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Course 2
Metabolism

Translated by Kamila Kočí

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Shaded paragraphs with a violet bar in the margin are additions from the lecture slides. Pictures taken from the slides are labelled "[slides]".

2.1 WHAT FUELS OUR CELLS?

doc. MUDr. Jan Trnka, Ph.D.

Energy

- = the ability of the system to perform work
- types of energy transfer - heat and work
  - Heat - disordered movement of energy from place to place
  - Work - an ordered movement of energy from place to place
- **internal energy of a system - U** = all energy (kinetic and potential) of all particles of the system
  - its value cannot be measured (too complex to calculate) - we can only measure its changes = ΔU

First law of thermodynamics – law of conservation of energy

= the internal energy of an isolated system never changes - it is not possible to produce / consume energy

- energy in an isolated system is neither produced nor disappears, but only transformed from one form to another
- we cannot construct a perpetual mobile (of first type) - a machine that generates energy from nothing
- **ΔU = W + Q**
  - ΔU - change of internal energy of the system
  - W - work received by the system
  - Q - heat received by the system

Enthalpy - H

- **It is an artificial definition created by maths which helps with calculations on internal energy**
- is defined as the sum of the internal energy of the system + the system pressure * the volume of the system
  - **H = U + pV**
- at constant pressure, the enthalpy change is equal to the heat received by the system: **$\Delta H_p = Q$**
  - if the system (for example, a mixture of solutions in a vessel) undergoes chemical reactions in which it releases heat into the environment (= exothermic reactions), Q will be less than 0 and the enthalpy of these reactions will be negative

Entropy - S

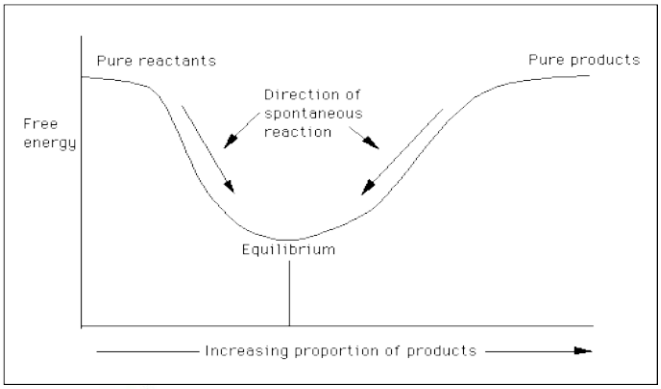
- **The notion of entropy was introduced by the German physicist Rudolf Clausius in the context of thermodynamics in order to explain why some processes are reversible and others are not**
- **ΔS = Q/T**
  - the change in entropy is equal to the heat "given out/donated"divided by the thermodynamic temperature at which the "giving/ donation" occurred

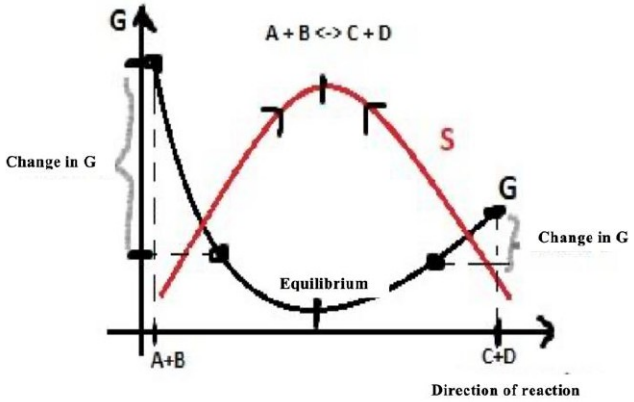
Second law of thermodynamics

- originates from knowledge on entropy
- **determines the natural direction in which natural processes take place**
- in an isolated system, the entropy must remain the same or increase (it can never decrease)
  - for reversible processes, their S = 0
  - in order for the process to run over and over in one direction without power supply (perpetuum mobile of the second type), the entropy of this process would have to be equal to 0
    - however, since T does not drop to 0 (ie absolute zero), each action will increase entropy, and therefore the perpetuum mobile of the second type cannot be assembled
- **virtually all processes taking place in nature can be considered irreversible -** because a part of the energy is always converted into waste heat, which increases entropy
- however, entropy can be decreased locally - e.g cells constantly decrease their entropy but increase entropy of their surroundings

Gibbs energy - G

- another fabricated quantity - this time to simplify the assessment of whether the process can run spontaneously or not
- is defined as enthalpy minus temperature multiplied by entropy:
- $G = H - TS$ => $\Delta G = \Delta H - T * \Delta S$ => $\Delta G = - T * \Delta S_{surrounding} - T * \Delta S_{system}$ => $\Delta G = - T * \Delta(Ss_{urrounding} + \Delta S_{system})$ => **$\Delta G = - T * \Delta S_{universe}$**
- change of Gibbs energy (ΔG) = the heat supplied minus the heat wasted to the surroundings
  - The change in Gibbs energy tells us the maximum amount of work you can get from that reaction
  - The change in Gibbs energy of both direct and feedback reactions has the same absolute value, with only a different sign
  - **Negative change in Gibbs energy = positive change in entropy – spontaneous process**
    - Not always – for example, if an obstacle needs to be overcome





ATP

- ATP drives our cells because the concentration of ATP / ADP is far from equilibrium = the potential for work is great
    - the more the reaction is from its equilibrium, the more work it can do = what determines the ability to do the work is only the distance from the equilibrium of the reaction
- the process of decomposing ATP to ADP is thermodynamically spontaneous

2.2 CHEMICAL REACTIONS IN METABOLISM

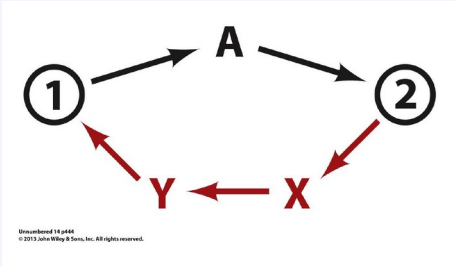
doc. RNDr. Ing. Petr Tůma, Ph.D.

Metabolism of macronutrients

- macronutrients = carbohydrates, lipids, proteins
- carbohydrates and lipids break down to CO_2 and H_2O
- proteins break down into CO_2, H_2O and NH_3
  - NH_3 is toxic to the body (mainly the brain) and is therefore converted to urea and excreted through urine
- all macronutrients have their own catabolic and anabolic pathways and some pathways in common (e.g Krebs cycle)
  - their pathways are linked through carboxylic acids

Metabolism – general principles

- living organisms are **not at equilibrium** – they require a continuous influx of free energy
- metabolism = overall process through which living systems acquire and utilize free energy
  - **exergonic reactions of nutrient oxidation are coupled to endergonic processes** (mechanical work, active transport, biosynthesis of complex molecules)
- **catabolic and anabolic pathways must differ**
  - if metabolite 1 → 2 is exergonic, the conversion 2 → 1 requires free energy, so it must go by a different route
  - separate routes allow **independent control** – the cell can "turn off" 2 → 1 while "turning on" 1 → 2



[slides] Catabolic and anabolic pathways between metabolites 1 and 2 differ

- metabolic pathways are **irreversible** – one highly exergonic reaction (large negative ΔG) makes the whole pathway irreversible
  - most reactions of a pathway are close to equilibrium
  - **committed step** – an irreversible reaction early in the pathway that commits its product to continue down the pathway
- pathways are regulated through key enzymes, usually the one catalysing the committed step
  - **rate-limiting step** – catalysed by the enzyme with the **lowest Vmax**
  - the rate-limiting enzyme and the committed-step enzyme can be the same or different
- in eukaryotic cells, pathways take place in specific cellular locations (compartments)

Main metabolic reactions

Catabolic reactions

- **glycolysis** - degradation of carbohydrates to pyruvate / lactate
- **glycogenolysis** - degradation of glycogen to glucose
- **lipolysis** - degradation of triglycerides to glycerol + fatty acids
- **beta-oxidation** - degradation of fatty acids to acetyl-CoA
- **ketone body breakdown** – in case of starvation, liver produces ketone bodies which are send off to other tissues for degradation
- **degradation of proteins and amino acids**

Anabolic reactions

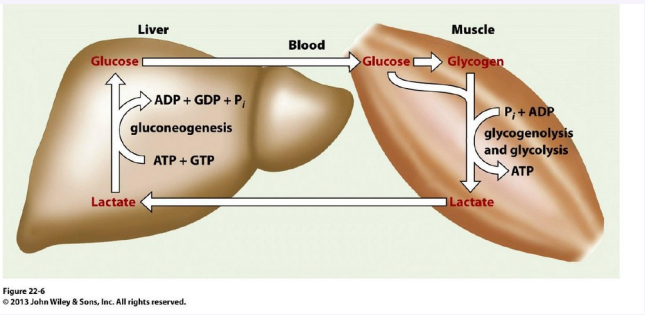
- **gluconeogenesis** – glucose synthesis
- **glycogenesis** – glycogen synthesis
- **Fatty acid synthesis**
- **lipogenesis** – synthesis of TAG from fatty acids and glycerol
- **ketogenesis** – production of ketone bodies in liver
- **proteosynthesis** – protein synthesis
- **ornithine cycle** – urea formation

Amphibolic reactions (some anabolic and some catabolic)

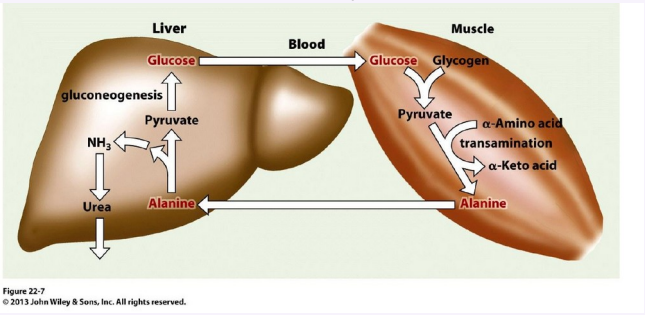
- **pyruvate dehydrogenase reaction** – converts pyruvate to Acetyl-CoA
- **Krebs or Citrate cycle** - consumes Acetyl-CoA to produce CO_2, NADH and $FADH_2$
  - **note**. NADH should be correctly written as NADH + H^+
- **electron transport chain** - consumes NADH and $FADH_2$ to produce ATP and H_2O

Organ specialization

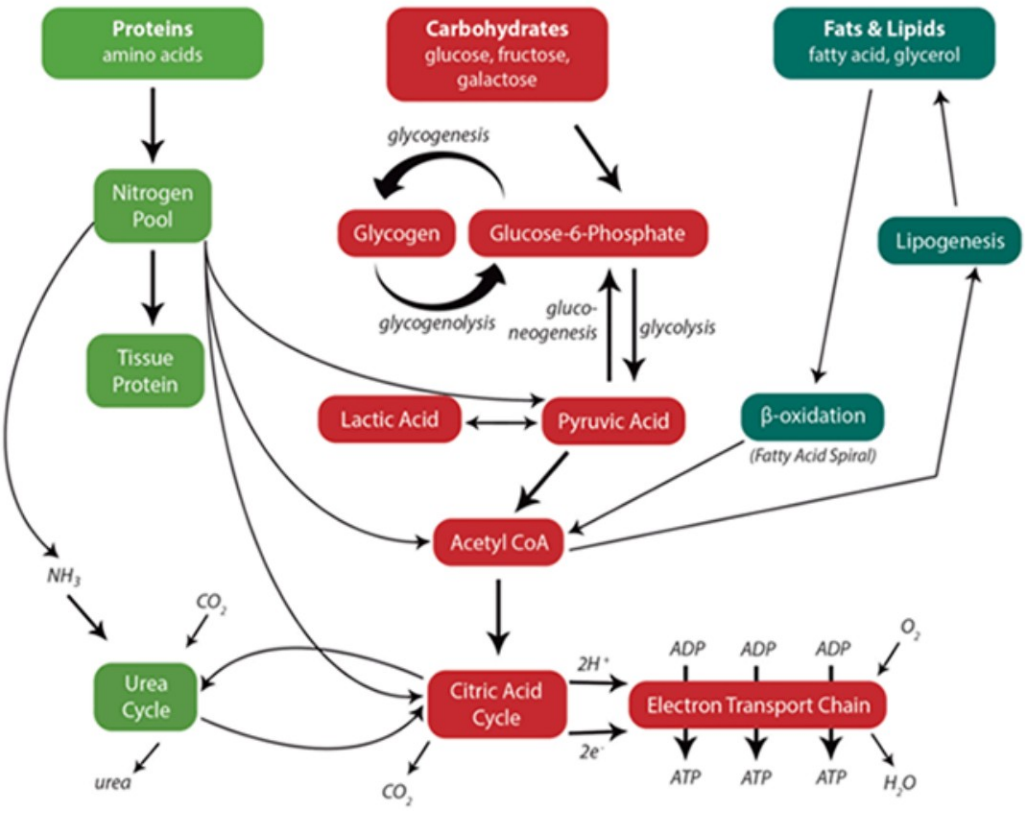
- the major pathways for glucose, fatty acids and amino acids centre on **pyruvate and acetyl-CoA**
- the Krebs cycle is the central pivot of intermediary metabolism – **amphibolic function**
- only a few tissues (e.g. the liver) can carry out all reactions; in a given cell only a part of the reactions runs at a significant rate
- primary fuels
  - **brain** – glucose; in starvation also ketone bodies
  - **muscle** – glucose (from glycogen), fatty acids, ketone bodies; it can produce ATP both anaerobically and aerobically
  - **heart** – fatty acids, ketone bodies, glucose, pyruvate and lactate
  - **adipose tissue** stores TAG and releases fatty acids as needed
  - **liver** makes all types of fuel available to other tissues
- some processes need cooperation between organs – e.g. the **Cori cycle** and the **glucose–alanine cycle**
  - Cori cycle – lactate from muscle → liver (gluconeogenesis) → glucose back to muscle
  - glucose–alanine cycle – muscle pyruvate is transaminated to alanine → liver: alanine → pyruvate → glucose; the NH_3 goes to urea



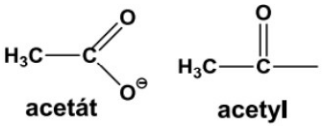
[slides] Cori cycle

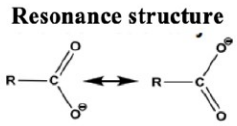


[slides] Glucose–alanine cycle



Carboxylic acids



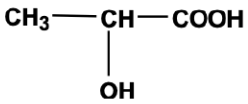


- have a dominant position in the whole metabolism
- **dissociate in the body** (due to pH) –**hydrogen is cleaved from -COOH group and anions are formed in a resonant structure, where two oxygen atoms exchange one electron**
  - e.g. acetic acid
    - under normal conditions - CH_3COOH = acetic acid
    - in the body - CH_3COO^- = acetate
    - the carboxylic acid anion names are derived from the Latin names of their respective acids
    - acetic acid in latin is acidum aceticum -> its anion is called acetate
- acyls are still very common in metabolism; these are carboxylic acids that lose OH

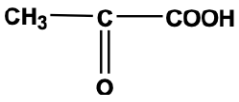
Systematický název	Triviální název	Latinský název	Aniont	Acyl
kys. metanová	kys. mravenčí	acidum formicum	mravenčan / formiát	formyl
kys. etanová	kys. octová	acidum aceticum	octan / acetát	acetyl
kys. propanová	kys. propionová	acidum propionicum	propionan / propionát	propionyl
kys. butanová	kys. máselná	acidum butyricum	máselnan / butyrát	butyryl
kys. etandiová	kys. šťavelová	acidum oxalicum	šťavelnan / oxalát	oxalyl
kys. propandiová	kys. malonová	acidum malonicum	malonan / malonát	malonyl
kys. butandiová	kys. jantarová	acidum succinicum	jantaran / sukcinát	sukcinyl
kys. pentandiová	kys. glutarová	acidum glutaricum	glutaran / glutarát	glutaryl

Hydroxy acids x keto acids

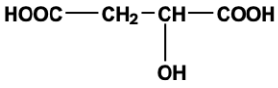
- in the body, hydroxy acids are constantly converted to keto acids and keto acids to hydroxy acids
- a classic example is lactate (hydroxyacid) and pyruvate (keto acid)
- **conversion of hydroxy acid to keto acid is oxidation –**hydrogen is removed
  - NAD^+ is needed to take both hydrogens and becomes a NADH + H^+
- **Conversion of keto acid to hydroxy acid is reduction –** hydrogen is added
  - NADH + H^+ is needed, which donates both of its hydrogens and becomes a NAD^+



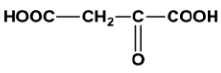
2-hydroxypropanoic acid = lactic acid = lactate



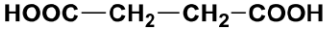
2-ketopropanoic acid = pyruvic acid = pyruvate x enolpyruvate



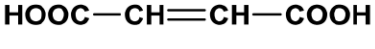
Malic acid (hydroxybutanedioic acid) = malate



Oxaloacetic acid (oxobutanedioic acid) = oxalacetate



succinic acid (butanedioic acid) = succinate

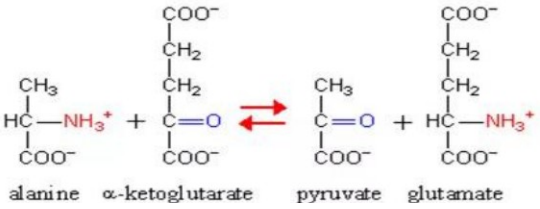


Fumaric acid (butenedioic acid) = fumarate

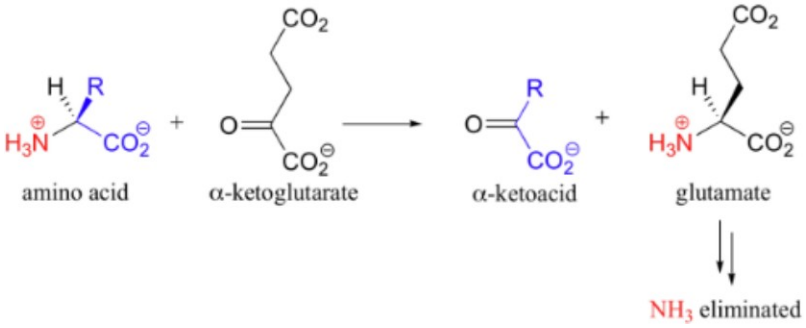
Detoxification of ammonia

- **transamination -> hydrolytic deamination / oxidative deamination -> urea synthesis**
- everything takes place in the liver, transamination can take place elsewhere, urea synthesis also takes place in the kidneys

- amino acids are degraded to CO_2, H_2O and NH_3
- however, ammonia is toxic to the body because it messes up the membrane potential and causes swelling
  - diseased liver loses (among other things) the function of detoxifying ammonia, causing brain swelling = liver encephalopathy
- literally the only difference between an amino acid and a keto acid is that one has NH_3 and the other has oxygen
- **transamination** - amino acid and keto acid exchange NH_3 and oxygen
  - $AMK_1 + KK_2 \rightarrow KK_1 + AMK_2$
  - Coenzyme of transamination is pyridoxal phosphate
  - alanine + α-ketoglutarate -> pyruvate + glutamate
  - aspartate + α-ketoglutarate -> oxaloacetate + glutamate
    - ALT (alanine transaminase) and AST (aspartate transaminase) enzymes are responsible for these two transaminations)
    - ALT and AST are also markers of liver function - their increased plasma concentration means that liver cells are breaking down

