Schedule of biochemical lectures
Faculty of Pharmacy, 2nd year

Definition and scope of biochemistry

Bioenergetics – Living organism is thermodynamically an open system

- Definition of the open system
- equilibrium state in biological systems, steady state condition
- definition of- enthalpy, entropy, Gibbs free energy
- endergonic processes
- exergonic processes
- role of high energy compounds, macroergic bond, examples for high-energy compounds (formation of macroergic compounds, phosphorylation at substrate and coenzyme level)
- central role of ATP

Protein structure

1. Functions of proteins in the organism

2. Protein composition/structure
    -amino acids
    -formation of peptide bond
        * release of water
        *alfa carbon atom
        * linear chain without branches
    - conformation - proper spatial structure for enzyme function
    - secondary bonds stabilizing conformation
        * exception: disulfide links at Cys residues
        * van der Waals interaction, * H bond
    - rotation at the peptide bond
    - secondary structures
        * α-helix,* β-sheet, * random structure

3. Protein conformation
    - primary
    - secondary
    - tertiary
        * three dimensional
        * determines the characteristics of the protein
    - quaternary
        * linkage of several polypeptide chains
        * formation of dimers, tetramers, polymers
    - importance of folding

Enzymes

1. Enzymatic action
    - catalyzators, biological catalyzators, biocatalyzators
        a/ materials catalysing access/joining
            * ensuring proper surface
            * protein surface: proper orientation, great concentration - speed increases
        b/ side chains of proteins actively participate in the reaction
            * separation of charges
            * catching the substrate temporarily
            * proper orientation
    - binding the active intermediate - not substrate specific!
    - molecular mechanism of catalysis

2. Models
    a/ lock and key model (Emil Fischer)
    b/ induced fit model
        - the real template is produced when the substrate is approaching
        - it can be highly regulated - negative and positive feed back

3. Coenzymes
    - role: transfer of energy, charge or acyl group
    - role of NAD, FAD, NADP and CoA (derivatives of ADP)
    - vitamins - water- and fat-soluble

4. Classification of enzymes
    a/ trivial names
    b/ International Union of Biochemists (IUB)
        - oxidoreductases
        - transferases
        - hydrolyses
        - lyases
        - isomerases
        - ligases

5. Isoenzymes, their clinical importance

6. Units of enzyme activity
    - international (standard) unit (IU)
    - catal, - specific activity, molar activity

7. Definition of multienzyme complexes

Enzyme kinetics

1. Role of enzymes during catalysis
    - reduction of activation energy
    - speeding up the approach of the equilibrium state

2. Steady state
    - the speed of the enzyme reaction depends on the substrate concentration
    - Michaelis-Menten equation
    - Briggs-Haldane equation
    - definition/determination of v_{max} and v
    - definition/determination of K_m

3. Order and conditions of reactions
    - first order
    - second order
    - pseudo first order
    - zero order kinetics

4. Kinetics of inhibition
    - competitive
    - non-competitive
    - uncompetitive

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

3

Citric acid cycle, terminal oxidation and oxidative phosphorylation (3 hours)

1. Citric acid cycle as the central pool of the intermediate metabolism
   - The main steps of the discovery of the TCA cycle; role of Albert Szent-Györgyi
   - Localization of the TCA cycle, steps, energy balance
   - Main entry and exit points in the cycle, anaplerotic reactions
   - Regulation of the cycle, its dependence on the general energy balance of the cell

2. Definition of terminal oxidation, redox systems in the organism
   - Thermodynamic characterization of redox reactions
   - Energy balance of NADH and FADH oxidation
   - The structure of mitochondria, mitochondrial transport systems
   - Steps of terminal oxidation (complex I-IV, coenzyme Q, cytochrome c)
   - Vectorial character of proton transport, the process of electron transport, functional groups of redox systems

3. Oxidative phosphorylation
   - Definition and mechanism of oxidative phosphorylation
   - The chemiosmotic theory
   - Structure and function of F_1F_0 ATPase
   - Steps of ATP synthesis in terminal oxidation, their efficacy
   - Action of uncoupling substances
   - Brown adipose tissue and its role in thermoregulation

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!

