxynitrite), main effects, signalization (cGMP)

BIOCHEMISTRY OF VISION

The biochemistry of the necessary structures of vision

- **Cornea**: structure and metabolism (clear, regular collagen structure (type VIII.), characteristic proteoglycans, avascular tissue, O_2 diffusion, aerob glucolysis, active HMP-shunt, role of GSH and NADPH)
- **Lens:** structure and metabolism (m. ciliaris, zonular fibres (fibrillin), collagen and proteoglycan composition, specific proteins (crystallines, intermedier filaments), the role of high water content, osmotic balance (Na^+-K^+ ATPase), avascular tissue, O_2 diffusion, anaerob glucolysis, active HMP-shunt, the role of GSH and NADPH)
- ❖ Patobiochemistry of cataract (free radicals, chrystalline-oxidation, decreased NADPH level, galactosaemias/ fructose-intolerance/ Diabetes (role of galactitol, sorbitol)
- **Retina**: structure and metabolism (layers, vascular tissue, active glucolysis, role of LDH)

The biochemistry of vision

- Structure of the photoreceptor types (rods and cons), (outer segment, inner segment, protein synthesis, mitochondria, membrane system), role of pigment cells
- The structure of rhodopsin (Opsin (Lys)+ 11-cys-retinal= Schiff-base)
- Light-induced changes in the retinal (cis-trans isomerization)
- Light-induced changes in the rodopsin (primary: photorodopsin, bathorodopsin, secundary: metarodopsin-I, metarodopsin-II, and opsin+ all-trans-retinal)
- Regeneration of rodopsin (isomerase: all-trans retinal to 11-cis-retinal + opsin), the storage and regeneration of retinal (role of the pigment cells, liver, retinol-dehydrogenase and isomerases)
- ❖ Vitamine-A deficiency (night blindness)
- ❖ Retinitis pigmentosa (crystalline/ periferin mutation)
- The signal transduction of vision in dark (Na^+-channels opened by cGMP, depolarisation (dark flow), neurotransmitter (Glu)-release)
- The signal transduction of vision at light (excited rhodopsin, metarhodopsin-II, transducin (Gt) activation, phosphodiesterase (PDE) binding and activation, degradation of cGMP, close of Na^+-channels, hyperpolarization, transmitter (Glu) release decreases
- Signal amplification (1photon - 1 activated rodopsin - 500 activated transducin -500 activated PDE (transport number of PDE: 4200 molecule/sec), during 1 sec: 4200 x 500 = 2 100 000 degraded cGMP)
- Molecular mechanism of light adaptation (Ca2+ regulation of cGMP level, rhodopsin-kinase, arrestin)
- Mechanism of color vision (types and functioning of cons)
- ❖ Red-green color blindness

ENDOCRINE SYSTEM

General characteristics of the endocrine system

- the essence of the integrated psycho-neuroendocrine-immune system
- endocrine systems under the control of the central nervous system (hypothalamo-hypophyseal systems, sympathetic - adrenal medulla system /SAS/)
- endocrine system under the control of blood plasma factors (glucose, Ca; blood pressure - osmolarity)
- growth factors, cytokines, tissue hormones, inflammatory factors
- endocrine and paracrine regulation

Hypothalamo-hypophyseal systems

- release and release-inhibitory hormones and their regulation
- function of the adenohypophysis (anterior pituitary)
- characteristics of the growth hormone (GH) and prolactin (PRL)
- GH: regulation of its synthesis, signaling, effects on the metabolism
- ❖ dwarfism, gigantism and acromegaly
- ▪ regulation of PRL synthesis and effects of PRL
- ❖ consequences of increased PRL production
- ▪ TSH and the gonadotropin hormone family (FSH, LH, CG): characteristics, their formation and their effects
- ▪ members of the pro-opio-melanocortin (POMC) family (ACTH, MSH, endorphins, lipotropins) and their formation
- ▪ regulation of ACTH production and effects of ACTH
- ❖ Cushing syndrome: main features
- ▪ endorphins: regulation of their synthesis, main effects
- ▪ MSH and lipotropins: main effects
- ▪ hormones of neurohypophysis (posterior pituitary): oxytocin and vasopressin/ADH), their characterization
- ▪ regulation of vasopressin/ADH secretion, effects and signaling
- diabetes insipidus: main symptoms
- ▪ oxytocin: regulation of its secretion and main effects