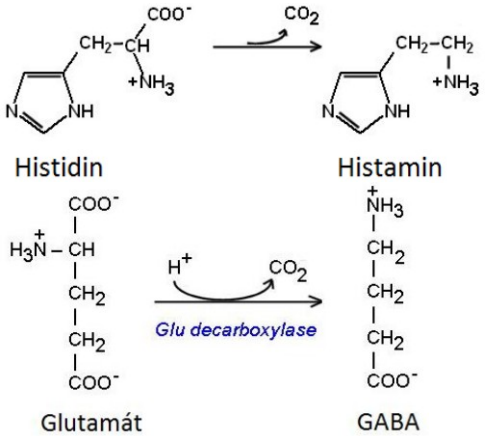


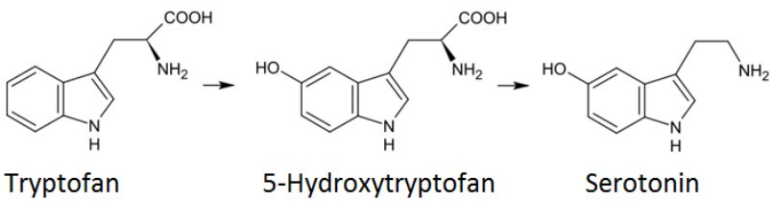
- **hydrolytic deamination** - glutamine -> glutamate + NH_3
- **oxidative deamination** - glutamate -> α-ketoglutarate + NH_3
- **urea synthesis (ornithine cycle)** - CO_2 + 2x NH_3 -> urea
- in the slides, 2-oxoglutarate = α-ketoglutarate



Decarboxylation

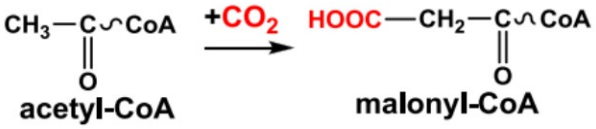
- removal of the carboxyl group in the form of CO_2
- amino acids become **biogenic amines**
  - **histidine -> histamine**, hormone and neurotransmitter
  - **glutamate -> GABA** (gamma-Aminobutyric Acid), inhibitory neurotransmitter
  - **tryptophan -> 5-hydroxytryptofan -> serotonin**, one of the hormones of happiness





Carboxylation

- opposite of decarboxylation - CO_2 is added
- for example, in the synthesis of fatty acids



Hydrogen as a source of energy

- very big respiratory chain simplification - $2H_2 + O_2 \rightarrow 2H_2O$ + energy (in the forms of ATP)

Dehydrogenation

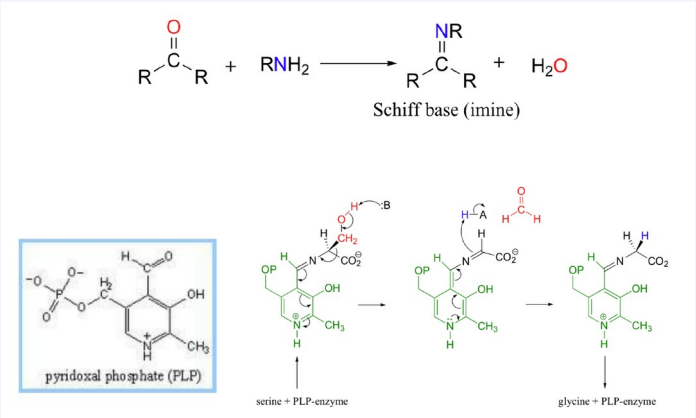
- **oxidation of single bond to double bond**
  - $-CH_2-CH_2- \rightarrow -CH=CH- + 2H^+ + 2e^-$
  - very frequent reaction in metabolism - for example in β-oxidation of fatty acids, Krebs cycle and synthesis of unsaturated fatty acids
- **dehydrogenation of 2-keto acids – it proceeds simultaneously**
  - R-CO-COOH + HCoA -> R-CO-CoA + CO_2 + $2H^+$ + $2e^-$
  - examples of the oxidation of a single bond to a double bond: **succinate → fumarate**, desaturation (synthesis of unsaturated FA)
  - examples of 2-keto acid dehydrogenation: **pyruvate → acetyl-CoA** + CO_2 + $2H^+$ + $2e^-$; **2-ketoglutarate → succinyl-CoA** + CO_2 + $2H^+$ + $2e^-$
- **dehydrogenation of alcohols**
  - **primary alcohols**
    - alcohol -> aldehyde -> carboxylic acids
      - methanol -> formaldehyde -> formic acid
      - ethanol -> acetaldehyde -> acetic acid
    - methanol itself is completely harmless, only after its metabolism to formic acid it becomes extremely toxic
      - 10 ml is enough to completely destroy the optic nerve (and cause permanent blindness), 15 ml is deadly
    - all alcohols are metabolized by one enzyme **(alcohol dehydrogenase)**, therefore ethanol is given as a treatment for methanol poisoning - the enzyme will have more work and metabolize methanol slowler, giving the liver and kidneys time to remove formic acid from the blood
  - **secondary alcohols**
    - alcohol -> ketone
  - **tertiary alcohols** – dehydrogenation does not occur
  - the slides also mention poisoning by **ethylene glycol** (also metabolized by alcohol dehydrogenase)

Reactions of monosaccharides

- esterification
  - glucose could theoretically leave at the moment of entry into the cell, therefore in all cells immediately after entry it is esterified with phosphate to **glucose-6-phosphate**, which no longer crosses the membrane
  - Only hepatocytes have an enzyme that can separate the phosphate again
- oxidation
  - oxidation of glucose on 6th carbon -> **glucuronic acid**
  - oxidation of glucose on 1st carbon -> **gluconic acid**
- reduction
  - glucose reduction produces an alcohol → **glucitol**
- epimerization
  - **fructose**, **mannose** and **galactose** can be converted to one another though glucose
- other reactions shown on the slides: **mutarotation** (α ⇌ β anomer), formation of **gluconolactone**, **glycoside formation** (e.g. α-methylglucoside)

Other reactions to remember

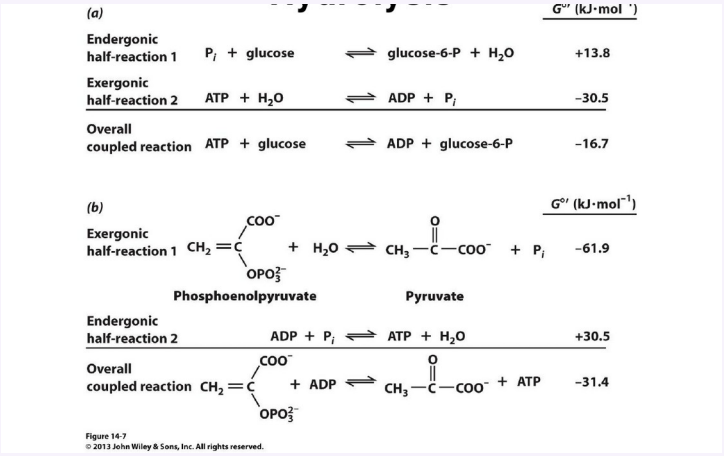
- **esterification** – carboxylic acid + alcohol → ester + H_2O
- **Schiff base (imine) formation** – carbonyl group + primary amine (R-NH_2) → imine (C=N) + H_2O
  - e.g. **pyridoxal phosphate (PLP)** binds amino acids through a Schiff base
- **aldol condensation** – carbonyl compound (enol/enolate form) + aldehyde → aldol → α,β-unsaturated carbonyl compound (–H_2O)
- **hydrolysis** – saccharides by **glycosidases**, proteins by **peptidases**, lipids by **lipases**



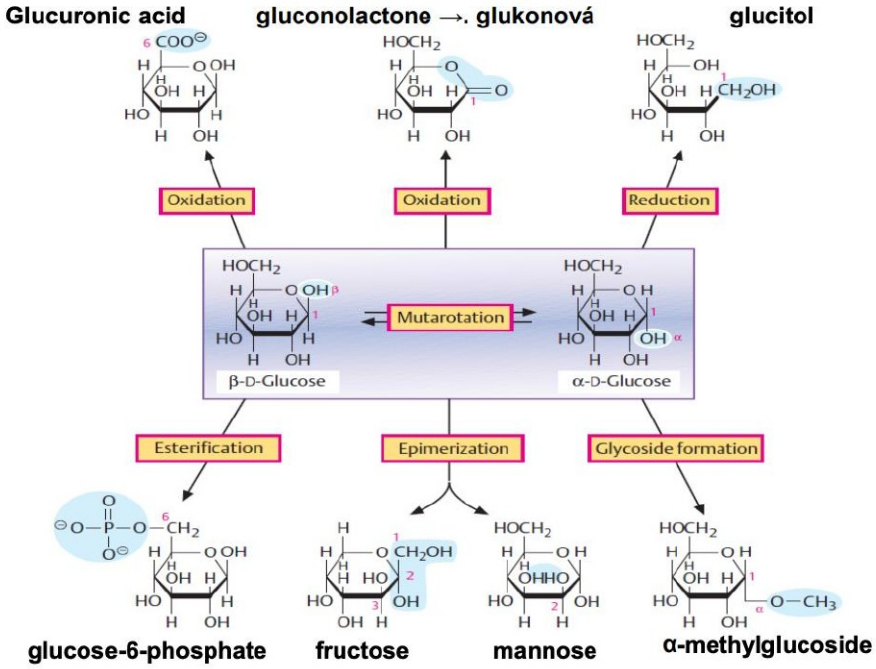
[slides] Imine (Schiff base) formation; PLP-enzyme with an amino acid

High-energy compounds

- organisms capture the free energy released by nutrient degradation as **"high-energy" compounds** such as ATP
- the "high energy" of ATP = the large negative ΔG of hydrolysis of its **phosphoanhydride bonds**
- **ATP hydrolysis can be coupled to an endergonic reaction so that the overall reaction is favourable**
  - e.g. P_i + glucose → glucose-6-P (+13.8 kJ/mol) coupled with ATP → ADP + P_i (−30.5 kJ/mol) = overall −16.7 kJ/mol
  - phosphoenolpyruvate has an even higher phosphoryl transfer potential (−61.9 kJ/mol) – it can make ATP from ADP

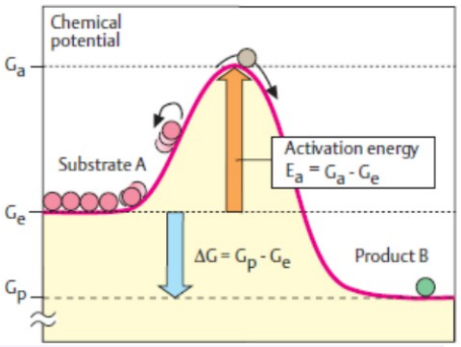


[slides] Endergonic reactions coupled to ATP hydrolysis



2.3 ENZYMES

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2.3.1 Thermodynamics and kinetics

- If a reaction can occur or not is determined by Gibbs energy
- Only reactions with negative Gibbs energy can take place
  - At the same time, however, they need enough energy to surpass the activation barrier – this is where enzymes help out

- thermodynamics tells us **whether** a process can occur spontaneously, but says little about its individual steps (mechanism)
- ΔG = the "driving force" of a reaction – it is not constant; it depends on the nature and concentration of reactants and on conditions (e.g. temperature)
- equilibrium: **$\Delta G^{o'} = -RT \ln K_{eq}$**
  - ΔG < 0 → products predominate; ΔG > 0 → reactants predominate
- most reactions of organic substances are very slow regardless of ΔG – molecules must first reach a minimum energy (G_a); $G_a - G_e$ = **activation energy**
- **kinetics** = the study of reaction rates and how they change with conditions (related to the reaction mechanism)
  - **enzyme kinetics** – the rate of an enzyme-catalysed reaction depends mainly on the properties of the catalyst

Speed of chemical reaction

- Change in the mass of reactant or products per unit of time
- example - reaction $3H_2 + N_2$ -> $2NH_3$
  - the formula is quite intuitive- the rate of chemical reaction is measured as the amount converted by the reaction over time
    - **v = concentration of product / time**
  - also works the other way around – the rate of reactants lost over time
    - **v = - concentration of reactants / time**
  - **however, we must be careful about the stoichiometric coefficients** – for H the formula needs to be adjusted by multiplying it by 1/3, for the NH_3 by multiplying by 1/2

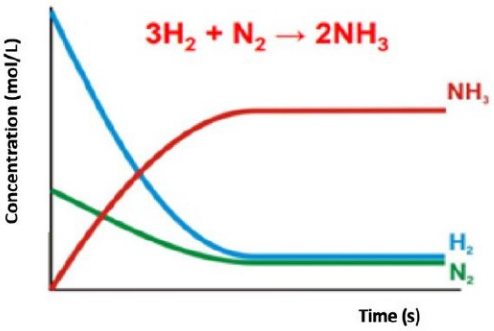
$$v = \frac{\Delta[\text{produkt}]}{\Delta t}$$

$$v = -\frac{\Delta[\text{reaktant}]}{\Delta t}$$

$$v = -\frac{1}{3}\cdot\frac{\Delta[H_2]}{\Delta t}$$

$$v = -\frac{\Delta[N_2]}{\Delta t}$$

$$v = +\frac{1}{2}\cdot\frac{\Delta[NH_3]}{\Delta t}$$

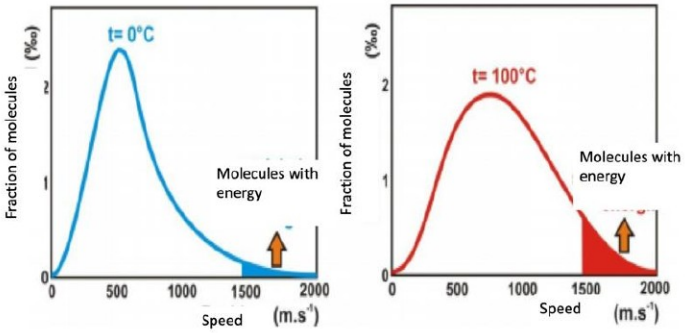


Dependence of reaction rate on concentration

- A + B -> products
- Particles in the system fly at great speed – by this movement the particles collide together; in order for a reaction to occur, particles A and B ,must collide, creating new bonds between the atoms and the old ones disappearing → product is formed
- **Collision theory** – the rate of reaction is proportional to the number of active collisions between molecules A and B per second
- **Kinetic equation v = k * [A] * [B]** // k is coefficient, [A] is concentration A, [B] is concentration B
  - Applies for reactions of I. and II. order
  - Reaction of I. order are monomolecular reactions – one molecule spontaneously disintegrates into 2 molecules
    - v = k * [A]
    - N_2O_4 -> $NO_2 + NO_2$
    - v = k * [N_2O_4]
  - reaction of II. order are bimolecular – two molecules react
    - v = k * [A] * [B]
    - a collision of a maximum 2 particles is assumed, a more complex reaction takes place via intermediates
    - example reaction - $4HBr + O_2$ -> $2H_2O + 2Br_2$
      - does not proceed directly but through intermediates
        a) $HBr + O_2$ -> HOOBr - slow
        b) HBr + HOOBr -> 2 HOBr
        c) HBr + HOBr -> $H_2O + Br_2$
      - $v = k \cdot [HBr] \cdot [O_2]$
    - the speed of the whole system is controlled by the speed of the slowest intermediate step

Activation energy and temperature

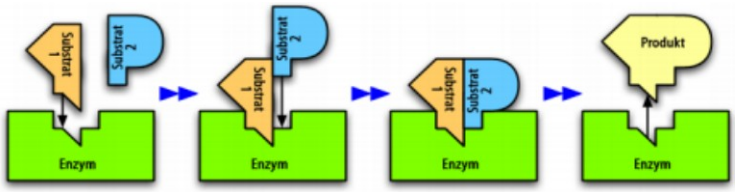
- **v = k * [A] * [B]**
  - k = proportionality constant = $k^{(-EA/RT)}$ – the so-called Boltzmann factor
    - expresses the fraction of molecules in the system with energy higher than the activation energy (EA)
- Boltzmann factor calculates which molecules have enough temperature to overcome the activation energy
- Effect of temperature – increasing the temperature by 10 °C increases the reaction by 2-3 x



  - (not always – enzyme reactions show more complex temperature dependence: higher temperature can cause conformational changes and denaturation of the enzyme)
- the rate constant k does not depend on the concentrations of the reactants, only on temperature
- **Arrhenius equation: k = A · e^{−Ea/RT}** (A = pre-exponential factor, RT = average kinetic energy)
  - high temperature and low activation energy → larger k → faster reaction; the effect is large because the terms are in the exponent

Principle of catalysis

- In the human body we cannot heat substances too much – we need another way to increase the rate of chemical reaction
- **catalysis = reduction of activation energy**
  - enzymes are catalytic proteins – they reduce activation energy without changing the ratio of reactants and products
    - captures reactant molecules on its surface, stabilizes them and allows them to orient themselves appropriately
- the catalyst may be a metal or an enzyme



- catalyst = increases the reaction rate **without being consumed** in the process
- example – 2 H_2O_2 → 2 H_2O + O_2: E_A without catalyst 75.4 kJ/mol, with Pt 48.6 kJ/mol, with **catalase 23.0 kJ/mol**

2.3.2 Enzymes

- high efficiency catalysts which increase the rate of chemical reactions by several orders of magnitude
- they are proteins, so they are easily regulated
- e.g. acetylcholinesterase- protein capable of cleaving 25 000 molecules of acetylcholine in one second
- allows reactions to proceed at low temperatures, neutral pH and atmospheric pressure

- enzymes differ from ordinary catalysts in **reaction rate, reaction conditions, reaction specificity and control**
- the rate enhancement of enzymes can be up to ~10^{17}-fold (e.g. orotidine/AMP nucleosidase, staphylococcal nuclease)
- mechanisms of enzyme catalysis: 1. proper orientation of the substrate, 2. removal of hydration shells, 3. **stabilization of the transition state**, 4. group transfer
- **turnover number (k_{cat})** – number of substrate molecules converted by one enzyme molecule per second (acetylcholinesterase ≈ 25 000 s^{-1})

History and nomenclature of enzymes

- 1810 – Gay-Lussac: yeast converts saccharides to ethanol and CO_2 (fermentation); Greek "en" = in, "zyme" = yeast
- historical names (pepsin, ptyalin); systematic: substrate + **-ase** (urease, saccharase)
- EC number X.X.X.X = class, subclass, sub-subclass, serial number
  - e.g. **1.1.1.27 lactate dehydrogenase**: 1 = oxidoreductase, 1.1 = e^- donor –CH-OH, 1.1.1 = e^- acceptor NAD^+; lactate + NAD^+ → pyruvate + NADH

Enzyme classes

- 1. **oxidoreductases** – oxidation-reduction reactions
- 2. **transferases** – transfer of functional groups
- 3. **hydrolases** – hydrolysis reactions
- 4. **lyases** – group elimination to form double bonds
- 5. **isomerases** – isomerization
- 6. **ligases** – bond formation coupled with ATP hydrolysis
- 7. **translocases (new class EC 7, since 2018)** – catalyse movement of ions or molecules across membranes (side 1 → side