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
- comparison of the aerobic and anaerobic degradation of glucose
- pyruvate dehidrogenase enzyme complex
- regulation

Gluconeogenesis

- steps, especially the 3 irreversibel steps (names and structures! of intermediates, enzyme names, cofactors, intracellular localization)
- role, source / precursors
- regulation (allosteric and hormonal), ATP requirement

Glycogen

- its role, characterization of stores
- synthesis and degradation (structures, enzymes, coenzymes)
- regulation of synthesis and degradation, role of hormones and allosteric regulation
- Adaptation (regulation of blood glucose level)

Pentose phosphate pathway (HMP-shunt)

- steps with enzymes, coenzymes and intermediates; hexose part with structures
- importance
- regulation of phases depending on NADPH/ribose requirement

Rappaport-Liebering-shunt

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

Special fate of glucose in tissues and organs

- RBC, brain, liver, muscle, adipose cells

Relationship between the carbohydrate metabolism and other metabolisms:

synthesis of amino acids:	glycerol phosphate - serine
	pyruvate - alanine
	oxaloacetate - aspartic acid
fatty acid biosynthesis:	source of NADPH + H^+
triacylglycerol synthesis:	the role of glycerol phosphate in the adipose tissue
citric acid cycle	pyruvate - acetyl-CoA
nucleotide synthesis	source of ribose-5-phosphate

LIPIDS

Chemistry, classification

Fatty acid derivatives

- glycerides
  - triacylglycerols
  - phospholipids
- sphingolipids

Isoprene derivatives

- steroids
- ubiquinone, dolichol

Common saturated and unsaturated fatty acids, essential fatty acids,

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; cholesteryl ester hydrolase
- role of bile acids
- absorption

Metabolism of lipoproteins

- classification and characterization of lipoproteins
- metabolism of lipoproteins

Lipid mobilization

- characterization of the lipid stores
- 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)

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)

Synthesis of triacylglycerols and phospholipids

- steps of TG and phospholipid synthesis (formulae, coenzymes)
- importance of TG and phospholipid synthesis

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

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

Amino acid metabolism

1. Classification of amino acids (essential - , non essential - , semiessential amino acids)
2. Removal of amino acid nitrogen: transamination
   oxidative deamination
   direct deamination
3. The role of glutamine in the transport of ammonia
4. Detoxification of amino acid nitrogen (the main steps of the urea cycle)
5. The fate of the carbon skeleton of amino acids (definition of glucogenic and ketogenic amino acids)
6. Conversion of amino acids to pyruvate
   to oxaloacetate
   to alfa - ketoglutarate
   to fumarate
   to acetoacetyl - CoA, acetyl - CoA
   to succinyl - CoA

7. Utilization of amino acids in:
   - energy generation (points of entry of the carbon from the amino acids into the TCA cycle
   - synthesis of lipid and cholesterol (acetyl - CoA)
   - synthesis of glucose (pyruvate, oxaloacetate)
   - synthesis of coenzymes (SAM, NAD)
   - synthesis of neurotransmitters and hormones (histamine, serotonine, GABA, DOPA, norepinephrine, epinephrine, thyroxine, triiodothyronine)
   - porphyrine synthesis
   - purine synthesis
   - the synthesis of other compounds (creatine, taurine)

Nucleotide metabolism

1. Chemical characterization of nucleic bases (purine - ,pyrimidine bases)

2. Participation of nucleotides in the metabolism:
   - energy storage (central role of ATP)
   - nucleotide units for DNA synthesis
   - nucleotide units for RNA synthesis
   - components of cofactors (NAD, FAD)
   - intracellular second messengers (cAMP, cGMP)
   - synthesis of activated intermediates (UDP - glucose, CDP - choline, CDP - diacylglycerol, GDP - mannose)
   - allosteric effectors

3. Some aspects of nucleotide metabolism:
   - de novo synthesis of purine nucleotides
   - purine salvage mechanisms
   - degradation of purin bases
   - synthesis of pyrimidine nucleotide
   - pyrimidin salvage mechanisms
   - degradation of pyrimidine bases

4. Drugs influencing nucleotide metabolism

Biochemistry of membranes

1. Structure of biological membranes, fluid mosaic model
   Composition of membrane lipids, their localization in the membrane
   The role of lipids in the maintenance of membrane fluidity, adaptation of membranes to changes in temperature
   The role of cholesterol in biologicaal membranes
   Dynamism of membrane lipids, rotation, flip flop, lateral movement
   Interactions between lipids and proteins; definition of anular and bulk lipid