Sympathetic – adrenal medulla system /SAS/

- hormones of the adrenal medulla (epinephrine, norepinephrine), their synthesis (structures), degradation (enzymes), effects
- adrenergic receptors and related signalization

Thyroid hormones

- ▪ main features of the thyroid gland, regulation of its functioning, the essence of signalization of thyroid hormones
- ▪ types of thyroid hormones (T3, T4, rT3), chemical features (structures)
- ▪ synthesis of thyroid hormones: characterization, importance of thyroglobulin, its synthesis, and regulation of its formation

- steps of thyroid hormone synthesis (with enzymes): iodide trapping, iodide oxidation, iodination of thyroglobulin, coupling, storage (colloid), release of hormones and its regulation, deiodination (formation of T3), T3 and T4 secretion
- ❖ inhibitors of T3, T4 synthesis (iodide transport, iodide oxidation)
- T3, T4 transport in blood (TBPA, TBG), characteristics of TBG
- fate of T3 és T4 (deiodination, inactivation, excretion)
- signalization of T3 in the target cell (nuclear receptors)
- metabolic effects (basal metabolic rate, metabolism of carbohydrates, lipids and proteins)
- tissue/organ effects (cardiovascular system, respiration, skeletal muscle, interaction with other hormonal effects, effects on the central nervous system)
- ❖ hyperthyroidism, Basedow-Graves disease: main symptoms
- ❖ hypothyroidism, myxedema; connatal hypothyroidism (cretenism): main features
- ❖ goiter: its essence, types; iodine defect, daily iodine requirement

Steroid hormones

General characterization of steroid hormones

- structure and availability of precursor cholesterol in hormone producing tissues
- classification of steroid hormones according to their structures
- classification of steroid hormones according to tissue distribution and action
- mechanism of action, uptake to peripheral tissues, characterization and action of steroid hormone receptors
- transport via blood stream, inactivation and secretion

Hormones of cortex of adrenal gland

- hormones produced in zones of the adrenal cortex
- steps of synthesis starting with the cholesterol (names of enzymes, names and structures of intermediers, in details!!!)
- mineralocorticoids: structure of aldosterone, main action
- regulation of aldosterone production: renin-angiotensin system (ACE: role of angiotensin converting enzyme)
- glucocorticoids: structure of cortisol, main action on metabolism (metabolic pathways, reactions, enzymes)
- sexual steroids: structure of DHEA and androstenedione
- ❖ deficiency of 21-hydroxylase, main characteristics of adrenogenital syndrome

Sexual steroids

- female hormone producing glands, cell types
- structure of progesterone and estrogen, steps of synthesis (names of enzymes, names and structures of intermediers, in details!!!)
- main actions of female sexual steroids (briefly)
- compartmentalization of synthetic pathways in steroid hormone producing tissues
- male hormone producing glands

- structure of testosterone and 5-dihidrotestosterone, steps of both synthetic pathways (names of enzymes), DHEA and androstendione as precursors (structures)
- main actions of testosterone (briefly)

Vitamin D

- structure
- steps of the synthesis in different tissues (names of enzymes, names and structures of intermediers)
- influence on Ca homeostasis and metabolism

❖ lack of vitamin D, its formation and consequences (rachitis, osteomalacia)

❖ overdose of vitamin D, its formation and consequences (calcinosis)

Blood glucose homeostasis and diabetes mellitus

Complex regulation of blood glucose level

- hormones and factors involved in the process, effect for increase and decrease in blood glucose level
- normal blood glucose level (reference range), importance of stable and balanced blood glucose level
- definition of hypo- and hyperglycemia, conditions associated with hypo- and hyperglycemia, occurrence and characterization of acute hypoglycemia
- pathways involved in restoration of blood glucose level regulation in different stages of starvation
- pathways involved in restoration of blood glucose level in hyperglycemia

Insulin

- synthesis, structure
- formation of C peptide, diagnostic importance
- secretion from the beta cells, target cells, type of insulin receptor and its action
- influence on glucose uptake, GLUT transporters
- influence on pathways of carbohydrate metabolism, regulation points and mechanisms (regulated pathways, enzymes, reactions)
- influence on pathways of lipid metabolism, regulation points (regulated pathways, enzymes, reactions)
- influence on pathways of amino acid and protein metabolism