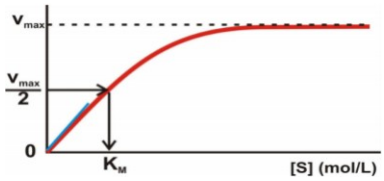
2), e.g. H^+, inorganic ions, amino acids, peptides

Chemical composition of enzymes

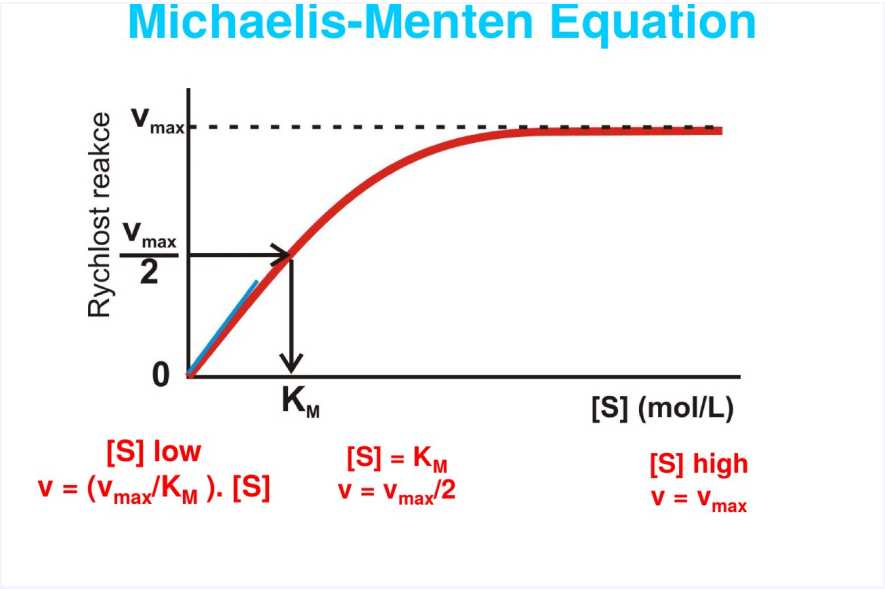
- **holoenzyme = apoenzyme + cofactor**; some catalysts are RNA – **ribozymes**
- cofactors
  - **metal ions** – Zn^{2+} (ADH, carbonic anhydrase), Mn^{2+} (arginase), Mg^{2+}, Fe^{2+} (phenylalanine hydroxylase), Cu^{2+}
  - **coenzymes (cosubstrates)** – NAD, ubiquinone, ATP, coenzyme A
  - **prosthetic groups** (tightly bound) – heme, FAD, lipoamide
- enzymes have an **active site** on their surface where substrates can bind
  - shape of the active site corresponds to the shape of the substrate
  - enzyme stabilizes the substrate – a product can be formed – reaction takes place and the product is released from the active site
- **isoenzymes** - enzymes having the same function but different structure (and hence physical and chemical properties)
  - if two different isoenzymes catalyse the dame reaction, same product is formed

Rate of enzyme catalysed reaction

- determined using the **Michaelis-Menten** equation
- K_M is **Michaelis constant** - expresses the concentration of the substrate at which the reaction will proceed at half maximum speed; that is, how much substrate is needed to feed 50% of the enzymes
  - determines the affinity of the substrate to the enzyme - the higher the constant, the lower the affinity
  - units - mol/l (because it expresses concentration)
- it is inversely proportional -> the Michaelis-Menten equation is hyperbolic



  - **low substrate concentration - [S] < K_M**
    - at low substrate concentration, the velocity is almost proportional to the substrate concentration
    - **$v = v_{max} * [S] / K_M$**
  - **substrate concentration equal K_M - [S] = K_M**
    - follows from the definition of Michaelis constant that in this case **$v = v_{max} / 2$**
  - **very high substrate concentration - [S] >>> K_M**
    - in this case, the enzyme is completely saturated with the substrate and everything is controlled only by the ability of the enzyme
    - **$v = v_{max}$**

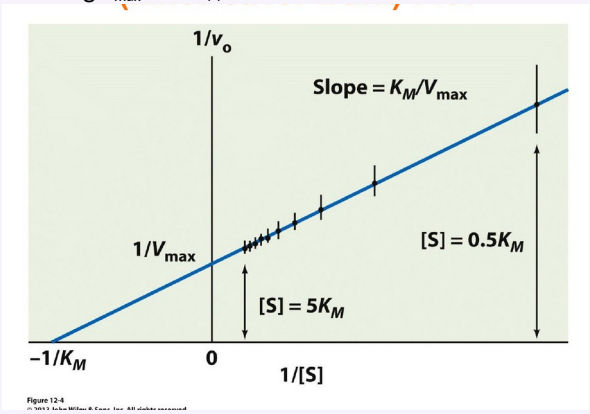


[slides] Michaelis-Menten curve at low [S], [S] = KM and high [S]

- the model behind the equation: **S + E ⇌ ES → P + E**
- v_{max = $k_{cat} \cdot [E]_T$} → $v = k_{cat}[E]_T[S] / (K_M + [S])$
- low K_M = **high affinity** for the substrate

Lineweaver-Burk (double-reciprocal) plot

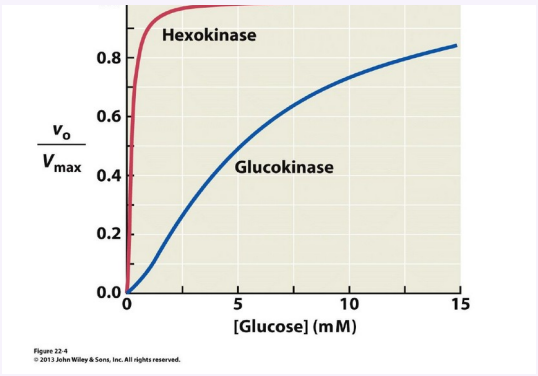
- $1/v = (K_M/v_{max}) \cdot 1/[S] + 1/v_{max}$ – a straight line
  - y-intercept = $1/v_{max}$; x-intercept = $-1/K_M$; slope = K_M/v_{max}
  - the best method for evaluating v_{max} and K_M from kinetic data



[slides] Lineweaver-Burk plot

Hexokinase vs. glucokinase

- **hexokinase** – low K_M (high affinity) → saturated already at low glucose; **glucokinase** (liver) – high K_M → works mainly at high glucose concentrations



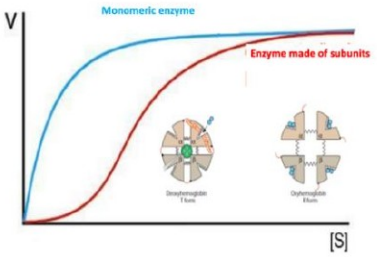
[slides] Hexokinase vs. glucokinase

Enzyme activity

- qualitative evaluation of enzyme - amount of substrate converted by enzyme per unit of time
- unit catal [cat] = $mol.s^{-1}$ - concerts 1 mole of substrate per second
- unit [U] = $micromol.min^{-1}$ - concerts 1 micromole per minute
- 1 U = 16.67 nkat
- enzyme activity is dependent on pH, temperature, concentration of activator and inhibitors in the reaction mixture …

Allosteric enzymes

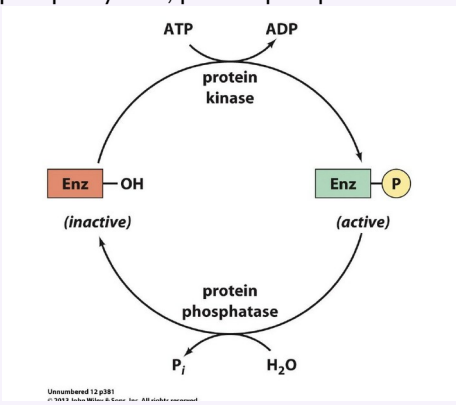
- besides the active site, they have another site- **allosteric (from Greek, allos = other)**, where modulators (activators or inhibitors) attach, which influences the affinity of the enzyme to its substrate
- most allosteric enzymes tend to be oligomeric (composed of multiple subunits)
- **homotrophic regulation of allosteric enzymes** substrate is also a modulator
  - eg. haemoglobin - has 4 sites for O_2, sites as soon as one oxygen connects, others connect more easily
  - the reaction rate curve is sigmoidal - nothing, nothing, nothing and suddenly shoots up to the maximum
  - function as regulatory enzymes of metabolic pathways
- **heterotrophic regulation of allosteric enzymes** - modulator and substrate are different molecules
  - in the case of haemoglobin, the heterotrophic regulator would be CO_2



- activity of allosteric enzymes is modulated by **non-covalent** binding of an activator or inhibitor
- enzymes made of subunits (e.g. haemoglobin) show a **cooperative effect**

Control of enzyme activity

- **allosteric control** – binding at a site other than the active site → conformational change → altered catalytic activity
- **covalent modification – phosphorylation / dephosphorylation** (e.g. **glycogen phosphorylase**) shifts the equilibrium between more and less active conformations
  - protein kinase (ATP → ADP) phosphorylates; protein phosphatase removes P_i



[slides] Control by phosphorylation

Specificity of enzymes

- enzymes are specific both by their substrate (sugar, alcohol…) and by their action (deamination, oxidation…)

- **substrate specificity**
  - **absolute** – one substrate only, e.g. **urease** (urea + H_2O → CO_2 + 2 NH_3; not methylurea)
  - **relative (group)** – several similar substrates, often with the same functional group, with different affinity (K_M): hexokinase (glucose/fructose), ADH (ethanol/methanol)
- **reaction specificity** – type of reaction, e.g. amino acids: deamination, decarboxylation, transamination; depends on the coenzyme and apoenzyme
- **stereospecificity** – D-monosaccharides, L-amino acids; e.g. **aconitase**: citrate → isocitrate gives only one stereoisomer

2.4 COENZYMES, VITAMINS, TRACE ELEMENTS

doc. RNDr. Ing. Petr Tůma, Ph.D.

2.4.1 Enzymes and coenzymes

- proteins with catalytic activity (accelerate reactions)
  - catalytically active RNA is not an enzyme but a ribozyme
- **the name of the enzyme is usually its substrate + reaction type which is catalyses + ase**
  - **e.g. enzyme catalysing dehydrogenation of lactate is called lactate dehydrogenase**
- enzymes have several specificities
  - **binding** – what substrate the enzyme works with
    - absolute - the enzyme processes only one molecule (eg urease)
    - relative - the enzyme processes one group of molecules (eg Hexokinase - phosphorylates all 6C sugars)
  - **effect** - what type of reaction does the enzyme catalyse
    - dehydrogenation / oxidation / decarboxylation / transamination…
  - **stereospecificity** - enzymes can only process one chiral type
    - monosaccharides must be D, amino acids L

- **simple enzymes** (protein only) – e.g. peptidases
  - **trypsin** (endopeptidase) – cleaves peptide bonds next to **Arg, Lys**
  - **chymotrypsin** (endopeptidase) – cleaves peptide bonds next to **Tyr, Trp, Leu**

- enzymes can be pure protein (so-called simple enzymes) or composed of protein and non-protein parts (complex enzymes)
  - protein part of a complex enzyme is apoenzyme, non-protein part is cofactor
  - cofactors are
    - organic – e.g. haeme
    - inorganic - metal ion - Zn, Cu, Fe, Mn
      - stabilize the active centre, aid redox reactions and polarize bonds
  - division of cofactors
    - **prosthetic groups** - firmly bound to the enzyme, part of the stable structures
    - **coenzymes** - only weakly bound to the enzyme, can completely detach

- why cofactors? – the functional groups of proteins can take part in acid-base reactions and charge-charge interactions, but are **poorly suited to redox reactions and many group-transfer reactions** – these need small cofactors
- some coenzymes are only transiently associated with the enzyme (e.g. NAD^+, $NADP^+$) = **cosubstrates**; prosthetic groups are permanently (often covalently) bound, e.g. heme in cytochromes
- **apoenzyme (inactive) + cofactor ⇌ holoenzyme (active)**
- metal ions: stabilize the native conformation of the active site, take part in redox reactions, polarize bonds in the substrate (e.g. Zn^{2+} in carbonic anhydrase)

Coenzyme	Reaction Mediated	Section Discussed
Biotin	Carboxylation	23-1A
Cobalamin (B_{12}) coenzymes	Alkylation	25-2E
Coenzyme A	Acyl transfer	21-2A
Flavin coenzymes	Oxidation–reduction	16-2C
Lipoic acid	Acyl transfer	21-2A
Nicotinamide coenzymes	Oxidation–reduction	13-2A
Pyridoxal phosphate	Amino group transfer	26-1A
Tetrahydrofolate	One-carbon group transfer	26-4D
Thiamine pyrophosphate	Aldehyde transfer	17-3B

Table 13-1

[slides] The common coenzymes and the reactions they mediate

Inorganic elements	Enzymes
Fe^{2+} or Fe^{3+}	Cytochrome oxidase Catalase Peroxidase
Cu^{2+}	Cytochrome oxidase
Zn^{2+}	DNA polymerase Carbonic anhydrase Alcohol dehydrogenase
Mg^{2+}	Hexokinase Pruvate kinase
Mn^{2+}	Arginase
K^+	Pyruvate kinase
Ni^{2+}	Urease
Mo	Nitrate reductase
Se	Glutathione peroxidase

Enzyme classes

- divided into 6 basic classes, depending on the type of catalysed reaction
- since 2018 there is a 7th class – **translocases (EC 7)** (see 2.3)

Oxidoreductases - $A_{ox} + B_{red} \rightarrow A_{red} + B_{ox}$

- catalyse intermolecular oxidation-reduction reactions; transfer of hydrogen, electrons, or reaction with oxygen
- types of enzymes - dehydrogenases, oxidases, peroxidases, oxygenases
- eg alcohol dehydrogenase, lactate dehydrogenase (lactate <-> pyruvate), phenylalanine hydroxylase (phenylalanine -> tyrosine)
- example reactions: pyruvate + $NADH/H^+ \rightarrow$ lactate + NAD^+ (dehydrogenase); phenylalanine + O_2 + $NADH/H^+ \rightarrow$ tyrosine + H_2O + NAD^+ (**monooxygenase**)
- coenzymes of oxidoreductases are
  - **NAD (nicotinamidadenine dinucleotide) and NADP ((nicotinamidadenine dinucleotide phosphate)**
    - participate in the transfer of hydrogen and electrons
    - $NADP^+$, NAD^+ - oxidized forms
    - $NADPH + H^+$, $NADH + H^+$ - reduced form
    - derived from nicotinamide
  - **FAD a $FADH_2$ - flavinadenine dinucleotide**
    - also participates in hydrogen and electron transfer, but in different reactions than NAD (usually in the formation/destruction of double bonds)
    - FAD - oxidized forms
    - $FADF_2$ – reduced form
    - derived from riboflavin (vitamin B2)
    - **FMN** (flavin mononucleotide) and FAD are **prosthetic groups** of dehydrogenases, oxidases and monooxygenases
  - **coenzyme Q - ubiquinone/ubiquinol**
    - part of the respiratory chain
    - prosthetic group – haem
    - **cytochromes** – heme coenzymes, transfer electrons by $Fe^{2+} \rightleftharpoons Fe^{3+}$

Transferases - A-x + B -> A + B-x

- transfer groups (-CH 3, -NH 2, phosphate) from the donor to the acceptor
- types of enzymes - C-transferases, glycosyltransferases, aminotransferases, phosphotransferases (or kinases)
- the coenzymes of transferases are
  - **ATP (adenosine triphosphate), GTP (guanosine triphosphate)**
    - carry phosphate groups
    - **kinases (phosphotransferases)** – glucose + ATP → glucose-6-P + ADP
    - **nucleotidyl transferases** – activation of metabolites by nucleoside phosphates: glucose-1-P + **UTP** → UDP-glucose + PP_i (glycogen synthesis); choline-P + **CTP** → CDP-choline (phospholipid synthesis)
  - **CoA - Coenzyme A**
    - transmits acyls
    - pantothenate (= pantoinate + β-alanine, the vitamin) + cysteamine
  - **TDP - thiamine diphosphate (also TPP - thiaminpyrophosphate)**
    - carries carbon groups
    - transfers **hydroxyalkyl** residues; part of **pyruvate dehydrogenase**
    - precursor - thiamine (vitamin B1)
  - **PALP - pyridoxal phosphate**
    - carries the NH_2 group
    - precursor - pyridoxine (vitamin B6)
  - **THF - tetrahydrofolate**
    - carries single-carbon residues; precursor: folic acid
    - transfers **formyl, methylene and methyl** groups; needed for the **synthesis of purines and pyrimidines** (target of sulfonamides)
  - **SAM – S-adenosylmethionine**
    - methylation reactions – norepinephrine → epinephrine, creatine biosynthesis, DNA methylation; after giving its methyl → homocysteine

Hydrolases - $A\text{-}B + H_2O \rightarrow A\text{-}H + B\text{-}OH$

- catalyse hydrolytic cleavage of the substrate = cleaves bonds with the help of water
- we divide them into groups

- Proteases - cleaves peptide bonds in protein and peptide molecules
- glucosidases - cleaves glycosidic bonds
- lipases - cleaves ester bonds in lipids
- phosphatases - remove the phosphate group
- amylases - cleaves bonds between glucose molecules in polysaccharides

- hey are mostly simple enzymes, or they have a metal ion - anyways, no coenzymes

Lyases - A-B -> A + B

- enzymes catalysing bond decomposition in a different way than hydrolysis or oxidation
- double bonds or cyclic compounds are often formed
- can cleave (or introduce) small molecules, e.g. H_2O, CO_2 or NH_3
- coenzymes similar to transferases

Isomerases - A -> A‘

- catalyse reactions within a molecule of one substrate, move atoms (groups) from one carbon to another
- e.g cis-trans-isomerase, ribose phosphate-isomerase, epimerase (changes the orientation of OH groups)
- most often they do not contain coenzymes

Ligases - A-b + C -> A-C + b

- they catalyse the synthesis of simple molecules to complex molecules
- often energy-intensive bonds with simultaneous energy consumption (mostly ATP -> ADP + Pi)
- often contain coenzymes of transferases
  - biotin (vitamin H) - carboxylation
    - biotin = transfer of CO_2 (**carboxylases**)
- ligase example: glutamate + NH_3 + ATP → **glutamine** + ADP + P_i

2.4.2 Trace elements

Element	Body		Blood	Plasma	RBC	Remarks
	mg	µg/g		mg total		
Iron	4200	60	2500	3.6	2400	70.5% in hemoglobin
Fluorine	2600	37	0.95	0.87	0.17	98.9% in bone
Zinc	2300	33	34	5.6	2.8	65.2% in muscle
Strontium	320	4.6	0.18	0.17	0.008	99% in bone
Copper	72	1.0	5.6	3.5	2.2	34.7% in muscle
Selenium	13	0.2	1.1	...	...	38.3% in muscle
Manganese	12	0.2	0.14	0.025	0.12	43.4% in bone
Iodine	11	0.2	0.29	2.6	0.35	87.4% in thyroid
Molybdenum	9.3	0.1	0.083	...	...	19% in liver
Chromium	1.7	0.02	0.14	0.074	0.044	37% in skin
Cobalt	1.5	0.02	0.0017	0.0014	0.00034	18.6% in bone (marrow)

2.4.3 Vitamins

- organic substances that we cannot synthesize
- mostly act as cofactors

- many organisms cannot synthesize some essential cofactors – they must be in the diet → **vitamins**
- vitamins were discovered as substances that cure deficiency diseases (e.g. nicotinamide / nicotinic acid (niacin) cures **pellagra**)
- **the vitamins that are coenzyme precursors are all water-soluble**; fat-soluble vitamins (e.g. A, D) are not components of coenzymes, but are also needed in trace amounts

Vitamin	Coenzyme	Human Deficiency Disease
Biotin	Biocytin	*a*
Cobalamin (B_{12})	Cobalamin (B_{12}) coenzymes	Pernicious anemia
Folic acid	Tetrahydrofolate	Megaloblastic anemia
Nicotinamide	Nicotinamide coenzymes	Pellagra
Pantothenate	Coenzyme A	*a*
Pyridoxine (B_6)	Pyridoxal phosphate	*a*
Riboflavin (B_2)	Flavin coenzymes	*a*
Thiamine (B_1)	Thiamine pyrophosphate	Beriberi

[a]No specific name; deficiency in humans is rare or unobserved.