2. Membrane proteins and transport systems
   Classification of membrane proteins, extra-, intra- and transmembrane proteins
   Dynamism of membrane proteins, rotation, lateral mobility, its role, experimental techniques
   Modes of transport, transport of lipid soluble materials, structure and function of ionophores, channels, active transport
   General structure of channel-forming proteins
   Classification of channels, according to gating mechanism: voltage-, ligand-, stretch-gated channels, examples
   Active transport systems: P type, F_1F_0 type, V type ATPases, structures and functions

Second messenger system
General principles of biochemical regulation, adaptation, limits of adaptation

1. Signalling systems
   Intracellular calcium as second messenger
   Factors influencing intracellular calcium level
   Calcium transport systems, intracellular transport stores
   IP3 as calcium release signal
   Structure and function of calcium-binding proteins

2. Cyclic nucleotide dependent signalling systems
   Structure, types and function of G proteins
   Effect of cholera and pertussis toxin
   ADP ribosylation
   Adenylate and guanylate cyclase systems
   Role of phosphodiesterase in the cyclic nucleotide dependent signalization

3. Phosphorylation and dephosphorylation as regulation mechanisms
   Types and function of protein kinases
   Role of protein phosphatases in signalization
   Phosphorylation and dephosphorylation cascades, their role in the coordinated regulation of physiological functions of the organism

Biochemistry of the liver and biotransformation

1. Structure of liver and its microcirculation system
   Metabolic pathways in liver, and their localization
   Role of liver in carbohydrate and in protein/amino acid metabolism
   Role of liver in lipid metabolism, mechanism fat liver formation
   Role of liver in steroid metabolism, synthesis and role of bile acids
   Formation and turnover of bile pigments
   Role of liver in the metabolism of ions and vitamins

2. Biotransformation
   Aim and phases of biotransformation
   Reactions of the first phase, structure and function of cytochrome P450
   Reactions of the second phase, conjugation systems
   Removal of the biotransformation products
   Induction of the biotransformation system
   Interaction of xenogen materials, biochemical mechanism of addiction

3. Biochemical effects of alcohols
   Metabolic pathways of alcohol
   The effect of ethanol on cell metabolism, acute and chronic effects
   Fate of methanol in the organism, effects
   Effects of long chain and multivalent alcohols
   Biochemical consequences of liver insufficiency

Biochemistry of the blood

PLASMA PROTEINS

- Serum protein electrophoresis
- Fractions of plasma proteins
- Serum total protein concentration-significance of decreased serum level
- Proteins: albumin
  alfa-1-antitripsin
  ceruloplasmin
  alfa-2-macroglobulin
  transferrin
  ferritin
  fibrinogen
  lipoproteins

RED BLOOD CELLS

- Special metabolism of red blood cells (metabolic pathways – glycolysis, HMP shunt, GLUT transporter, ATP synthesis and usage, fate of lactate)
- Role of glutathione
- Iron metabolism
- Oxygen transport of hemoglobin (Hb types)
- Factors influencing oxygen binding capacity of hemoglobin
- Synthesis of heme group
- Degradation of heme group

WHITE BLOOD CELLS

- Classification of leukocytes
- Special metabolism of neutrophil granulocytes
- Oxidant and antioxidant factors and mechanisms
- Killing mechanisms of neutrophil granulocytes
- Respiratory burst in neutrophil granulocytes

BLOOD CLOTTING-COAGULATION CASCADE

- Characterization of platelets and their role in blood clotting
- Coagulation factors
- Role of serine proteases and transglutaminases
- Vitamin K dependent factors
- Intrinsic and extrinsic pathways of coagulation cascade
- Inhibitors of coagulation
- Fibrinolysis

Chemical composition and metabolism of the neural tissue

Composition: 40-80 % lipid, cholesterol (25 % of the amount in the organism)
20-60 % protein (turnover is fast - 80 hours on average)
Main characteristics:
- no energy store (fat, glycogen)
- main glucose consumer of the organism (60 % of the total glucose), its use for ATP and neurotransmitter synthesis
- in the absence of glucose ketone bodies but not fatty acids are metabolized

Blood-brain barrier
Lipids: synthesis of phospho- and sphingolipids in situ

Amino acids: 8-10-fold more are free as pool (75% are Glu and Asp, the rest are mainly glutathion, cystathione and taurine (the functions of these last ones are not known)
Many ketoacid: oxalacetate, □-ketoglutarate (used for NH_4^+ detoxification)
Nucleotides: purines but not pyrimidines are synthetised (there is no carbamoyl-P-synthetase)
Characteristic proteins: S-100 - sclerosis multiplex, appican (amyloid precursor protein) - Alzheimer - disease; neuron-specific enolase - tumor diagnostics