Glucagone

- structure, synthesis, target cells, type of glucagone receptor and its action
- influence on pathways of carbohydrate metabolism, regulation points and mechanisms (regulated pathways, enzymes, reactions)
- influence on pathways of lipid metabolism, regulation points (regulated pathways, enzymes, reactions)
- influence on pathways of amino acid and protein metabolism

Biochemical background of diabetes mellitus

- main types of diabetes mellitus (DM-I: IDDM, DM-II: NIDDM)
- mechanism of diabetic hypoglycemia
- mechanism of diabetic hyperglycemia
- characterization of acute metabolic failure, biochemical background, tissues are involved (ketoacidosis, enhanced gluconeogenesis, hyperosmosis, dehydration), coma
- biochemical background (molecular mechanism) of long term consequences of diabetes (protein glycolysation, lipid peroxidation) and its role in patomechanism of angiopathies; involved tissues
- basics of treatment in case of Type I. and II. diabetes
- formation of hypoglycemic coma in diabetes

Clinical biochemical diagnostics of diabetes mellitus

- measurement of blood glucose level (traditional (wet diagnostics)and POCT (dry diagnostics))
- essence and importance of OGTT testing in diagnostic of DM
- role hemoglobin A1c in diagnostics, measurement of this parameter

Calcium metabolism

- forms and appearance of calcium, its importance in the body; effect of pH on the appearance of the ionized form; total and ionized calcium concentration in blood plasma
- parathyroid hormone (PTH): its characteristics and formation; regulation of its formation, degradation and secretion
- effects of PTH (bone, kidney, synthesis of calcitriol)
- ❖ hyperparathyroidism: main types (primary and secondary) and main symptoms
- ❖ hypoparathyroidisms: main features
- formation and activation of vitamin D in the body, regulation of its activation in the kidney
- effects of vitamin D (calcitriol) om tissue and cellular level (intracellular receptor)
- ❖ D vitamin defect (hypovitaminosis), main symptoms of rickets and osteomalacia; renal osteodystrophy
- ❖ vitamin D overdose (hypervitaminosis); main symptoms of calcinosis
- calcitonin: its formation and main effect

Growth factors, cytokines and tissue hormones

- main characteristics of growth factors, cytokines and tissue hormones, paracrine/autocrine secretion, general effects on tissue and cellular level

Growth factors

- importance of growth factors (GF) in embryogenesis and during regeneration
- ❖ significance of growth factors in the formation of malignant tumors and in other chronic diseases (e.g. fibrosis, atherosclerosis)
- main growth factors (EGF, FGF, IGF-I, IGF-II, /insulin/, PDGF, NGF, VEGF, TGF-beta) and their main special effects
- main characteristics of GF receptors, domains
- steps of GF signalization (according to EGF): initial signalization at the Tyr-kinase receptor (adapter proteins, importance of small monomeric G proteins)
- steps of GF signalization (according to EGF): MAP-kinase cascade and other signalizations
- signaling of insulin and IGF-I
- FGF-2: its various actions and signaling pathways
- VEGF as the main stimulatory factor of angiogenesis (effect of hypoxia)
- down regulation of GF signalization and its regulation; connection and network of signaling pathways
- ❖ proto-oncogenes and oncogenes: characteristics and their connection to GF signaling, their role in the formation of malignant tumors; retrovirus oncogenes and sarcomas

Cytokines

- characteristics of cytokines, their importance, examples (interleukins, TNF, interferons)
- signalization of cytokines (receptor-associated Tyr-kinase)

Tissue hormones, inflammatory factors

- gastrointestinal hormones: types, their effects
- ANP (heart) and erythropoietin (kidney); their effects
- eicosanoids: main groups and effects (PG, PGI, TX)
- histamine and serotonin as tissue factors
- central (white) adipose tissue (WAT) as endocrine tissue, main produced factors, hormones (e.g. leptin, steroids, IGF-I, IL-6, prostaglandins)