Table 13-2

[slides] Vitamins that are coenzyme precursors and their deficiency diseases

Water soluble vitamins

Vitamin B1 - thiamine

- involved in carbohydrate metabolism
- active form - **coenzyme TPP (thiaminpyrophosphate)**
- metabolic function - transfer of hydroxy-alkyl residues = oxidative decarboxylation
  - eg, oxidative decarboxylation of pyruvate and α-ketoglutarate
- symptoms of deficiency - fatigue, convulsions, digestive disorders, nerve disorders, beri-beri nerve disease
- sources - cereals, yeast, lentils, offal, yolk, pork
- RDI - 1,5 mg

Vitamin B2 - riboflavin

- components of flavoproteins, enzymes involved in oxidative reduction processes (respiratory chain)
- active form – **coenzyme FAD**
- symptoms of deficiency - nflammation of oral corners, lips, damage to mucous membranes and skin, growth arrest
- sources - meat, milk, eggs, liver, yeast, beer
- RDI - 1,7 mg

Vitamin B3 - nicotinic acid

- Also known as niacin form NIcotinic ACid vitamIN
- Active form – **nicotinamide nucleotides NAD and NADP**
- symptoms of deficiency - convulsions, nervous disorders, pellagra disease
- sources - meat, fish, yeast, multigrain cereal, lentils
- RDI - 10 mg

Vitamin B5 – pantothenic acid

- basis of coenzyme A, contribution to protein synthesis and oxidation reduction processes
- symptoms of deficiency - nervous disorders, convulsions
- sources - meat, cheese, eggs, liver, yeast, lentils
- DDD - 6-8 mg

Vitamin B6 - pyridoxine

- part of enzymes involved in amino acid metabolism (transaminases)
  - **pyridoxal phosphate** -> transamination and decarboxylation of AA
- symptoms of deficiency - disorders of haemoglobin production, inflammation of skin and mucous membranes, epileptic inflammation
- sources - liver, whole grain cereal products, egg yolk, yeast
- RDI - 1,5-2 mg

Vitamin B7 = Vitamin H = coenzyme R = Biotin

- has a bunch of names because it was discovered by many scientists in different areas of metabolism and each gave it their own name before it was discovered that it was only one and the same molecule
- significant coenzyme, promotes cell growth and division
- symptoms of deficiency - skin diseases, anorexia, fatigue
- sources - eggs, liver, vegetables, yeast, formed by intestinal bacteria
- RDI - 0,5-1 mg

Vitamin B9 – Folic acid

- t is often called **folate** from Latin acidum folicum
- affects the amino acid metabolism necessary for the formation of red blood cells
- active form - **tetrahydrofolate**(THF) - transfer of monocarbon residues
    - carries methyl residues and alters deoxyuridine phosphate
- symptoms of deficiency: disorders of protein synthesis, anaemia
- sources - eggs, leafy vegetables, yeast
- RDI - 0,4 mg
- deficiency → **megaloblastic anaemia**

Vitamin B12 - cobalamin

- does not occur in plants, it is formed only in animals
- ensures normal haematopoiesis
- consists of a tetrapyrrole skeleton (similar to haemoglobin) with a cobalt atom attached inside
- metabolic function - transport of methyl groups
- symptoms of deficiency - anaemia, degeneration of spinal nerves
- cobalamin coenzymes mediate **alkylation**; deficiency → **pernicious anaemia**
- sources - liver, meat, intestinal bacteria
- RDI - 3-31 µg

Vitamin C - Ascorbic acid

- Allows for iron absorption, formation of collagen and erythrocytes, promotes blood clotting, production of antibodies, is an antioxidant
- oxidoreductase cofactor (electron donor)
- symptoms of deficiency - gingivitis, bleeding, decreased resistance to infections, scurvy - the most severe stage of avitaminosis
- sources - vegetables (Brussels sprouts, peppers), fruits (blackcurrants, strawberries), potatoes, internal organs of animals
- RDI - 60-200 mg - should be higher in smokers, the elderly and women using oral contraceptives

Fat soluble vitamins

Vitamin A - retinol

- component of visual pigment, important for epithelial formation, antioxidant
- active forms
    - **retinal** - vision, carbohydrate transport
    - **retinoic acid** - signalling molecule, ensures development, differentiation, growth
- symptoms of deficiency - night blindness, drying of cornea and conjunctiva, rough skin, stopping growth
- sources - liver, egg yolk, butter, cheese, sea fish fat, provitamin (β-carotene) in plant foods (carrots)
- RDI - 1 mg

Vitamin D – calciferol

- group of vitamins, most important - D3 (cholecalciferol) and D2 (ergocalciferol)
- controls the metabolism of calcium and phosphorus, promotes their absorption from the small intestine and bone deposition
- effective form - hormone calcitriol
- hypervitaminosis - increase of calcium absorption, its deposition in tissues and formation of kidney stones
- symptoms of deficiency - softening and deformation of bones - rickets (rachitis)
- sources - fat of sea fish, butter, liver, egg yolk, also due to UV radiation of the skin
- RDI – 0.02mg

Vitamin E - tocopherol

- antioxidant, supports the activity of the gonads
- protection of the organism against cancer (together with A and C)
- symptoms of deficiency - muscle weakness, vascular system disorders
- sources - vegetable oils, cereal sprouts
- RDI - 10-15 mg

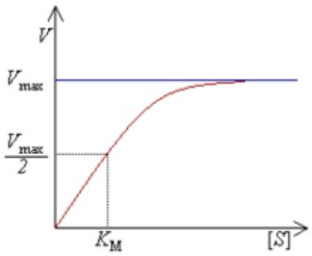
Vitamin K - phylloquinone

- supports the process of blood clotting, promoting the synthesis of prothrombin in the liver
- active form - phylohidroquinone
- functions in the **carboxylation of plasma proteins** (clotting factors)

- symptoms of deficiency - impaired blood clotting
- sources - leafy vegetables, made up of intestinal bacteria
- RDI - 0.08 mg

2.5 INHIBITION OF ENZYMES, ROLE IN PHARMACOLOGY

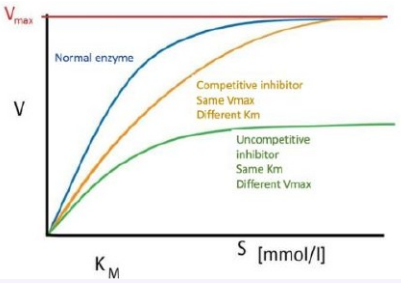
doc. MUDr. Jan Trnka, Ph.D.



2.5.1 Inhibition of enzymes

Michaelis-Menten equation

- how fast the reaction will proceed is described by the Michaelis-Menten model - it suggests that the reaction rate depends on the substrate concentration (higher concentration = higher speed)
  - however, this does not work indefinitely; there is a finite maximum speed
- $v = \frac{v_{max} * [S]}{K_M + [S]}$
- [S] = substrate concentration, written in brackets because it is an equilibrium quantity
- **Enzyme + Substrate <-> Enzyme-Substrate complex -> Enzyme + Product**
  - The reaction to produce a product is also reversible in some cases, but we do not account for it for simplicity
- **Maximum, speed is achieved when the enzyme is saturated** - at an infinite substrate concentration
  - in practice this speed can be achieved, it is a real value
- **Michaelis constant (K_M) = substrate concentration at which half of the maximum reaction rate is reached**
  - the concentration of the enzyme does not matter, only the substrate concentration
  - we can imagine that the constant describes the affinity of the enzyme to the substrate - the lower the constant, the higher the affinity

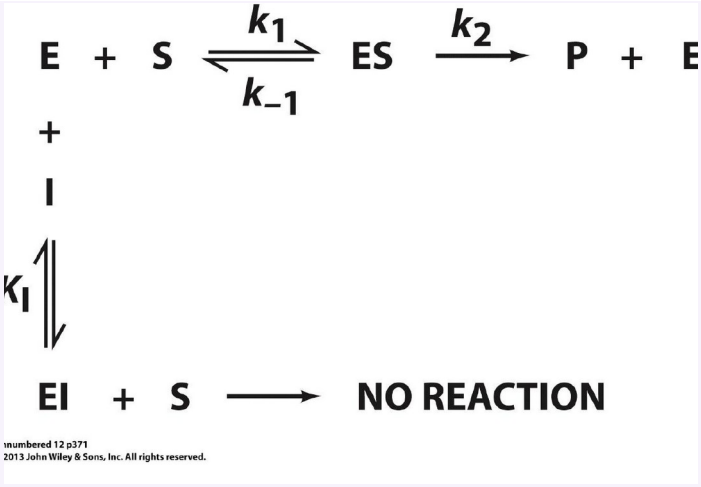


Types of inhibitors

- most enzyme inhibitors act **reversibly** (no permanent change of the enzyme); **irreversible inhibitors** permanently modify the target enzyme
- inhibitors alter K_M and/or v_{max} – best evaluated with the **Lineweaver-Burk (double-reciprocal) plot** (see 2.3)

Competitive inhibition

- competitive **inhibitors "compete" with substrate molecules for the active site** of the enzyme
  - Successful binding results in a dysfunctional enzyme-inhibitor complex
  - these inhibitors often **resemble** the substrate - they must fit in the same place as the substrate, so they must look similar
  - **E + I <-> EI**
- it is a reversible action
- **by increasing the substrate concentration, it is possible to decrease the efficiency of inhibition**- by increasing the substrate concentration we decrease the chance that the enzyme will pair with the inhibitor
  - **Vmax will remain the same – at infinite concentration of substrate competitive inhibitors will not be effective**
- the action of a competitive inhibitor **increases K_M**, because we need more substrate for the same reaction rate
- in the Lineweaver-Burk plot: lines intersect on the **y-axis** (same $1/v_{max}$), the slope increases
- example: **ethanol as treatment of methanol poisoning** – ethanol competes with methanol for alcohol dehydrogenase (see 2.2)

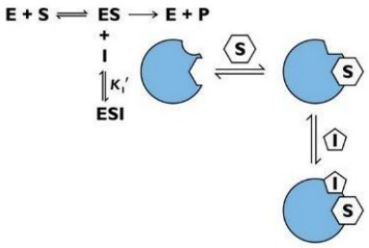


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[slides] Competitive enzyme inhibition

Uncompetitive inhibition

- ⚠️ **terminology differs between the script and the slides** – the slides (Samcová/Tůma) use:
  - **uncompetitive inhibition** – inhibitor binds **only to the ES complex**, not to the free enzyme; it distorts the active site → **both K_{M} and v_{max} decrease**; significant mainly for multisubstrate enzymes (= what the script describes below as "non-competitive")
  - **mixed inhibition (= non-competitive)** – inhibitor affects both substrate binding and catalysis; binds to sites involved in both; **v_{max} decreases, K_{M} may increase or decrease**
  - **pure non-competitive inhibition** – only v_{max} is affected, K_M unchanged (= what the script describes here as "uncompetitive")
- the uncompetitive inhibitor is in all cases **allosteric** (= another binding site on the enzyme other than the active site)
  - binding of the inhibitor causes a conformational change in the enzyme which is then unable to bind its substrate
- the inhibitor binds to the enzyme completely regardless of whether or not the substrate is bound to the enzyme= t**he concentration of the reactants does not affect the potency of the inhibition**
- **v_{max} decreases -** we have less functional enzyme molecules
  - the inhibitor does not look at the substrate concentration at all - even if we increase the substrate concentration, there will always be some of the enzyme molecules in the solution that will be inhibited
- **K_M stays the same**- the inhibitor only takes out several molecules of enzyme, it does not change its affinity to the substrate



Non-competitive inhibition

- Inhibitors that bind only to the **enzyme-substrate complex**
  - they are often allosteric
  - **E + S + I -> ES + I <-> ESI**
- this inhibition is often observed with enzymes that bind multiple substrates - it works by binding the first substrate to the enzyme, causing a change in the conformation of the enzyme and revealing an additional binding site to which the inhibitor subsequently binds
- **the inhibitor is very poor at low substrate concentration** - it does not have enough ES complexes to bind to
  - high substrate = lots of ES complexes= a lot of opportunities for the inhibitor to bind to an enzyme
- substrate concentration does not affect the binding of the inhibitor to the enzyme-substrate complex
- **v_{max} and K_M decrease** (enzyme appears to have higher affinity for substrate)

TABLE 12-2 Effects of Inhibitors on Michaelis–Menten Reactions[a]

Type of Inhibition	Michaelis–Menten Equation	Lineweaver–Burk Equation	Effect of Inhibitor
None	$v_o = \frac{V_{max}[S]}{K_M + [S]}$	$\frac{1}{v_o} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$	None
Competitive	$v_o = \frac{V_{max}[S]}{\alpha K_M + [S]}$	$\frac{1}{v_o} = \frac{\alpha K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$	Increases K_M^{app}
Uncompetitive	$v_o = \frac{V_{max}[S]}{K_M + \alpha'[S]} = \frac{(V_{max}/\alpha')[S]}{K_M/\alpha' + [S]}$	$\frac{1}{v_o} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$	Decreases K_M^{app} and V_{max}^{app}
Mixed (noncompetitive)	$v_o = \frac{V_{max}[S]}{\alpha K_M + \alpha'[S]} = \frac{(V_{max}/\alpha')[S]}{(\alpha/\alpha')K_M + [S]}$	$\frac{1}{v_o} = \frac{\alpha K_M}{V_{max}}\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$	Decreases V_{max}^{app}; may increase or decrease K_M^{app}

[a] $\alpha = 1 + \frac{[I]}{K_I}$ and $\alpha' = 1 + \frac{[I]}{K'_I}$.

Table 12-2

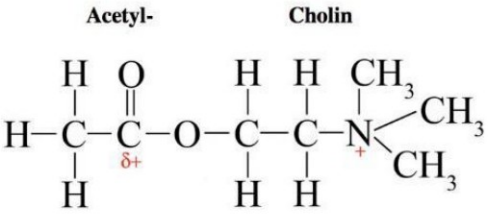
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[slides] Effects of inhibitors on the Michaelis-Menten and Lineweaver-Burk equations

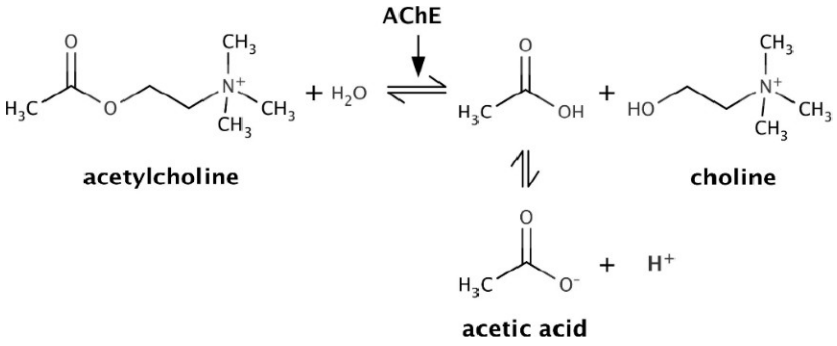
2.5.2 The role of inhibitors in pharmacology

Acetylcholinesterase (AChE)

- AChE is an enzyme **responsible for the hydrolytic cleavage (degradation) of acetylcholine** in the synaptic cleft between two neurons
  - **is a hydrolase and contains serine in its active site**
- acetylcholine is a minor but very important neurotransmitter found on neuromuscular plates, in the CNS and in the PNS
  - It plays a role in maintaining consciousness, attention, memory formation and muscle signaling
  - it is an ester of acetic acid and choline

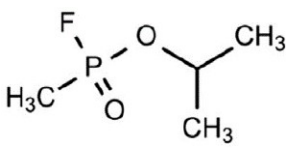


- **AChE causes cleavage of the ester bond between acetyl and choline** - acetic acid and choline is formed
- if the neuron is stimulated and the acetylcholine is released into the synaptic cleft, it must also be removed - acetylcholine cannot act permanently, it would cause hyper stimulation
  - for this reason, we directly breakdown acetylcholine via AChE
    - the enzyme attacks the carbonyl carbon (C=O)



Inhibitory acetylcholinesterase

- amplify signal on synapses that use acetylcholine as a neurotransmitter (cholinergic synapse)
- **irreversible inhibitors**
  - **carbachol** - similar to acetylcholine, has many mechanisms of action, including inhibition of acetylcholinesterase
    - contains carbamoyl group -NH_2-CO-
  - **sarin – nerve gas**
    - banned gas that is currently being used in the war in Syria
    - irreversible inhibition of AChE -> increase in acetylcholine -> uncoordinated muscle contractions -> muscle paralysis, including respiratory ones -> suffocation death
    - works by **serine** (which is located in the active site of **AChE**) attacks **sarin** -> fluorine is cleaved from **sarin** and the rest of the **sarin** then binds to serine and inhibits **AChE**



Sarin

        - belongs to organophosphate compounds - substances that are not used in medicine, but its modified forms are used as pesticides and insecticides in agriculture
- **reversible inhibitors**
    - **edrophonium** - competitive inhibitor
        - it is very fast and reversible inhibitor (its effect is only in a matter of minutes), therefore it is used for diagnostics
        - **myasthenia gravis** - an autoimmune disease attacking acetylcholine receptors
            - he main symptom is muscle weakness - after delivery of edrophonium to patients this symptom disappears temporarily (for a few minutes) and then reappears
    - **donepezil** - a substance used to improve the cognitive abilities of people with **Alzheimer disease**
        - blocking of AChE basically amplifies the signal that acetylcholine sends - muscle is shouting instead of contracting
        - there are fewer neurons and thus less acetylcholine in people with Alzheimer's disease - inhibition of acetylcholinesterase thus amplifies the signal that this neuron residue still emits

2.6 EPITHELIAL TISSUES II - GLANDULAR EPITHELIA

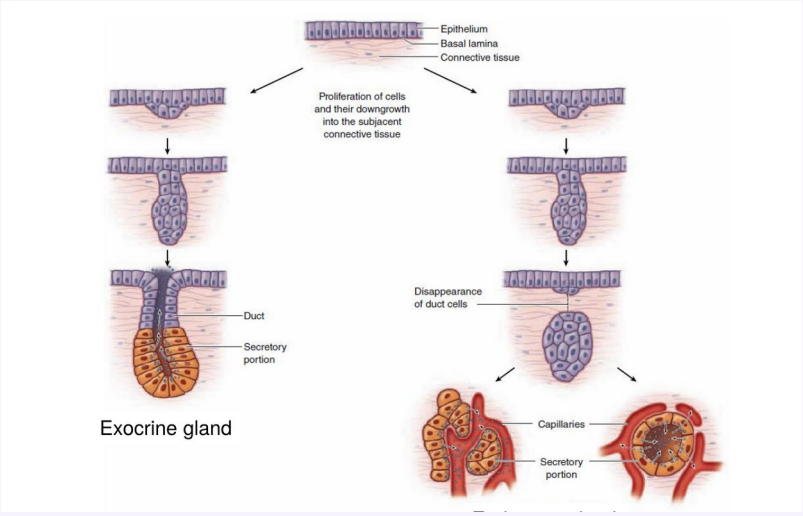
MUDr. Eva Maňáková, Ph.D.