Synthesis, storage, exretion aand inactivation of neurotransmitters:

1. Acetylcholine:
    synthesis:
        Ser -- decarboxylation -- cholamine + 3 SAAM -- choline
        choline + acetyl CoA ---acetylcholine + CoA
    stored in the presynaptic vesicles (10-60 000/vesicle) together with ATP
    effect: on nicotinic and muscarinic type aacetylcholine receptors
    neurotoxins: receptor inhibitors - cobra toxin, d-tubocurarine
    breakdown: acetycholine esterase
        types of acetylcholine esterase, inhibitors, organic phosphate esters, pesticides

2. Catecholamines:
    synthesis: sympathic nerve terminals + adrenal medulla
        Tyr -- dihydroxy-phenylalanin (DOPA) -- decarboxylase -- dopamine
        oxidation -- norepinephrine -- SAM -- epinephrin
    Parkinson's disease
    secretion: to the serum via sympathic terminals innervating smooth muscle
    effect: via receptors (□- and □-adrenergic receptors)
    inactivation: through methylation of phenyl-3-OH, COMT (astroglia)
    MAO - NH_4^+ cleavage in addition to formation of COOH group -
        3,4-dihydroxyphenylglycolaldehyde is produced

3. GABA:
    synthesis: astroglia - neuron cooperation
        glutamate -- decarboxylase -- GABA
    effect: via GABA-receptors (Cl^- channel) - inhibitory neurons
    inactivation: transamination
        GABA -- succinate semialdehyde -- succinate

4. Serotonine
    synthesis:
        Trp --hydroxylation on the 5th carbon atom -- decarboxylase --
            5-hydroxytryptamine (serotonine)
    effect: vasoconstrictor + neurotransmitter of the smooth muscle of the intestine
    inactivation: MAO --- NH_4^+ + 5-hydroxyindolacetate

5. Histamine
synthesis: His -- decarboxylase -- histamine
effect: vasodilatator, stimulates HCl secretion, mast cells also prodice it (allergy)

6. Glutamate
synthesis: astroglia (Glu) neuron interaction
receptors: ionotrop - ion chaannels (NMDA, AMPA)
metabotrop - G- protein - adenylate cyclase
their role (learning processes)
receptor desensitization - phosphorylation

Biochemical characteristics of the connective tissue and the cytoskeleton

Main components:
1. Fibrillar proteins (collagen, elastin, fibrillin)
2. Proteoglycans (core protein, glycosaminoglycans)
3. Adhesive glycoproteins (fibronectin, laminin, tenascin etc.)

Connective tissue, cytoskeleton, extracellular matrix

Main components: 1. Fibrillar proteins (collagen, elastin, fibrillin)
2. Proteoglykans (coreprotein, GAG)
3. Adhesive glycoproteins

1. Fibrillar proteins

Collagen:
synthesis: Preprocollagen - hydroxylation, glycosylation - formation of alfa superhelix (hsp 47) - procollagen -secretion - N- and C-terminal peptidases - tropocollagen - arrangement of fibres - maturation-aging, formation of cross-links (Lys oxidase) aldol condensation, Schiff base
Collagen types:
fibrillar collagens (skin, bone, cartilage, reticular fibre, cell surface)
facit collagens: IX, XII (fibrillar collagen arrangement)
basal membrane collagens: IV, VIII (transparency of cornea)
short collagen
long collagen: VII (epidermolysis bullosa)
collagens with non-collagen functions

Elastin:
synthesis - proelastin - loose structure - beta helix formed from beta sheets
cross-links between 4 chains (1 Lys + 3 Lys aldehydes), desmosin, isodesmosin

Fibrillin:

2 genes: 5. and 15. chromosomes
component of microfibrils at the periphery of the elastic fibres

2. Proteoglycans

Characterisation
classification
GAG types:
- hyaluronic acid
- chondroitin sulfate
- dermatan sulfate
- heparan sulfate + heparin
- keratan sulfate

New classification according to core protein:
1. Great aggregating proteoglycans
2. Small, Leu-rich proteoglycans: fibromodulin, decorin
3. Membrane-bound proteoglycans:
   a/ transmembran PG: syndecan, fibroglycan
   b/ GPI-bound PG: glipican