BIOLOGICAL SIGNALLING, SECOND MESSENGER SYSTEMS

General characteristics of signal transduction

- communication problems of multicellular organisms
- types of signal transduction (synaptic, endocrine, paracrine, autocrine, direct cell-cell connection)
- levels of intracellular signalization: signal, reception (hydrophilic signal: membrane-bound receptor, hydrophobic signal: intracellular receptor), transduction/amplification, elicited response
- general characteristics of receptors (ligand- and cell specificity, up- and down-regulation, adaptation, desensitization, internalization), receptor pattern characteristic of cells
- types of receptors (membrane-bound: ion channel receptors /ligand-dependent neurotransmitter receptors/, heterotrimeric G-protein-linked receptors, monomeric G-protein-linked receptors /GF and cytokine receptors/), other, special receptors: guanylate cyclase and adhesion receptors; cytoplasmic/cell nuclear receptors)
- general characteristics of signal transduction (amplification, complexity, signalling networks, molecular switches, adapter proteins, second messengers, protein kinase cascades, termination; cell specificity)
- types of second messengers (cAMP, cGMP; Ca^{2+}; PIP_2, DAG, IP_3)
- special signal transduction: inflammatory reaction and blood clotting

Receptors

Ion channel receptors

- characteristic structure and function (transmembrane domain, ligand-dependent fast (ms) signal transduction, importance of ion gradient)
- examples: neurotransmission (e.g. nicotinic acetylcholine receptor, NMDA receptor)

Heterotrimeric G-protein-linked, 7-transmembrane domain receptors

- structure of receptors (7-transmembrane helix) and characteristic function
- examples (muscarinic acetylcholine receptor, adrenergic receptors etc.)

Monomeric G-protein-linked, 1-transmembrane-domain receptors

- structure of GF receptors (modular structure; extracellular domain: ligand binding, intracellular domain: TK activity); examples (insulin-R, VEGF-R, PDGF-R, EGF-R, NGF-R, FGF-R)
- activation of TK receptors (autophosphorylation)
- characteristics of the insulin receptor (2 α and 2 β subunits, binding of 1 insulin), target proteins (IRS-1 and -2), effector functions (glucose/glycogen metabolism: PIP_2/ protein kinase B (Akt) pathway; regulation of gene expression, cell proliferation: ras/MAPK pathway)

- main characteristics of cytokine receptors (no TK activity), examples (interleukin receptors)

Special receptors
- guanylate cyclase receptors (ligands: NO, ANP/BNP; membrane- /ANP/BNP/ or intracellular, soluble /NO/ receptors)
- main types of adhesion receptors (integrins, cadherins, Ig-family, selectins)

Intracellular/ nuclear receptors
- ligands (T3/T4, steroids, retinoic acid)
- receptor structures, domains and functions of steroid receptors, hormone-binding DNA sequences (TRE: thyroid hormone responsive element, GRE: glucocorticoid responsive element etc.)

<u>Signal transduction</u>

Signaling pathways linked to heterotrimeric G-proteins
- mechanism of the heterotrimeric G-protein cycle (activated receptor, heterotrimeric G-protein: alpha, beta, gamma subunits, GTP/GDP exchange and its importance, effectors: adenylate cyclase, phospholipase C, phosphodiesterase)
- characteristics of the heterotrimeric G-proteins, main classes according to the α subunit with examples ($G\alpha_s$ stimulatory: cAMP↑ /epinephrine/adrenalin/β receptor/, $G\alpha_i$: inhibitory: cAMP↓ /epinephrine/adrenalin/α2-receptor/, $G\alpha_{q:}$ PLC-IP_3-DAG↑ /epinephrine/adrenalin/α1 receptor/, $G\alpha_t$ transducin, PDE-6 cGMP↓ /vision/)
- structure and function of adenylate cyclase and protein kinase A, formation and degradation of cAMP
- function of the $G\alpha_q$: PLC-IP_3-DAG system (Ca^{++} release), importance of protein kinase C
- ❖ heterotrimeric G proteins and related diseases: cholera toxin and pertussis toxin: mechanism of action (ADP-ribosylation)

Small (monomeric) G proteins
- characteristics of small (monomeric) G proteins, their signalling pathways (MAPK cascade), function of ras protein
- ❖ mutant ras protein and tumour formation

Guanylate cyclase receptors
- mechanism (cGMP, protein kinase G), effector functions (blood vessel walls: smooth muscle relaxation, kidney: water retention etc.)
- NO synthesis (NOS isoenzymes, their characteristics and regulation)
- NO signal transduction in blood vessel walls
- NO and oxidative stress (reactive oxygen /ROI/ and nitrogen /RNI/ intermediers)

Adhesion receptors
- signal transduction of integrins during cell adhesion (clustering, focal adhesion)