- glands are sets of cells or individual cells producing a substance with biological function - proteins, lipids, complex compounds of sugars and proteins = secrete
  - goblet cells - separate gland cells (technically it is a gland consisting of one cell)
    - forms mucus mucin
    - are in the GIT, the airway, the conjunctiva in the upper eyelid…

- products of glands: **proteins** (digestive enzymes), **lipids** (sebum), **glycoproteins** (mucin), **water/electrolytes** (sweat)
- epithelia by organisation: **surface** (one surface on the basement membrane, the opposite one free – most epithelia), **trabecular** (cells in branching cords – liver, endocrine glands), **reticular** (cells in contact by long protrusions, wide intercellular slits – thymus)
- the polarised epithelial cell: **apical** surface (microvilli, cilia), **lateral** (intercellular junctions), **basal** (hemidesmosomes, basal labyrinth)

Development of glands

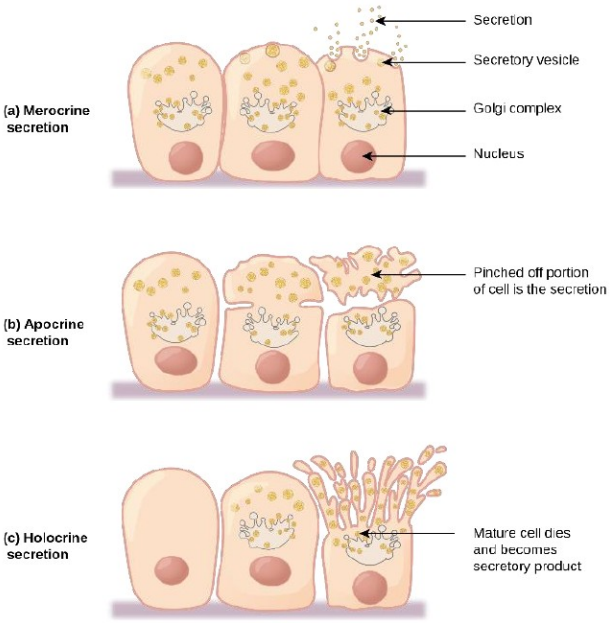
- glands develop from covering epithelium – cells proliferate and grow down into the underlying connective tissue
  - **exocrine** – the proximal part forms the **duct** (connects the secretory part with the surface epithelium), the distal part differentiates into **secretory cells**
  - **endocrine** – the proximal part (duct) **disappears**; the distal part forms islands of secretory cells surrounded by blood capillaries



[slides] Development of exocrine and endocrine glands

Division of glands

- **exocrine**- secretion is produced on the body surface either directly or through the duct
  - secretion exits the cell through its apical surface
  - sweat glands, mammary glands, sebaceous glands, salivary glands…
- **endocrine**- secretion remains in the body, most often goes from the cell to the surrounding connective tissue and then into the bloodstream
  - secretion from the cell via basal surface
  - pancreas, pituitary, adrenal gland, thyroid gland…
- **amphicrine**- combination of exocrine and endocrine glands
  - e.g. **pancreas** (exocrine acini + endocrine islets of Langerha
- endocrine glands have **no ducts** and **fenestrated capillaries**



2.6.1 Exocrine glands

- we distinguish several qualities of exocrine glands
  - mechanism of excretion of secrete
  - position of glandular cells in relation to superficial epithelium
  - structure of the secretory portion
  - architecture of the gland

- the slides add the division **according to the number of cells** – unicellular / multicellular
    - type of secrete

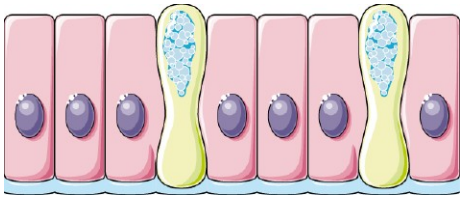
Mechanism of secrete excretion

- **merocrine secretion**
    - secretory granules are excreted by exocytosis
    - secretion synthesis is continuous but its secretion is not - secretion is stored inside the cell
    - The merocrine cell has a lot of RER, GA and secretory granules
- **apocrine secretion („reverse phagocytosis ")**
    - the cell secretes secretions by cleaving the portion of the apical cytoplasm in which the secretions are located
    - typical for the mammary gland - fat droplets accumulate at the apical pole and the whole piece of the cell breaks apart
    - frequent in lipid secreting cells
- **Holocrine secretion**
    - the cell secretes secretion so in a way that it disintegrates completely and disappears by apoptosis
    - typical for sebaceous glands
- **eccrine secretion**
    - secretion is secreted by individual molecules through the cell membrane (either by itself or via carriers) and is passively followed by water on the basis of osmosis
    - Hydrophilic and ionic secretion needs transporters to get through the cell membrane
    - the result of eccrine cell activity is mucus - solution of secrete, water and ions isotonic with cytoplasm
        - from this solution, the ions are gradually pumped back into the cells
    - **Cystic fibrosis** - caused by a malfunction of the membrane transporter for Cl^-
        - the consequence of a higher concentration of chloride ions leads to excessive reabsorption of sodium from mucus
        - because water follows sodium, mucus dehydrates and increases its viscosity
        - too thick mucus explains the symptoms of cystic fibrosis - thin tubes (pulmonary alveoli, vas deferens) clog and excessive mucus density interferes with the ability of antimicrobial peptides, leading to frequent infections (mainly respiratory)

- merocrine = most exocrine glands (e.g. salivary glands – parotid); the cell stays intact
- apocrine – the lipid droplets have no membrane; they meet in the apical cytoplasm and are released with it → the cell gets smaller
- holocrine – the cell first differentiates and stores lipid droplets, then undergoes apoptosis and its content and remnants are released into the duct
- eccrine – cell stays intact, ions go through transporters and channels, water follows (e.g. **sweat glands**)

Position of glandular cells

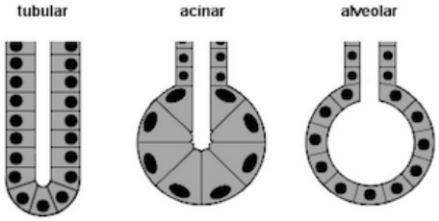
- intraepithelial glands
    - are built directly into the epithelium
    - they do not have an outlet/ duct system- they open directly to the surface of the epithelium
    - **goblet cells**- mucin mucus production; is oppressed by other cells, hence the shape of the cup
        - have RER, large GA, apical cytoplasm filled with secretory granules
    - in some organs, the whole surface epithelium is composed of mucin producing cells (stomach, uterus)



- other single-cell (intraepithelial) glands: **Paneth cells**, **gastro(entero)endocrine cells**
- extraepithelial glands
    - are placed under the epithelium of origin
    - consist of a secretory section and a duct
        - if they are far from the epithelial surface, they have a long and branched duct system

Structure of the secretory portion

- one layer of secretory epithelial cells which sit on the lamina basalis and surround the lumen where the secretion is drained
- simple types of secretion
    - **tubular** - tube shape, most common, mucinous glands (see below)
    - **acinar** -round shape, narrow lumen
        - for these glands, serous secretion is typical - proteins, enzymes
    - **alveolar** - shape of a bladder, have a wide, well recognizable lumen
- compound type of secretory portion



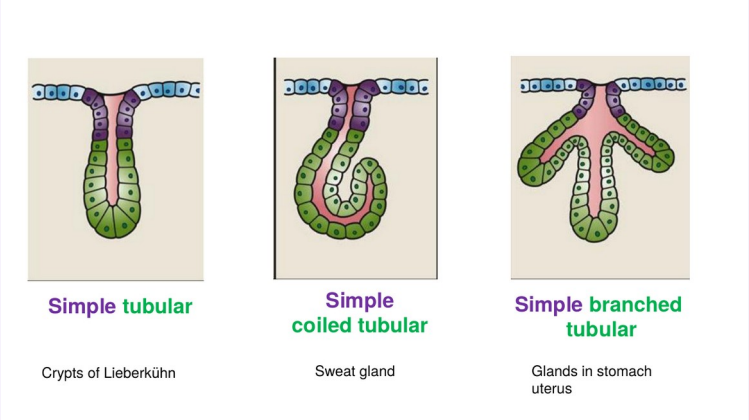
- **tuboacinous**- the secretory compartment has the shape of a tube with a round end
- e.g. submandibular and sublingual
- **tuboalveolar**- there is a wide lumen at the end of the tube
  - e.g. mammary glands in lactation, sweat glands

- examples (slides)
  - **tubular** – mucinous glands, eccrine sweat glands
  - **acinar** – pancreas, parotid gland
  - **alveolar** – sebaceous glands
  - tuboacinar – submandibular and sublingual gland; tuboalveolar – lactating mammary gland, apocrine sweat glands

Architecture of glands

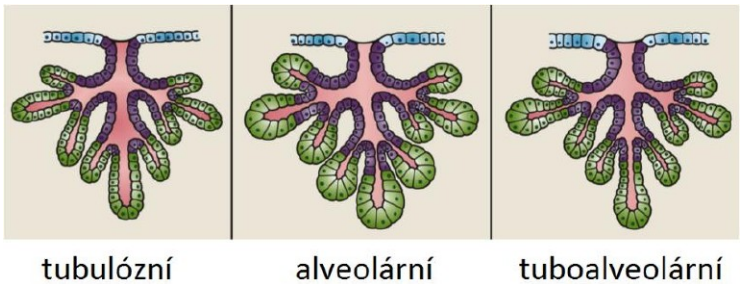
- simple tubular glands - do not need a duct, they open directly to the surface (e.g crypts in the stomach)
- compound glands have a branching duct system that gathers secretions from multiple sites and gathers them into one
  - they look like a river - small springs run down to bigger and eventually run into the sea (=the surface of the epithelium)

- **simple** gland = the duct does not branch (1 duct) – but the secretory part can be **branched**
  - simple tubular – crypts of Lieberkühn; simple **coiled** tubular – sweat gland; simple **branched** tubular – glands of the stomach and uterus
- **compound** gland = ducts from several secretory units fuse into larger ducts (tree of ducts); e.g. compound acinar – exocrine pancreas



[slides] Simple tubular, simple coiled tubular and simple branched tubular glands

- basic structure: **parenchyma** (secretory parts + ducts) and **stroma** (connective tissue – capsule, septa dividing the gland into lobes/lobules)
  - types of ducts in compound glands
    - **intralobular**- surrounded by epithelial structures, inside the lobule, leads directly from acinus to interlobular duct
      - **intercalated duct –** forms part of the intralobular duct
      - **striated**- connects the intercalated duct to the interlobular duct. Also, part of interlobular ducts
    - **interlobular**- outlet between lobule
    - **main** - connects to the interlobular ducts, it is the last duct before the secretion exits the gland



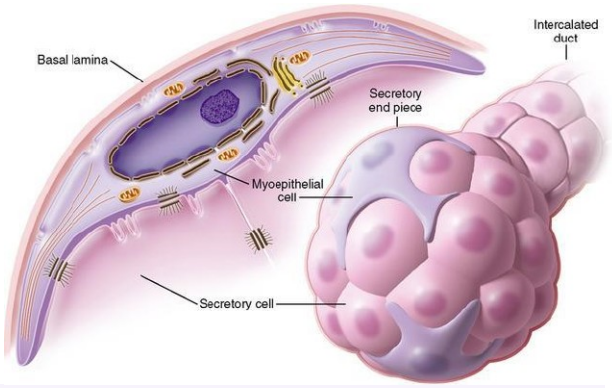
- duct epithelium (salivary glands)
  - **intercalated duct** – simple squamous to cuboidal cells
  - **striated duct** – simple cuboidal to columnar cells; the "striation" = **basolateral labyrinth** (folds of the basal membrane + mitochondria) → ion-transporting cells that change the composition of saliva (make it **hypotonic**)
  - **interlobular ducts** – within connective tissue; the epithelium changes with the position of the duct in the organ
- function of ducts
  - the removal of the secrete
  - secretion modification - so-called primary secretions can enrich or otherwise change to a secondary secretion that is secreted to the surface

Properties of secretions

- exocrine gland secretions (except sebaceous glands) are watery
- **serous secretion - is produced by alveolar and acinous cells**
  - is basophilic - most often it is peptide hormones, proteins and enzymes
  - Serous secreting cell (aka serous cell) has round nucleus, RER, GA and secretory granules
    - **pyramidal shape**, forms **acini**, basophilic, apical microvilli
- **mucinous secretion - is produced by tubular cells**
  - goblet cell - Produces mucus rich in mucin
  - mucinous cell has an active nucleus, GER, GA and a secretory granule
  - mucin secretory granules are well stainable by PAS dye, which is sensitive to polysaccharides
    - mucinous cell (slides): cuboidal/columnar, **flat nucleus in the basal part of the cell**, forms **tubules**, mucin is very light (pale) in the microscope, viscous secretion
- **mixed secretion** - seromucinous
  - both types of secretory compartments occur side by side and also in direct combination - e.g. tuboalveolar
  - Serous semilumen - a cap from serous cells at the end of the mucinous tubule
  - serous demilune – a group of moon-shaped serous cells attached to mucinous tubules; produce **α-amylase, lysozyme**; found in the **submandibular gland**

Myoepithelial („basket“) cells

- something between smooth muscle and epithelium
- contractile epithelial cells used to expel secretions from secretory compartments and into ducts
- slender star-shaped cells (basket cells) which are found in glandular epithelium as a thin layer above the basement membrane but generally beneath the luminal cells
- they contain cytokeratin's as epithelial cells and actin and myosin as smooth muscle cells



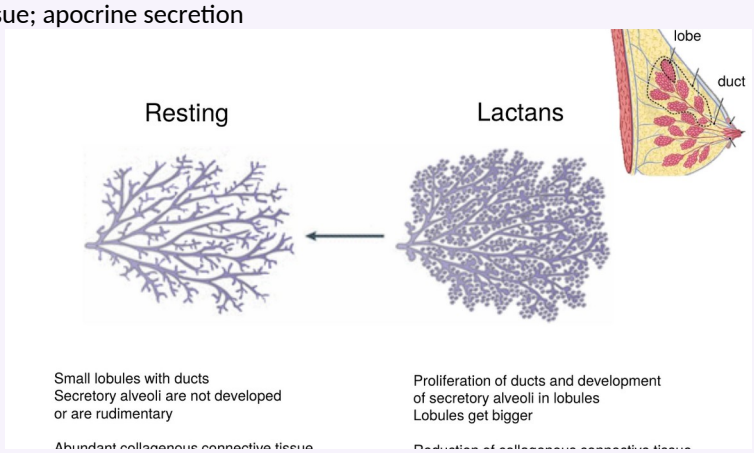
- found in sweat, mammary, salivary and lacrimal glands, glands of the oesophagus and respiratory tract
- **no myoepithelial cells in the pancreas and sebaceous glands**

Regulation of secretion

- the secretory activity of the exocrine glands is controlled by the nervous vegetative system and sometimes hormonal
- under pathological conditions, other substances, such as inflammatory mediators, may affect the quality and quantity of secretion

Examples of exocrine glands

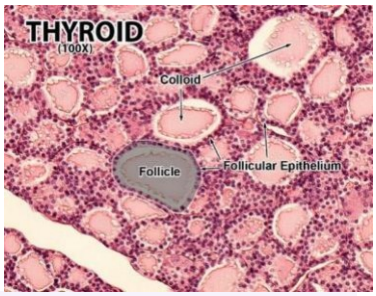
- **salivary glands**
  - **parotid gland** – compound acinar gland, **serous** secretion (merocrine)
  - **submandibular gland** – compound tuboacinar gland, **seromucinous** secretion (serous dominant), serous demilunes
- **mammary gland**
  - **resting** – small lobules with ducts, secretory alveoli not developed or rudimentary, abundant collagenous connective tissue
  - **lactating** – proliferation of ducts and development of secretory alveoli, lobules get bigger, less collagenous connective tissue; apocrine secretion



[slides] Mammary gland – resting vs. lactating

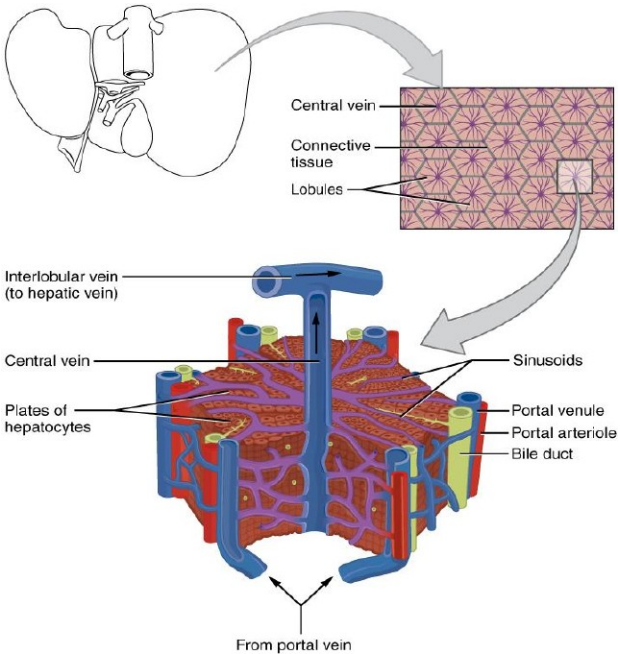
- **sebaceous gland** – holocrine secretion
- **pancreas** – divided into lobules by septa
  - exocrine part – **serous acini** + ducts (intercalated, intralobular, interlobular)
  - endocrine part – **islets of Langerhans**

2.6.2 Endocrine glands



- reverse polarization - the cell secretes secretion towards the basalis lamina
- construction of endocrine glands - always trabecula of cells, only in the thyroid gland we find follicles (due to storage of iodine)

- **steroid-synthesizing cells** – lipid droplets, **smooth ER**, **tubular mitochondria**, no secretory vesicles
- **peptide-synthesizing cells** – **rough ER**, Golgi apparatus, secretory vesicles; synthesize larger molecules (pre-pro-hormones)
- endocrine glands have **fenestrated capillaries** and **no ducts**
- endocrine structures: 1. **single cells** (DNES), 2. endocrine structures as **parts of bigger organs** (pancreas, ovary), 3. **"true" glands** (hypophysis, thyroid, adrenal glands)
- types of secretion: **endocrine** (into blood), **paracrine** (to neighbouring cells), **autocrine** (on the cell itself)
  - **DNES - Diffusion Neuro Endocrine System** - endocrine cells scattered everywhere along the GIT, secrete signalling molecules to regulate digestive system function
  - types of endocrine glands according to hormones produced
    - polypeptides and proteins - adenohypophysis, parathyroids, islets of Langerhans...
    - catecholamines - adrenal medulla
    - steroid hormones - adrenal cortex, testis, ovary
    - thyroxine – thyroid gland



Thyroid Gland

- Cuboidal epithelium arranged around the follicle
- inside the follicle is colloid - thyroglobulin - a storage form of thyroid hormones

Adrenal gland

- cortex + medulla (**different origin**); capsule of connective and adipose tissue; cells in trabeculae between fenestrated capillaries
- cortex
  - **zona glomerulosa** (15 %) – mineralocorticoids
  - **zona fasciculata** (65 %) – glucocorticoids
  - **zona reticularis** (7 %) – androgens, glucocorticoids
- medulla – **chromaffin cells** producing catecholamines

Endocrine pancreas

- **islets of Langerhans** – cords of endocrine cells among the serous acini of the exocrine pancreas

Hypophysis

- anterior lobe = **adenohypophysis**, posterior lobe = **neurohypophysis** (pars nervosa) + **pars intermedia**
  - pars intermedia – rudimentary, highly basophilic, contains follicle-like cysts filled with colloid
- adenohypophysis – cells in trabeculae, fenestrated capillaries; by staining:
  - **chromophobes** (unstained) = undifferentiated cells
  - **chromophils** – **acidophils** produce non-glandotropic hormones (**prolactin, growth hormone**); **basophils** produce glandotropic hormones (**ACTH, MSH, TSH, FSH, LH**)

Liver

- characteristic arrangement
- amphicrine character
  - Exocrine function - forms bile
  - endocrine function - involved in the metabolism and production of plasma proteins
- the basic morphological unit of the liver is the lobule of the central vein - the hexagonal prism consists of the plates of hepatocytes
  - The central vein sits right in the middle of the whole prism
  - fenestrated capillaries and bile ducts lead from the central vein towards the periphery of the lobules
- at the point of contact of the three adjacent lobules there is **a triad - 1 vessel, 1 vein and 1 bile duct**

- other ways to describe the liver unit: **hepatic lobule, portal lobule, hepatic acinus**
- hepatocytes are arranged in trabeculae (plates) between **sinusoids**; other cells: **Kupffer cells** (macrophages), **stellate cells**; **space of Disse** between the sinusoid and hepatocytes

2.7 STRUCTURE AND ORIGIN OF MITOCHONDRIA

MUDr. Eva Maňáková, Ph.D.