3. Adhesive glycoproteins

glycoproteins with several different binding domains
1. fibronectin
2. laminin
3. tenascin
4. nidogen/entactin:

4. Adhesive receptors

Integrins (receptors)
their role in the reorganization of the cytoskeleton following activation

5. Cytoskeleton

- Microfilaments
- Intermedier filaments
- Microtubules

Biochemistry of nutrition

1. Makronutrients
   - Proteins, carbohydrates, lipids
   - Daily requirement (quality and quantity)
2. Dietary fibers
   - Types
   - Diet rich in fibers
3. mikronutrients
   - vitamins
   - minerals

Biochemistry of contractile tissues (2 hours)

1. Types of contractile tissues
   Structure and components of the skeletal muscle
   Structure and function of the contractile system, sliding filament theory
   Characteristics of myosin, composition of thick filament
   Components of the thin filament: actin, tropomyosin, troponin complex
   Formation and dissociation of the actin myosin complex, cross-bridge cycling, the role of ATP, ADP+Pi
   Regulatory role of the troponin complex
   Main processes generating energy for muscle contraction

2. Regulation of muscle contraction by calcium
   Extra- and intracellular membrane systems of the muscle fibre, role of volume-surface ratio
   Connection of the T tubule and the junctionaal SR
   Structure and function of the dihydropyridine receptor and the ryanodin receptor
   Mechanism of calcium release
      in heart muscle: calcium induced calcium release
      in skeletal muscle: intramembrane charge movement
   Structure and function of sarcoplasmic reticulum calcium ATPase

Biochemistry of hormones

1. Chemical and biochemical classification of hormones
   Classification of hormones according to action
   Hormones of the hypothalamic- hypophyseal system
   The somatomammotrop family and its biochemical effects
   The glycoprotein family and its biochemical effects

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POMC gene products and biochemical effects
Biochemistry of the neurohypophyseal system

2. Biochemistry of thyroid and parathyroid hormones
Mechanism of thyroid hormone production
Iodine turnover of the organism
Role of plasma proteins in the biological effect of thyroid hormones
Transport of T3, T4 and iodine, metabolic effects
Gene regulatory effects of thyroid hormones
Regulation of extracellular calcium
Action of the parathyroid hormone (biochemical mechanism)
Factors influencing extracellular ionized calcium level

3. Hormonal regulation of blood glucose level
Structure and synthesis of insulin
Effect of insulin on the intermediaate metabolism
Insulin receptor: structure and function; somatomedins
Early metabolic consequences of diabetes mellitus
Pathobiochemical basis for late complications of diabetes mellitus
Pathobiochemical and diagnostic significance of protein glycosylation

4. Biochemistry of steroid hormones
Biosynthetic pathways, enzymes and compartmentalization of steroid hormones
Important metabolits of the different steroid groups
Action of steroid hormones, steroid receptors
Metabolic effects of steroid hormones

Regulation of gene expression

The structure of DNA, the structure of chromosome, euchomatin, heterochromatin, regulation of transcription, enhancer, silencer, the difference between prokaryotic and eukaryotic gene expression.
Histones and their function, nonhistone proteins, regulation of replication of the eukaryotic cell: exit from G0 phase, protoonkogenes, ciklines, tumor suppressor genes.
Repair mechanisms, tumorous cell proliferation, apoptosis, tumor sensitivity of knock out mutants of the p53 gene.
The structure of eukaryotic genes, exon, intron, splicing, coding and noncoding fragments, classification of genomic sequences by repetitive character, IRE and UTR
RNA types, RNA polymerases, the transcription process, maturation of mRNA, the mechanism of splicing, tissue specific and developmental dependent splicing, thalasemias, antisense RNA.
Transcription factors, HLH proteins, Zn-finger proteins, Leu-ziper, intracellular hormone receptors, fos and jun proteins, p53, hox proteins, the connection of

transcription and the chromatin structure.
The mechanism of prokaryotic translation, the initiation complex, the three steps ofelongation; termination, the signal peptide and signal recognition particle

General principles of biochemical regulation

Theoritical differences of control and regulation
Behaviour of open equilibrium systems
Definitions of normal value, normal intervallum
Biochemical adaptation, loading tests
Adaptation on the level of the individual, species, animal kingdom; biochemical evolution
Limits of adaptation, biochemical interpretation of illness, role of drugs in the extension of the limits of biochemical adaptation
Thermodynamic interpretation of death