- connection to GF signaling („anchorage-dependent cell proliferation")

Special signal transduction: inflammatory reaction and blood clotting

- platelet receptors and related signal transduction
- PLA_2 and functions of eicosanoids

Ca^{2+} as second messenger

- intra- and extracellular calcium concentration, „horror calcii", role of calcium in cell damage and in cell death
- structure and function of PMCA (plasma membrane Ca^{2+}ATP-ase), Na^{+}-Ca^{2+} exchange systems, intracellular calcium buffer systems, calcium-binding proteins, calcium-dependent regulatory proteins (calmodulin, Ca^{2+}-calmodulin-kinase /CamK/, troponin C), structure of EF hand-domain; intracellular calcium sequestration, structure and function of SERCA enzymes
- calcium signal: initiation, types of surface calcium channels
- mechanisms of intracellular calcium release, calcium-dependent calcium release (myocardium), mechanism of IP_3-dependent calcium release
- Ca^{2+} as an allosteric enzyme regulator (e.g. glycogen phosphorylase kinase)

Phosphorylation, dephosphorylation in biological signal transduction

- physicochemical properties of the phosphate group, its advantages from the aspect of biological regulation, advantages of ATP (special molecular structure)
- protein phosphorylation: protein kinases, serine/threonine kinases, tyrosine kinases, cyclic nucleotide dependent, calcium dependent, diacylglycerol dependent, and other protein kinases
- protein dephosphorylation: its regulatory role, protein phosphatases
- role of phosphorylation and dephosphorylation in the regulation of life functions, and in the integrated regulation of metabolic pathways
- coordinated regulation of phosphorylation and dephosphorylation systems in the metabolism (balance and hormonal regulation of glycogen synthesis - breakdown) and in other cell functions (e.g. smooth muscle contraction)

MOLECULAR BIOLOGY, REGULATION OF GENE EXPRESSION

The structure of the DNA

- central dogma of the molecular biology (one-way expression: DNA-RNA-protein)
- the chemical structure of the DNA nucleotides (A, G, C, T)
- connection of the nucleotides: alpha-helical primary structure, hydrogen bonds (G-C, A-T), the melting point of the DNA, minor and major groove, conformations of the double helix (A, B, Z) and their characteristics
- DNA packaging: the structure of the nucleosomes, types and functions of histone proteins, solenoid (30 nm) structure, the role of H1 histone, chromatin loops, the endpoint of the compression of the chromatin: chromosomes, non-histone proteins
- the definition of genome, its size, the human genome, repeating sequences in the genome, telomere, centromere, the function of the telomerase
- types of chromatin: heterochromatin (constitutive, facultative) and euchromatin
- epigenetic modifications of the DNA: histone modifications (acetylation /HAT enzyme/, deacetylation /HDAC ezymes/, phosphorylation, methylation), methylation of the DNA (C) and its importance, the definition of genomic imprinting and maternal effect, their importance

❖ Fragile X syndrome

DNA replication

- semiconservative mechanism: role of the helicase, replication fork, the mechanism of the replication in the two cases: leading and lagging strand (helicase, DNA-polymerase, RNA primer, primase, Okazaki-fragments, DNA-polymerase, ligase)

Modifications in the sequence of the DNA: Mutations

- types: in functional/non-functional cells, somatic/germ cells, point mutations (the definition of SNP: single nucleotide polymorphism)/ chromosome mutations (alterations in the number /euploid, aneuploid/ and in the sequence /translocation, deletion, inversion/ of the chromosomes), endogenous/exogenous mutations, spontaneous/induced mutations
- Point mutations: missense, nonsense, deletion, insertion (non-frame-shift and frame-shift mutations), mechanism (depurination, deamination /C-U alteration/, formation of thymine dimers, methylation of the purine bases)

Repair mechanisms

- base pairing errors during the replication: correcting function of the DNA polymerase (proof-reading)
- mutated nucleotides: base and nucleotide excision repair, mechanism (base: glucosidase, AP endonuclease, polymerase, ligase; nucleotide: endonuclease, polymerase, ligase)