2.7.1 Structure

- mitochondria are a membrane semi-autonomous (semi-independent) organelles
- **two membranes** - **inner** (bent) **and outer** (smooth), intermembrane space between them
  - inside the inner membrane is the mitochondrial matrix
- size - 0,5-1 µm wide and 3-5 µm long
  - membrane thickness 6-8 nm and intermembrane space is +- 20 nm
- microtubules move through towards where they are needed - to the surrounding places with high ATP consumption
  - usually are concentrated around the nucleus or basolateral labyrinth
- active cells have the most mitochondria - hepatocytes 800, oocytes up to 100 000, cardiomyocytes up to 30 - 40% of their volume

Outer membrane

- contains many porins - channels that allow molecules to pass through the membrane
  - as a result, the outer membrane non-selectively permits all molecules smaller than 10 kDa
- Bcl proteins - part of outer mitochondrial membrane - **important proteins in the regulation of apoptosis**
- Contains a few enzymes of fatty acid metabolism and phospholipids
- unlike the cell membrane, it contains almost no cholesterol

Intermembrane space

- similar composition to cytosol (much less protein than matrix)
- there are proapoptotic proteins, cytochrome C and a lot of protons

Inner membrane

- the original membrane of the former prokaryotic cell
- almost impermeable and very selective - everything except for small uncharged molecules needs a carrier
- it incorporates the respiratory chain complexes
  - **ATP synthase** forms elemental bodies on the inner mitochondrial membrane
- folds (cristae) - sometimes they are modified and form tubules (in cells producing steroid hormones)
- is composed of 75% proteins and 25% phospholipids
  - contains specific phospholipids cardiolipins that help with membrane elasticity

Mitochondrial matrix

- has a gel like consistency because of its high protein concentration (500 g protein per 1L matrix)
- contains enzymes of Krebs cycle, β-oxidation of fatty acids and ornithine cycle, various nucleotide coenzymes, inorganic ions (Ca), mtDNA, relevant tRNA and mRNA, mitochondrial ribosomes, chaperones and chaperonins

Import of proteins into mitochondria

- most proteins are imported from the cytoplasm - they are synthesized on ribosomes in the cytosol
- chaperones ensure that proteins remain unpacked so that they can be transported through the mitochondrial membrane
- on the inner and outer membrane are translocators for proteins - Tom and Sam complexes external, Tim complexes internal
  - **Tom (transporter of outer membrane) complex** gets protein into intermembrane space
  - **Sam complex** is for proteins to remain integrated into the outer mitochondrial membrane
  - **Tim (transporter of inner membrane) 23 complex** pushes proteins from the intermembrane space into the matrix
  - **Tim 22 complex** integrates proteins from the intermembrane space into the inner mitochondrial membrane

Mitochondrial DNA = mtDNA

- small (16.5 kbp) circular double helix without introns
- encodes 2 rRNA, 22 tRNA and 13 proteins
  - most of the genome has moved into the nucleus of the host cell, making the mitochondria nucleus dependent and unable to live independently - 600-1000 mitochondrial proteins are encoded by nuclear DNA
- in the matrix of mitochondria there is also a proteosynthetic apparatus and 70S ribosomes that can produce molecules encoded by mitochondrial DNA

- mitochondrial ribosomes resemble bacterial ribosomes, which means that some antibiotics targeting bacterial ribosomes may also affect mitochondria

2.7.2 Mitochondrial function

- production of ATP by oxidative phosphorylation
- Krebs cycle, β-oxidation, ketogenesis, steroid synthesis, ornithine cycle, gluconeogenesis, iron and calcium metabolism (regulation of intracellular calcium, triggering of apoptosis or necrosis, synaptic plasticity and thermogenesis
- basolateral labyrinth - transfer of water and ions using Na / K ATPase with high consumption of ATP
  - Insufficient activity of mitochondria leads to transmission disorders and thus to cell oedema

Apoptosis

- programmed cell death requiring gene expression, proteosynthesis, and ATP
- triggers in response to apoptotic signal, stress, cell damage...
- mitochondria contribute by releasing proapoptotic factors from the intermembrane space, interrupting the supply of energy to the rest of the cell, and overproducing ROS (reactive oxygen species)

Defects in membrane permeability

- membrane damage is one of the main causes of cell death
- the initial phase of cell damage is disruption of the membranes - their ability to selectively permeability is impaired
  - this harms ATP production, leading to ATP deficiency, non-functional membrane pumps and cell swelling

Mitochondrial theory of aging

- mitochondria naturally form oxygen radicals that may damage mtDNA or host cell DNA
- accumulation of mutations in mtDNA with age gradually decreases the function of mitochondria and respiratory complexes
- mitochondrial function impairment affects long-lived cells - neurons, cardiomyocytes, muscle cells
- consequence - heart failure, muscle weakness, diabetes mellitus, dementia, neurodegeneration...

2.7.3 Mitochondrial diseases

- **heteroplasmia** - very variable symptoms - different distribution of mutant mitochondria throughout the body, symptoms/consequences of disease will depend on where the mutant MIT is located
- mutation in MIT are classified according to where they occur

Mutations in mitochondrial DNA

- maternal inheritance - inherited along the maternal line because paternal mitochondria are not passed on to the offspring
- **most variable** - there are several tens of copies of mitochondrial DNA and at the same time we have tens of thousands of copies of mitochondrial DNA in the cell
- **DAD** - **diabetes and deafness**
  - Mutated **leucine** gene synthesizes abnormal proteins
  - damage of hair cells and pancreatic β cells
- **LHON** - **Leber's hereditary optical neuropathy**
  - begins in middle age with optic nerve dying and ends with complete blindness
  - can be caused by multiple mutations **in complex 1** - mitochondria do not work well and their production of ATP is not enough for powerful and energy-intensive nerves

Mutations in nuclear DNA

- onset of the disease in prenatal development or just after delivery
- inherited by Mendelian inheritance, because the mutated genes are found in the nucleus and not in the mitochondria
- **Leigh syndrome** - progressive degenerative brain disease

Therapy

- with the help of classic medicines very difficult, practically at all
- possibility of so-called **mitochondrial transplantation** - the child then has 3 parents, from one egg, from the second sperm and from the third mitochondria
  - Legal only in UK
- Unfortunately, the CRISPR method does not work on mitochondria because the DNA can no longer cross the membrane

Division and fusion

- there is a balance in the cell between fission and fusion mitochondria
- fusion - content mixing, protein replenishment, mtDNA repair, distribution of metabolic intermediates

- division - increase of mitochondria

Mitochondrial inheritance

- mitochondria reproduce exclusively asexually
- mtDNA is inherited maternally - all mitochondrial embryo genetic information comes from the egg and not from the sperm
- mtDNA mutates faster than nuclear genome - more susceptible to mutation accidents
    - heart and nerve tissue (brain, retina) - most often mitochondrial diseases
    - the cell is able to recognize and destroy mitochondria with defective mtDNA or trigger apoptosis
- mitochondria in the cell can exchange mtDNA but do not combine it

2.7.4 Evolutionary origin of mitochondria

- mitochondria have evolved from independent bacteria (rickettsia), which have entered other bacteria (archebacteria)
    - The bacteria contribute the ability to synthesize ATP and the host cell delivers the substrates
- two hypotheses as to how this connection occurred

1) Eukaryotes developed first and then symbiosis occurred with mitochondria
2) entry of mitochondria into the cell resulted in eukaryotes
    - some eukaryotic organisms do not have mitochondria, or just residues
- most of the genome has moved into the nucleus of the host cell, making the mitochondria dependent on it and unable to live independently of the organelle
- all eukaryotic cells are descendants of a single cell that has absorbed bacteria with oxidative phosphorylation capacity

2.8 ELECTRON TRANSPORT CHAIN AND ATP FORMATION

doc. MUDr. Jan Trnka, Ph.D.

- **summary of glucose metabolism = $C_6H_{12}O_6 + O_2 \rightarrow H_2O + CO_2$ + Energy**
  - during this time, the oxygen is reduced from 0 to -2 and carbon is oxidized from 0 to +4
  - glucose degradation can conveniently be divided into two phases
    - the first phase is the transfer of electrons to the transmitters (NAD, FAD) and the formation of CO_2 – ie. glycolysis, Krebs cycle
    - the second phase is the transfer of electrons to the final acceptor (oxygen) and the utilization of the energy we get
      - Only in the second phase we need oxygen
      - The process of the second phase of glucose metabolism is the so-called Respiratory Chain

2.8.1 Respiratory chain

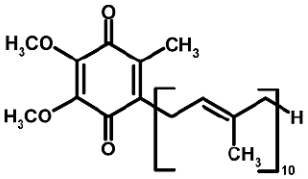
- „mitochondrial electron transport chain “
- is located in the inner mitochondrial membrane
- **consists of 4 large enzymes (complexes), coenzyme Q and cytochrome C**
- the respiratory chain is explained from the end, for better understanding

Complex IV (cytochrome C oxidase; ferrocytochrome)

- the last respiratory chain complex
- **takes electrons from cytochrome C and passes them to oxygen**
  - oxygen is reduced from 0 to -2 = 4 electrons are needed for each O_2
- **cytochrome C** - a relatively small protein containing one molecule of heme
  - heme contains an iron atom that can pass between Fe^{2+} and Fe^{3+} - this is how electron transfer functions
  - the whole cytochrome C can therefore carry only one electron at a time
  - is electrostatically attached to the inner membrane from outside (not part of the membrane)
- complex IV is inhibited it cyanide

Complex III (ubiquinol-ferricytochrome C oxidoreduktase)

- **transfers electrons from coenzyme Q to cytochrome C**
- contains FeS (iron-sulphur) centre - an evolutionary ancient electron carrier
- **coenzyme Q (ubiquinone)** - electron transporter inside the inner mitochondrial membrane



  - it's not a protein, it's just a small molecule (on picture)
  - has two forms - ubiquinone (oxidized form) and ubiquinol (reduced form)
  - there are many other types of coenzyme Q - mammals have coenzyme Q10
    - 10 because it contains 10 isoprene units
  - contains two oxygen in its structure, which can be reduced and then re-oxidized - it can carry two electrons at the same time
    - these two electrons can be obtained from many different sources - complex I, complex II, ETF dehydrogenase...

Complex I (NADH oxidase; NADH-ubiquinone oxidoreductase)

- a giant complex composed of 45 subunits - it is much larger than the membrane itself
- **oxidizes NADH + H^+ to NAD^+**
  - 2 electrons are released which travel to the coenzyme Q through complex I

Complex II (succinate dehydrogenase)

- embedded in the inner mitochondrial membrane, but not completely - partially communicating directly with the mitochondrial matrix
- **active also in Krebs cycle – oxidizes succinate to fumarate**
  - In this reaction, 2 electrons are released and go through FAD to coenzyme Q
  - FAD (unlike NAD) is not a separate molecule, it is just a part of enzymes (like a human hand)

Proton pumping

- Complexes I, III and IV are large proteins that cross the entire inner membrane and pump protons from the mitochondria matrix into the intermembrane space during electron transfer, **creating a strong proton gradient**

Complementary proteins of the respiratory chain

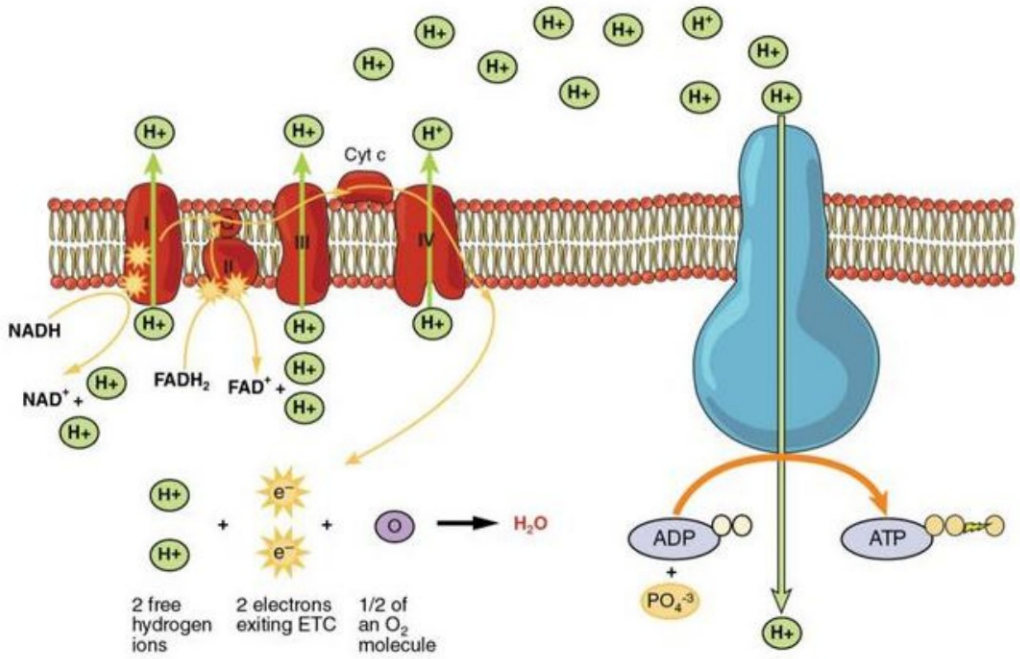
- serves mainly as an alternative pathway of electron transport to coenzyme Q

- ETFDH (ETF dehydrogenase, electron-transferring-flavoprotein-dehydrogenase)
  - ETF dehydrogenase contains FAD in its structure, thanks to which it can take electrons from ETF (electron transfer flavoprotein) and then pass them on to coenzyme Q
- GPDH (glycerol- phosphate dehydrogenase)
  - NADH, that is formed outside the mitochondria cannot spontaneously cross the mitochondrial membrane → it is transmitted by GPDH and subsequently reaches the respiratory chain where it donates its two electrons

2.8.2 Creating ATP

ATP synthase

- an enzyme not part of the respiratory chain but also in the inner mitochondrial membrane
- **whole protein works like a turbine**
  - has a proton channel that allows protons to pass from the intermembrane space to the matrix according to the concentration gradient
  - As it passes through the proton channel, it turns ATP synthase - like water flowing through a hydroelectric power station
- rotation of ATP synthase connects ADP and Pi into ATP
- oligomycin - bacterial product, ATP synthase inhibitor



Uncouplers - uncoupling proteins (UCP)

- eg. **thermogenin**
- transfers protons from the intermembrane space directly across the membrane and **bypasses ATP synthase**
  - uncouplers can be thought of as a channel that passes through the inner mitochondrial membrane, and allow protons to flow without any resistance
- heat is a by-product - in this way brown adipose tissue allows mammals to warm up or bears to survive during hybernation
- they are called uncouplers because normally oxygen consumption and ATP production are interconnected
  - when production of ATP is stopped, oxygen production stops - proton gradient will accumulate in the intermembrane space until it is so strong that respiratory chain complexes simply no longer push protons there
  - uncouplers will disturb this - protons will still flow, the respiratory chain will work, but ATP will not be produced
- there are other substances that can act as uncouplers
  - **dinitrophenol** - once used as a very effective weight loss medicine, but it was virtually impossible to prescribe the correct dosage and very often the use of dinitrophenol led to extreme weight loss and death
    - Nowadays we are beginning to experiment with dinitrophenol again

2.9 KREBS CYCLE

doc. RNDr. Ing. Petr Tůma, Ph.D.

- also, known as citric acid cycle, citrate cycle and tricarboxylic acid cycle
- occurs in the matrix of mitochondria
- 'crossroad of metabolic pathways' - the interconnection of carbohydrate, lipid and protein metabolism
- amphibolic pathway (ie anabolic and catabolic pathway)
  - **catabolic function -** energy source**, ATP synthesis**
  - **Anabolic function** - formation of precursors for the synthesis of glucose, lipids, amino acids, porphyrins
- burns nutrients to water, CO2 and reduced coenzymes that continue into the respiratory chain

- runs **only under aerobic conditions**; **does not occur in erythrocytes** (no mitochondria)
- final step of nutrient oxidation – acetyl~CoA is oxidized to 2 CO_2 with reduction of cofactors (reoxidized in the respiratory chain); direct gain of **1 GTP (= ATP)**
- different parts of the cycle can run with different intensity
- amphibolic examples: α-ketoglutarate → **glutamate** (most abundant excitatory neurotransmitter in the CNS) by transamination; succinyl~CoA → **tetrapyrroles (heme)**
- discovered by Sir Hans Adolf Krebs – Nobel Prize 1953

2.9.1 Krebs cycle

1) synthesis of citrate (starting substance, hence citrate cycle)) - **oxaloacetate + acetyl-coA -> citrate + CoA**
2) dehydrogenation and hydrogenation -> formation of **isocitrate** (so called citrate activation)
3) dehydrogenation and decarboxylation -> formation of **2-oxoglutarate** (or α-ketoglutarate)
- here CO_2 is split off and NADH if formed

4) dehydrogenation, decarboxylation and binding of CoA -> formation of **succinyl-CoA**
- CO_2 is slip off again and NADH is formed

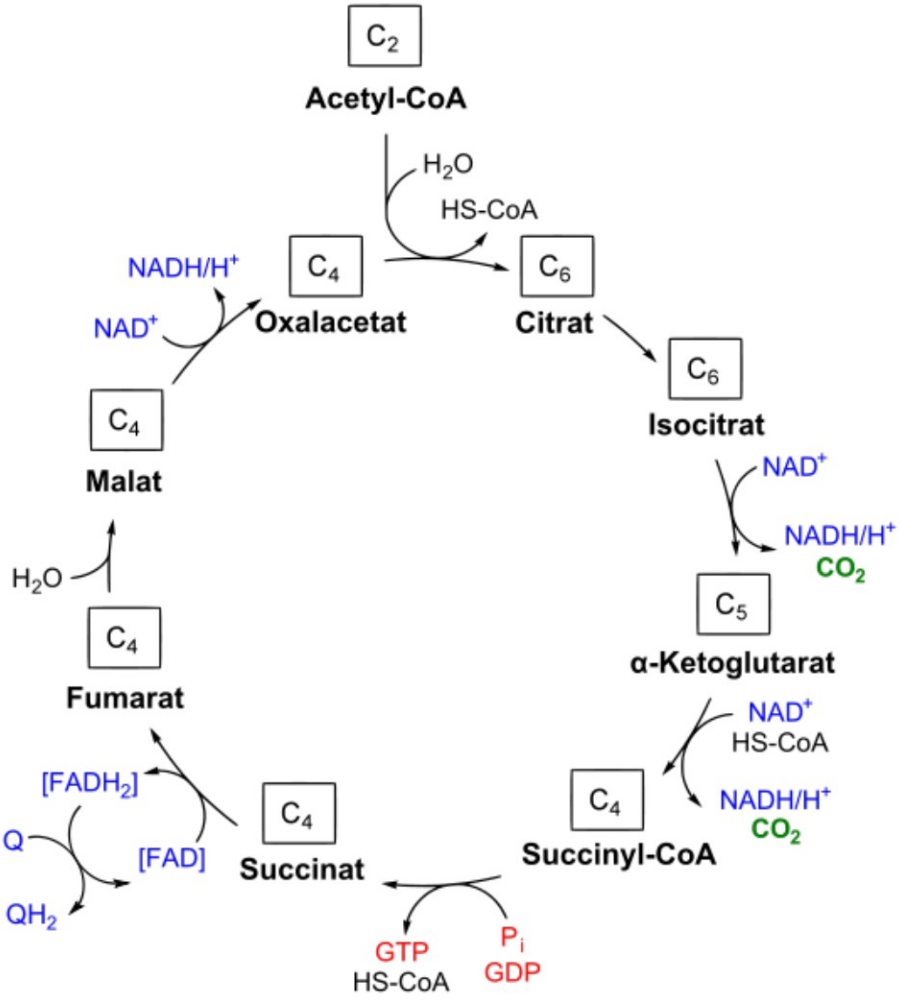
5) cleavage of CoA -> formation of **succinate**
- GTP is formed

6) dehydrogenation -> formation of **fumarate**
- FADH is formed

7) hydration -> formation of **malate**
8) dehydrogenation -> formation of **oxaloacetate**
- NADH is formed

- total yield from **1 acetyl-coA = 2 CO_2, 1 GTP, 3 NADH and 1 $FADH_2$**
  - the use of cofactors in the respiratory chain is about 10 ATP

- **overall equation**: CH_3-CO~SCoA + 3 NAD^+ + FAD (ubiquinone) + GDP + P_i + 2 H_2O → 2 CO_2 + 3 NADH + 3 H^+ + $FADH_2$ (ubiquinol) + GTP
- complete oxidation of pyruvate (PDH + Krebs cycle): CH_3-CO-COOH + 4 NAD^+ + FAD + ADP + P_i + 2 H_2O → **3 CO_{2} + 4 NADH + 4 H^{+} + FADH_{2} + ATP**



Enzymes of Krebs cycle

- **- regulatory enzymes - citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase -** enzymes that are inhibited by high concentrations of ATP, preventing excessive consumption of acetyl-CoA

- **pyruvate dehydrogenase or pyruvate dehydrogenase complex** - an enzymatic complex that catalyses the oxidative decarboxylation of pyruvate, which produces acetyl-CoA, which subsequently enters the Krebs cycle
  - a number of coenzymes participate in this reaction along with the complex
    - pyruvate + NAD^+ + CoA -> acetyl-CoA + NADH + H^+ + CO_2
    - **pyruvate dehydrogenase reaction is irreversible**
    - **the PDH complex = 3 enzymes**: **pyruvate dehydrogenase (E1** – main rate-limiting step), **dihydrolipoyl transacetylase (E2)**, **dihydrolipoyl dehydrogenase (E3)**
    - located in the mitochondrial matrix – pyruvate enters via the **MPC (mitochondrial pyruvate carrier)**
    - **five cofactors**: NAD^+ (vit. B3), FAD (vit. B2), coenzyme A (vit. B5), TPP (vit. B1), lipoamide (lipoic acid)
    - steps: 1. **decarboxylation** (pyruvate binds to TPP, CO_2 released) → 2. **oxidation** (hydroxyethyl → acetyl, bound to lipoamide = acetyl-dihydrolipoamide) → 3. **acetyl transfer** to CoA → 4. **lipoamide regeneration** by FAD (→ $FADH_2$) → 5. **FAD regeneration** by NAD^+ (→ NADH)

Regulation of the PDH complex

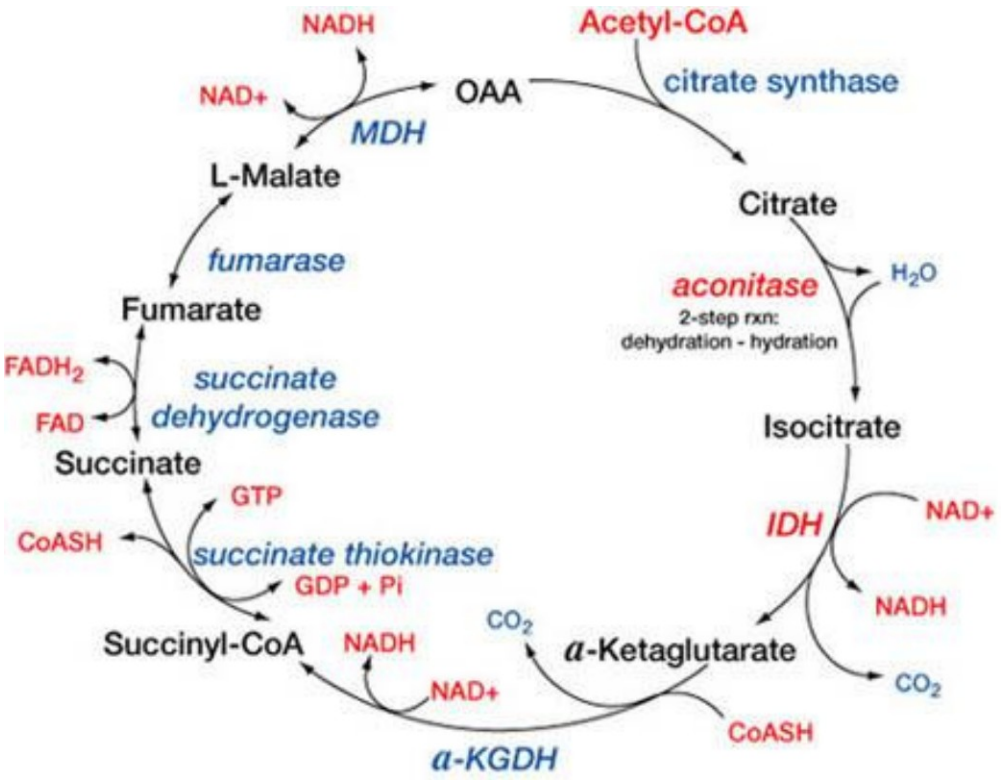
- important regulatory step of energy metabolism; main site of regulation = **E1 subunit**
- **inhibited in the phosphorylated form** (PDH kinases / PDH phosphatases)
  - **insulin activates** PDH (dephosphorylation)
  - in starvation, some tissues (e.g. skeletal muscle) change PDK/PDP levels → less pyruvate catabolism, more use of lipids
- inhibited when **ATP/ADP and NADH/NAD^{+}** ratios rise; allosteric inhibition by the product **acetyl-CoA**

Defect of the PDH complex

- a **mitochondrial disease** (mutations in nuclear genes for mitochondrial enzymes or in mtDNA); several forms, most often E1, different inheritance
- **lactic acidosis**, damage to the nervous system
- limited therapy – increase energy intake from lipids: **ketogenic diet**

- **citrate synthase** - catalyses the first reaction of the Krebs cycle – **condensation of oxaloacetate and acetyl CoA**
- ATP is not required for the condensation of oxaloacetate and acetyl-CoA under citrate synthase, on the contrary - if ATP is present, the Krebs cycle is generally inhibited (with sufficient energy, the cycle is not necessary)

- **aconitase** - converts citrate into **cis-aconitate (dehydration)** and then cis-aconitate to **isocitrate (hydration)**
- **Isocitrate dehydrogenase** - **dehydrogenates** isocitrate to **α-ketoglutarate** (= **2-oxoglutarate**)
  - the rate of reaction depends on the concentration of ATP and NADH -> excess of ATP or NADH means that the reaction will proceed slowly (again the excess of ATP and NADH indicates enough energy and therefore no need to produce more)
  - this reaction generates NADH + H
- **α-ketoglutarate dehydrogenase**- α-ketoglutarate -> **succinyl-CoA + NADH + H^+**
  - catalyses **oxidative decarboxylation** with simultaneous binding of α-keto carbon to CoA and formation of NADH + H +
  - the same mechanism as the oxidative decarboxylation of pyruvate
  - coenzyme **thiamine pyrophosphate** is required
- **succinyl -CoA-synthetase** – cleaves attached CoA and at the same time generates **GTP** (GDP+Pi -> GTP)
  - stupid nomenclature of the enzyme, because it does not synthesize succinyl-CoA, but breaks it down
  - = **substrate-level phosphorylation** – cleavage of the thioester bond of Suc~CoA with water
  - can be **bypassed** in the degradation of ketone bodies: acetoacetate + Suc~CoA → succinate + acetoacetyl~CoA (no GTP, but the ketone bodies are activated)
- **succinate dehydrogenase** - **Flavin enzyme** with firmly bound z**FAD** and non-heme **iron**
  - succinate dehydrogenase produces **fumarate**
  - the reaction is carried out with simultaneous transfer of hydrogen to FAD, which is reduced to $FADH_2$
  - succinate dehydrogenase is also **complex II in the respiratory chain**
  - practically irreversible in the body; transfers 2 e^- to coenzyme Q, then to complex III; cofactors **FAD (vit. B2) and Fe-S clusters**
- **fumarate hydratase** - **hydrates** fumarate into **malate**
- **malate dehydrogenase** - **dehydrogenates** malate into **oxaloacetate**
  - oxaloacetate and fumarate link the citrate cycle to the urea cycle
  - oxaloacetate can also be used for glucose synthesis and aspartate (aspartic acid) synthesis



2.9.2 Involvement in intermediate metabolism





Sources of acetyl-coA

- **Coenzyme A** is only a carrier of acetyl, it does not degrade during metabolism and can occur freely
- source of acetyl-CoA may be, for example, glycolysis occurring in the cytoplasm
  - 1 glucose -> 2 pyruvates that enter the mitochondria and pyruvate dehydrogenase create acetyl-coA from them (see above)
- **β-oxidation of fatty acids** occurring in mitochondria
  - carries much more energy than glycolysis
- **amino acids** - degradation of some AA produces pyruvate, others produce directly Krebs cycle intermediates
  - alanine -> pyruvate; aspartate -> oxaloacetate...

Cataplerotic reactions

- these are reactions that **deplete** the Krebs cycle intermediates
- if ATP is not needed, the Krebs cycle intermediates will begin to convert to other useful compounds
  - **citrate** - exits from mitochondria into the cytoplasm where it cleaves to acetyl-CoA + oxaloacetate
    - **acetyl-CoA** is used for the synthesis of fatty acids and steroids
    - **oxaloacetate** returns to mitochondria or is transaminated to aspartate
  - **α-ketoglutarate** -> its transamination forms **glutamate** (also the formation of glutamine, histidine ...etc.)
  - **succinyl-CoA** -> serves for **porphyrin** synthesis (=cyclic organic compound consisting of 4 pyrrole nuclei)
  - **malate, oxaloacetate** -> either converted to **AA** (aspartate), or gluconeogenesis produces **glucose**

Anaplerotic reactions

- anaplerotic reactions are reactions that **produce citrate cycle intermediates**
  - typical example is the **synthesis of oxaloacetate from pyruvate** - pyruvate + CO_2 + ATP -> oxaloacetate + ADP + Pi
    - catalysed by the enzyme **pyruvate carboxylase** with the participation of **biotin cofactor**
    - this is also the **1st step of gluconeogenesis**; biotin (vit. B7) is the cofactor of carboxylases
- the Krebs cycle is connected to the respiratory chain, amino acid metabolism, urea cycle, heme synthesis, gluconeogenesis and fatty acid synthesis – anaplerotic reactions replace the intermediates that are "lost" to these pathways
  - **metabolism of amino acids**
    - aspartic acid (Asp), asparagine (Asn) -> **oxaloacetate**
    - glutamic acid (Glu), glutamine (Gln), histidine (His), proline (Pro), arginine (Arg) -> **α-ketoglutarate**
    - valine (Val), threonine (Thr), methionine (Met) -> **succinyl-CoA**
    - phenylalanine (Phe), tyrosine (Tyr) -> fumarate + **acetylCoA**
    - alanine (Ala), serine (Ser), cysteine (Cys), glycine (Gly) -> **pyruvate**
  - **Fatty acids with an odd number of carbons** -> after β-oxidation remains **propionyl-CoA**, which is converted to **succinyl-CoA**

Regulation of Krebs cycle

- is closely related to the respiratory chain, the consumption of ATP and the consumption of reduced cofactors
- **activation - low** ATP/ADP or NADH/NAD
- **inhibition - high** ATP/ADP or NADH/NAD
- Krebs cycle regulatory enzymes = **citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase**

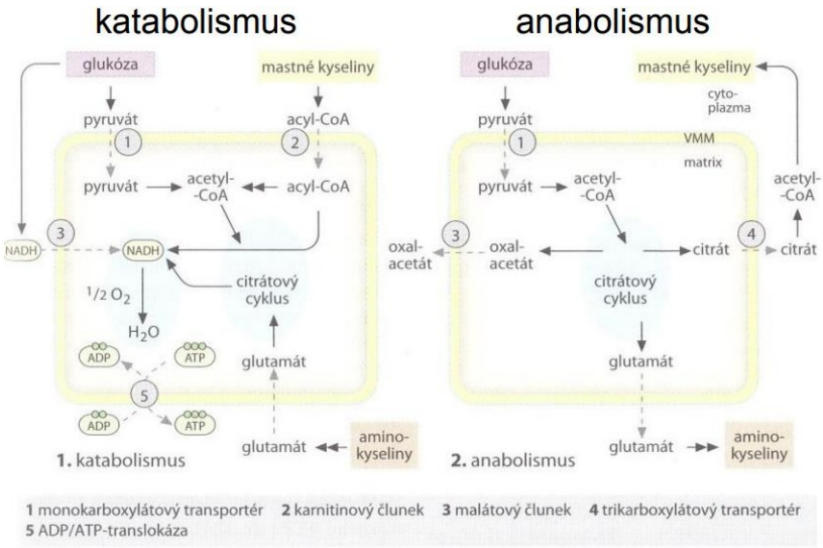


- **energy control (ATP/ADP)** – excess energy inhibits isocitrate DH and α-ketoglutarate DH (**ATP inhibits, ADP activates**)

- **respiratory control (NADH/NAD^{+})** – accumulation of NADH + H^+ (when the RC does not reoxidize it) inhibits isocitrate DH and α-ketoglutarate DH
- **substrate control of citrate synthase** – it produces as much citrate as it is supplied with OAA and acetyl-CoA
- Krebs cycle activity is closely linked to **oxygen availability**

2.9.3 Transport of metabolites

- outer membrane of mitochondria has a lot of pores, which makes it quite permeable
  - inner membrane tightly regulates transport of substances and is almost impermeable -> carriers are used for transport of larger molecules
- matrix is charged negatively (OH groups), intermembrane space charged positively (proteins)
    - the membrane potential ranges from 180 to 200 mV
  - molecules that can cross the membrane
    - **free diffusion - O_2, CO_2 a H_2O**
    - **antiport - pyruvate and H_2PO_4 for OH^-, ATP for ADP, Ca^{2+} for $2H^+$**



Shuttles

- **specific mechanism by which substances that would not normally pass through the inner mitochondrial membrane are transferred**

- the inner membrane has transporters for only about **50 substances**
- shuttles transfer **reducing equivalents (e^{-})** from cytosolic NADH + H^+ into mitochondria – NADH itself is not transferred (indirect pathway)
  - **malate-aspartate shuttle** – more important; forms NADH + H^+ in the mitochondria
  - **glycerol-phosphate shuttle** – less important, less efficient; transfers e^- to ubiquinone (via $FADH_2$)

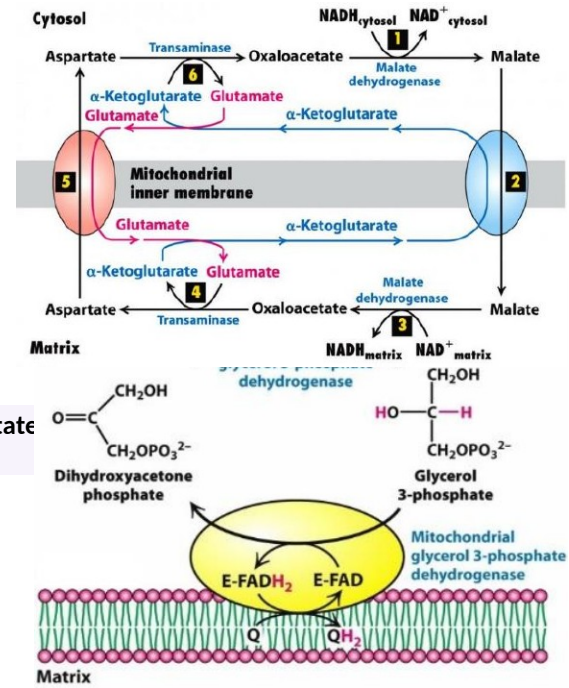
Malate-aspartate shuttle

- ensures the transfer of NADH from the cytoplasm to the mitochondria

1) in the cytoplasm NADH transfers hydrogen to oxaloacetate, resulting in malate formation
2) malate enters the matrix by antiport with α-ketoglutarate
3) malate passes hydrogen to NAD, oxaloacetate is formed again
4) transamination → oxaloacetate becomes aspartate and glutamate becomes α-ketoglutarate
5) aspartate enters the cytoplasm with the glutamate antiport
6) second transamination → aspartate becomes oxaloacetate and α-ketoglutarate becomes glutamate

- **result = in the cytosol less NADH and in the matrix more NADH**

- enzymes: **malate dehydrogenase** (cytosolic and mitochondrial), **aspartate** compartments; amino group donor glutamate/α-ketoglutarate



Glycerol phosphate shuttle

- the way by which cytosol rapidly regenerates NADH to NAD in glycolysis
  1) NADH in cytoplasm converts dihydroxyacetone phosphate to glycerol-3-phosphate
  2) glycerol-3-phosphate transfers hydrogen to mGPD (mitochondrial glycerol-3-phosphate dehydrogenase)
     - mGPDH has FAD as a prosthetic group, which then reduces coenzyme Q in the respiratory chain
- **result = oxidized NAD in the cytoplasm and reduced coenzyme Q in the respiratory chain**

- used e.g. in **skeletal muscle and CNS**
- glycerol-3-phosphate passes through the outer membrane into the intermembrane space, where it is reoxidized by the enzyme of the inner membrane (FAD is part of the enzyme)
- = alternative entry of NADH electrons into the RC, **bypassing complex I** → lower energy yield

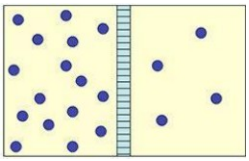
2.10 EPITHELIAL TISSUES III - BARRIERS AND TRANSPORT

MUDr. Eva Maňáková, Ph.D.