❖ xeroderma pigmentosum

- the role of p53 protein in the mechanism of repairing

The course of gene expression, regulation of the transcription

- fundamental differences between eukaryotic and prokaryotic gene expression
- levels of regulation of the gene expression in eukaryotes (epigenetic factors, transcriptional regulation, mRNA maturation, post transcriptional regulation, translational regulation, post translational modifications)
- definition of gene, the structure of the eukaryotic genes (exon, intron, promoter, 5' and 3' untranslated regions /UTR/)
- mechanism of transcription, coding and non-coding strand, direction of the transcription; types of eukaryotic RNA polymerases (I-II-III) and their description
- the most important types of RNA in the eukaryotic cells (mRNS, tRNS, rRNS) and their description, other RNAs (hnRNS, snRNS, snoRNS, siRNS, microRNS, antisense (as) RNS) and their importance
- the structure of the eukaryotic promoter (Start, TATA-Box, CAAT-Box, GC-Box), cis and trans regulatory elements, enhancer and silencer regions
- transcription factors, their structure: DNA-binding, signal-binding, trans-activating, dimerization domains, their classification based on the DNA-binding domain: HLH (helix-loop-helix) factors (c-myc, myogenic regulatory factor family), zinc finger factors (steroid receptors, SP1), leucine zipper factors (c-jun, c-fos), Hox transcription factor family
- the course of transcription: initiation, elongation, mechanism of termination, formation of preRNA (hnRNA)
- formation of mature mRNA: 5'-cap, splicing, 3' poly-A tail, importance of the modifications, mechanism of splicing, the structure of the spliceosome (importance of snRNAs, the lariat structure of the mRNA), mechanism of alternative splicing and its importance (tissue and progression specific gene expression e.g. calcitonin/CGRP)
- ❖ thalassemias
- mechanism of RNA editing (pl. ApoB48, ApoB100), its importance
- factors influencing the stability of mRNA, regulation of stability (e.g. iron dependent reciprocal regulation of the stability of ferritin and transferrin receptor-coding mRNAs)

Protein synthesis and its regulation, post translational modifications

- characteristics of the genetic code (universal, degenerate; start and stop codons), codon-anticodon interaction, wobble in the genetic code (the connection between the 3rd base of the codon and the 1st base of the anticodon is less strict)
- the structure of the tRNA, binding the specific amino acid, function and operation of aminoacyl-tRNA-synthetase
- structure of procaryotic and eukaryotic ribosomes, large and small subunit, rRNAs and ribosomal proteins

- detailed mechanism of the prokaryotic protein synthesis, its energetics, initiation (initiation factors, fMet as the first amino acid), elongation (elongation factors, A and P sites of the ribosome, formation of the peptide bond, movement of the ribosome, role of GTP), termination (releasing factor, stop codon)
- the mechanism of the effects of the different antibiotics (streptomycin, erythromycin, tetracycline, chloramphenicol) on the procaryotic translation, the inhibitors of the eukaryotic translation (puromycin, cycloheximide), mechanism of the effect of diphtheria toxin
- post translational mechanisms, the importance of the endoplasmic reticulum, the signal peptide and the SRP (signal recognition particle)

The regulation of the cell cycle, the molecular theory of tumor formation

- description of the cell cycle: G1, S, G2, M phases, the importance of G0 phase (e.g. neurons, muscle satellite cells), characteristic events of the different phases, regulatory points: R-point (in G1 phase), G2-M control point
- regulation of the cell cycle: types, structure and operation of the cycline dependent kinases (CDK) (catalytic and regulatory subunit; their activity can be regulated by phosphorylation and cyclins), types of cyclins, phase-dependent expression of CDKs during cell cycle
- regulation of the cell cycle: cyclin-dependent kinase inhibitors (CKI) (e.g. p21, p27, p16), mechanism of effect of p21 protein, regulation of the R-point: the mechanism of the effect of retinoblastoma (Rb) protein
- the definition of proto-oncogenes, the consequence of the (dominant) mutation of the proto-oncogenes: oncogens, malignant cell division, types of oncogens (growth factors /sis/, growth factor receptors /erbB/, G-proteins /ras/, protein-kinases /raf/, transcription factors /myc, fos, jun/, cell-cycle regulators /ciklinD/).
- the definition of tumor suppressors, their recessive mutations (in the case of both allels): malignant cell division (e.g. p53, p21, Rb), the molecular theory of tumor formation: the disruption of the balance of the oncogenic/tumor suppressor effects, the mechanism of the effect of tumor suppressor protein p53 (the 'protector' of the genome, activator of the repair, inhibition of G1-S phase transition)

Apoptosis

- definition (programmed cell death), its aim (morphogenesis (e.g. neuronal cell networks), tissue differentiation, protection against harmful effects)
- comparison of necrosis and apoptosis, the characteristics of the apoptosis (intact organelles, fragmented chromatin, the inner content of the cell is not released)
- the inducing factors of the apoptosis (intracellular, extracellular), the phases of the apoptosis (determination/induction, cell death, phagocytosis, degradation), its importance

ADAPTATION IN HUMAN BODY (MOLECULAR, CELL, TISSUE/ORGAN, ORGANISM)

Principles and levels of regulation

- regulation at the level of enzymes, role covalent modification and allosteric regulation
- basic principles of regulation at the level of genes
- basic principles of regulation at intracellular level: role of compartmentalization
- principles of regulation at tissue and organism level (integrated neuroendocrine regulation)

Adaptation mechanism in physiological and pathological conditions

Adaptation in starvation

- biochemical definition and stages of starvation
- hormones involved in adaptation
- pathways and tissues are involved in restoration of blood glucose level in different stages of starvation
- main shifts in lipid and amino acid/protein metabolism (metabolic pathways, regulated reactions, enzymes); affected tissues and organs
- consequences of chronic starvation, metabolic conditions in limitation of stravation

Adaptation in case of enhanced food intake

- hormones involved in adaptation regulation
- adaptation in case of enhanced food intake (main shifts in carbohydrate, lipid and amino acid/protein metabolism (metabolic pathways, regulated reactions, enzymes); affected tissues and organs
- adaptation in case of continuous enhanced food intake (changes in carbohydrate, lipid, protein metabolism, affected tissues), obesity, limitations of enhanced food intake
- possible consequences of obesity (metabolic syndrome and its consequences)

Adaptation in active muscle work

- characterization of metabolism of different muscle fibers
- energy supplying pathways for muscle contraction
- shifts in metabolic pathways, affected tissues in case of short and long term muscle work (metabolic pathways, regulated reactions, enzymes); affected tissues and organs
- long-term adaptation in muscle training

Adaptation in stress

- Selye stress theory (stress as adaptation mechanism) characterization of acute and chronic stress situations
- hormones and their action involved in stress adaptation

- steps of stress response, metabolic changes (metabolic pathways, regulated reactions, enzymes); advantages of stress adaptation
- harmful effects of intensive and chronic stress (neuroendocrine and metabolic changes)

Adaptation in lactation

- characterization and action of involved hormones
- mechanism of adaptation (metabolism)

Supraindividual regulation and general principles of biochemical regulation

Principles of regulation of metabolic pathways

- definition of control and regulation, flow of information in controlled and in regulated systems, stability of controlled and regulated systems
- behaviour of steady-state open systems during changes in the environment
- feedback, feed forward, definiton of negative and positive feed back
- main regulatory principles of metabolism, definition of convergences, divergences, examples, definition linear, circular and shunt pathways with examples
- definiton of normal value/range of biochemical parameters, probability of occurrence of "pathological" value in the normal population, its medical significance, definition of set point
- role of compensation mechanisms in the maintenance of normal values, importance of loading tests in the exploration of reserve compensating capacity, risks of these
- shifts in biochemical (laboratory) parameters, principles of their regulation, limits of their adaptation (examples)
- adaptation around the set point, limited adaptation with examples (changes in adaptation in ageing)
- types of networks, living organism and biochemical systems as networks, characteristics of scale-free networks, possible "Hub" elements of metabolic networks, methods and fields of Omics, genomics, proteomics, metabolomics, foldomics, interactomics

Supraindividual regulation

- examples for factors around the human individuals influencing adaptation (fix and shifting factors, conditions of living place)
- highly organized systems influencing the survival of humans (social, health and economical organization, effect of psychosocial environment)
- examples for high social organization not respecting the individual's own preferences (groups of ant and human society)

Biochemical definition of health and disease

- role of balance of the two facts in the biological evolution, selectional advantages and drawbacks
- importance of life style and prevention in maintenance of health

- illness as the relative insufficiency of the regulating mechanism, supraindividual (social) factors in the maintenance and recovery of regulative capacity
- medicine as supplementation of the regulative capacity, its limits
- health care system: regulation is replaced by control, perspectives of drug screening and biosensors
- loss of steady-state, irreversible, auto-generating processes
- death of the living system as thermodynamic phenomenon