Characteristics of epithelial tissue

- Predominantly cells over extracellular matrix
- high adhesivety of cells
- polarization of cells
- avascular tissue - tissue nourishes by diffusion from the connective tissue under the basement membrane
- Strong nerve innervation
- high regenerative ability due to stem cells in the basement membrane
- originates from all 3 germ layers - ectodermal epidermis, endodermal glands, mesodermal endothelium

Diffusion barrier



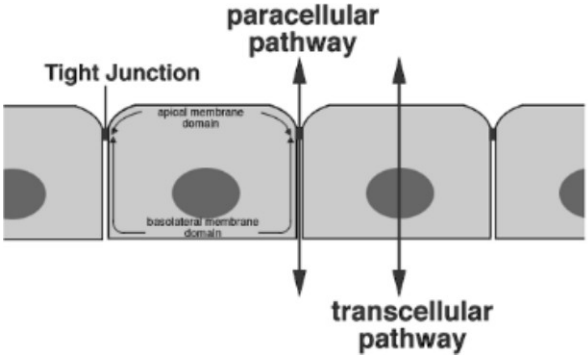
- one of the most important functions of the epithelium is to create compartments in the body and prevent free diffusion
- examples - intestine, kidneys, exocrine glands, brain capillaries (haematoencephalic barrier), plexus choroideus (barrier blood-liquor), thymus, testes

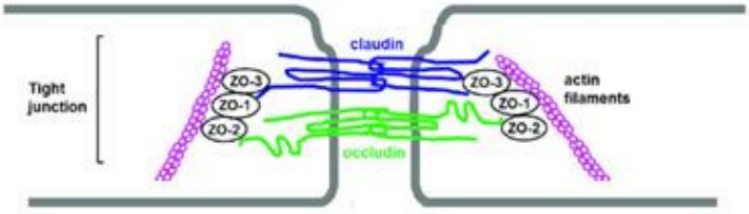
Junctional complex

- **zonula occludens (tight junction)** cells leave no space between themselves
  - serves for compartmentalization of surface plasmalema in resorptive epithelial cells
- zonula adherens (adhesive connection) - strong and mechanically resistant band connection using cell actin fibres
- macula adherens (desmosome) - strong and mechanically resistant point connection using intermediate filament cells
- other junctions: **gap junction** (communication) and **hemidesmosome** (anchoring the cell to the basement membrane)

Zonula occludens

- proteins claudins and occludins via adapter proteins connected to actin filaments of neighbouring cells
- **prevents the paracellular passage of substances => all substances must travel transcellularly**
- permeability varies among different epitheliums
  - impermeable - collecting ducts of kidneys, urothelium, capillaries in the brain
  - almost permeable - small intestine, proximal tubulus of the kidney





Basement membrane

- mediates interaction between cell and matrix
- anchors cells
- attached to connective tissue via anchor fibrils
- consists of proteins and proteoglycans
  - **lamina basalis** ensures attachment of cells to the basement
    - **lamina rara (lucida)** - transmembrane proteins syndecan or integrins connect cell and rara lamin
    - **lamina densa** - it consists of collagen IV, laminin, perlecan
  - **lamina fibroreticularis** - contains microfibrils and anchor fibers (fibrillin and collagen VI and VII), which fixate the basal lamina to the fibres of collagen III (connective tissue) under the basement membrane
- special basement membranes are made of two basement membranes (kidneys, lungs)

2.10.1 Diffusion barriers

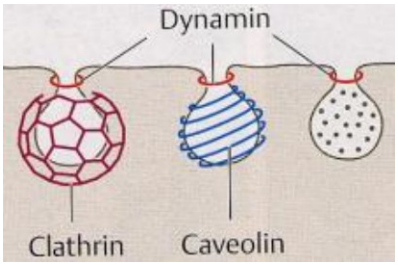
Types of epithelial transmission

- simple diffusion - O_2, CO_2, NO
- passive transport (facilitated diffusion) - carriers, ion channels, aquaporins (for water)
- active transport - pumps (ATPases), the most important is Na / K ATPase
- for larger molecules - endocytosis (pinocytosis, phagocytosis), transcytosis (transport of substances from the apical membrane to the basal or vice versa) and exocytosis
- **aquaporins** - transmembrane proteins with a hydrophilic tunnel that facilitate diffusion of water
  - example in collecting duct in kidney, large intestine

Diffusion barrier in transporting epithelium

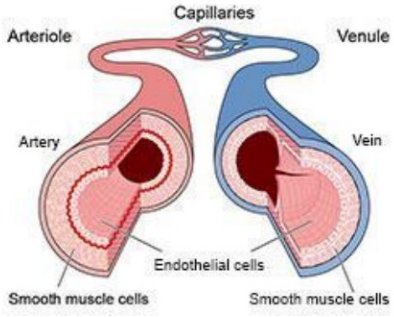
- impermeable junctions - ileum and colon, water and ions must pass transcellularly (or controlled)
- permeable junctions - duodenum and jejunum, there is an absorption of ions and nutrients
  - water passes paracellularly according to the concentration gradient

Pinocytosis



- runs through pinocytic vesicles -> the cytoplasmic membrane in invaginates and then gets excised
- some proteins trigger invagination of the plasmalema and the protein dynamin closes the sac
- clathrin-mediated endocytosis
  - Clathrin coat - adapter protein
  - the ingested molecule (LDL, transferrin) binds to the receptor
- Clathrin-independent endocytosis
  - caveola vesicles - numerous on endothelial cells
  - protein caveolin envelops the membrane
  - functions - endocytosis of viruses, transcytosis, start of signalling cascades (eg insulin)

Transcytosis



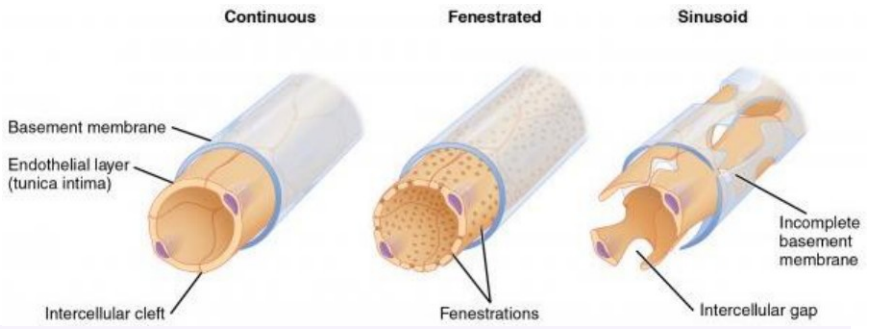
- transfer of macromolecules across barriers - endothelium
- transmission of antibodies across the placenta into the fetus - transplacental transmission, passively from the mother's body
- transmission of IgA antibodies through mucous membranes

Endothelium

- vascular lining - barrier between blood and tissues
- simple squamous epithelium
- it has different permeability - from very permeable in bone marrow to impermeable in brain
- endothelial function
  - gas exchange (diffusion), transport of substances, synthesis of vasoactive substances (mainly NO), vascular growth control, regulation of immune responses (facilitate the transfer of white blood cells to the site of inflammation) and participation in haemostasis

Transfer of substances through the endothelium

- transcellular - blood-brain barrier
- paracellular - intestinal epithelium
- **types of capillaries**
  - **continuous -** almost or completely impermeable
  - **fenestrated** - the endothelium has proteins that create holes and allow selective passage of substances
  - **sinusoid** there are large holes in both the endothelium and its basement membrane that allow free passage of virtually all substances in the blood

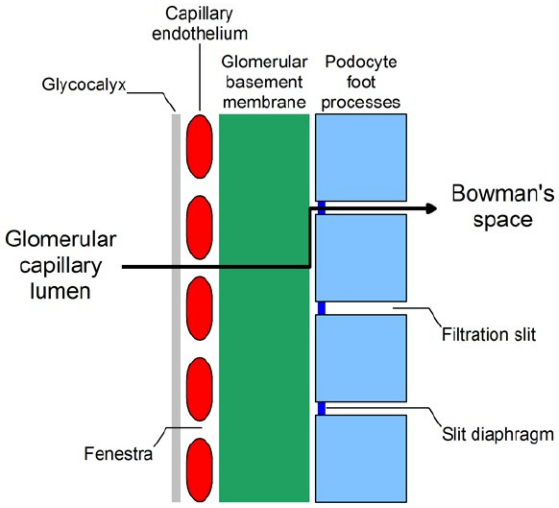


- capillaries by permeability (slides)
  - **continuous** (muscle, brain) – zonulae occludentes prevent paracellular transport; transcellular transport (transcytosis) possible
    - totally impermeable type (~100× less permeable + paracellular seal) – blood-brain (with glial cells), **blood-testis**, **blood-thymus** barrier
  - **fenestrated** (endocrine glands, kidney) – permeable for water and small hydrophilic molecules; filtration barrier (blood-

urine)
- **sinusoids** (liver) – permeable for all plasma components

Glykokalyx

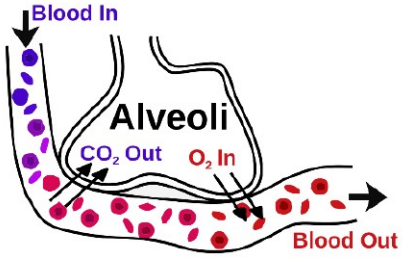
- glycoprotein and glycolipid layer, which covers the plasma membrane of some epithelium and other cells
- prevents blood clotting and white blood cells sticking
- allows cells of the immune system to pass to the sites of inflammation



2.10.2 Blood Barriers

Blood - primary urine barrier

- **on one side is a fenestrated endothelium**
- **on the other hand, there are cytoplasmic processes of podocytes**
- basement membranes of both are pivoted and pressed together - one thick basement membrane is formed, which forms most of the barrier
  - there is alot of heparan sulphate in this membrane, which is negatively charged, which repels proteins (as they are also negatively charged) and helps keep them in the blood
- the gaps between the protuberances of the podocytes are quite small and the vast majority of proteins have no chance of getting through
- albumin is just below the permeability limit - if the kidneys do not work as they should, albumin will get through and can be detected in the urine - albumin thus acts as an excellent marker of kidney function



- the basement membrane contains **laminin and heparan sulphate**

Blood-air barrier

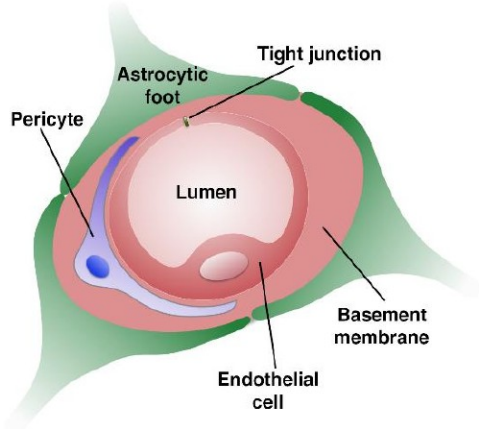
- **thin-walled barrier - two epithelial cells (alveoli epithelium + endothelium) attached to each other, connected by basement membranes**
- alveoli epithelium - single-layered flat epithelium (type I pneumocytes)
- continuous capillaries
- capillaries are continuous **somatic** type; **surfactant** is produced by **type II pneumocytes**

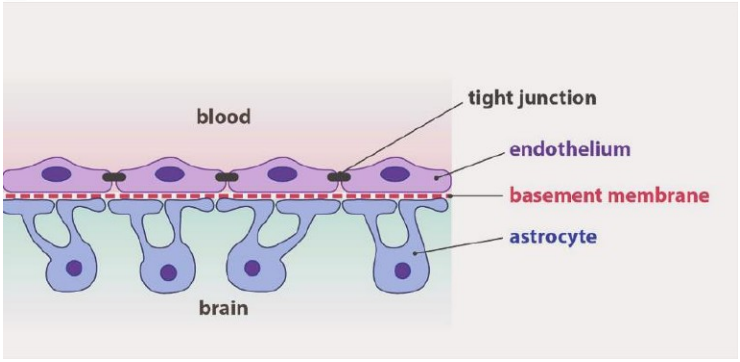
The blood-brain barrier

- BBB (Blood-Brain Barrier) - a barrier between the blood and the internal brain environment
- **several layers**
  - **endothelium**- continuous capillary
  - **basement membrane**
    - there may be pericytes in the basement membrane - a special cell type only for this occasion, helping to maintain BBB functionality and brain homeostasis
  - **astrocyte processes**- supporting glial cells, play a role in BBB and neuronal sheath formation
- only water, a few gases and fat-soluble molecules can pass through the BBB, everything else needs carriers
- is due to well-formed zonulae occludentes and a small number of caveol
- hydrophilic substances (glucose) cross only by carriers – **GLUT1**; the barrier also prevents crossing of neurotransmitters

Blood-testis barrier

- formed by **Sertoli cells** – creates a special microenvironment for the maturation of sperm cells



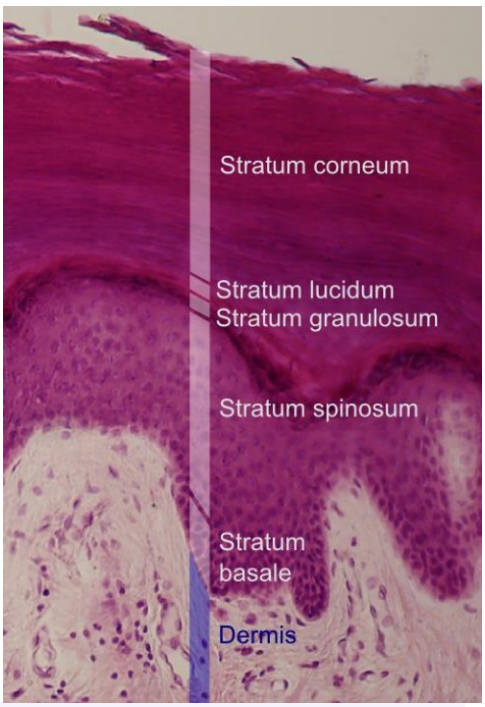


2.10.3 Multilayered epithelia as a barrier

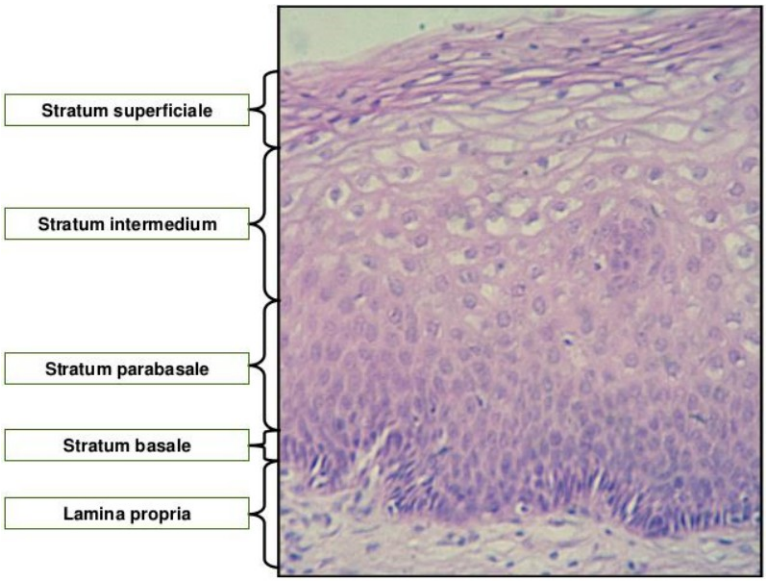
- multiple layers - basal, intermediate and superficial
- gradual cell differentiation - in the basal layer there are stem cells that proliferate and differentiate towards the surface
- tight junctions are present between the cells of the upper third of the epithelium (when they are up, they are not needed below)
- thick basement membrane
- the basal layer is of cubic or cylindrical cells
- **there is a fibrous layer under the basement membrane - either lamina propria or (in the skin) dermis**

Layered squamous epithelium

- **keratinised** - epidermis skin
  - stratum corneum - lucidum - granulosum - spinosum - basale
  - keratin is insoluble in water -> prevents water loss -> only lipophilic substances pass through the skin
    - in the stratum granulosum highest keratinization takes place and there is also a large number of zonulae occludentes
    - cytokeratin filaments and profilaggrin are involved in the production of keratin



- cells of the epidermis: **keratinocytes, melanocytes, Merkel cells, Langerhans cells**
- **non keratinized** oral cavity, oesophagus, vagina, cornea, conjunctiva, larynx
  - stratum basale - parabasale - intermedium - superficiale
  - **high glycogen content in superficial cells** bacteria metabolize glucose anaerobically to lactate and ensure low pH



Melanocytes

- protects against damage from UV radiation by melanin synthesis
- The melanocytes are present in the epidermis of the pars basalis
- melanin-containing granules are passed to cells in the stratum spinosum in which the granules disintegrate, and the melanin is released into the cytoplasm
- melanosomes (organelles) - from GER and GA - start of melanin formation
    - synthesis - dopa (enzyme tyrosinase) is synthesized from tyrosine, which polymerizes to melanin

Transient epithelium - urothelium

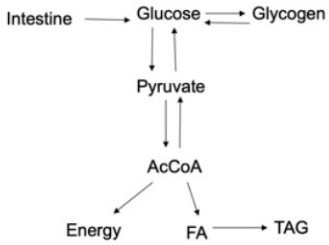
- location - urinary tract
- lots of tight junctions
- **surface cells (umbrelocytes)** are the largest cells in the urothelium, they have differently bent membrane
- the rest of the epithelium - low cubic cells
- Special protein in tight junctions - uroplakin - even increases their effectiveness
- The surface of the cells (umbrelocytes) becomes more stained due to the presence of cytokeratin filaments
- uroplakin - in the membrane
    - takes part in sealing as well

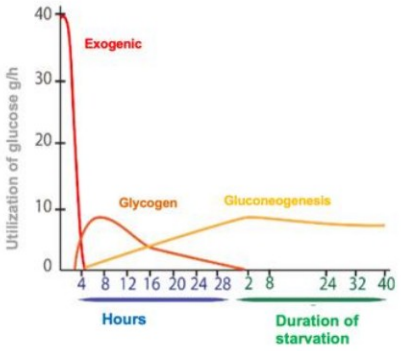
2.11 DEGRADATION AND SYNTHESIS OF GLUCOSE

MUDr. Josef Fontana

2.11.1 Glucose

- Central position in carbohydrate metabolism – all carbohydrates can be converted to glucose and vice versa
- Energy can be obtained from it even in the absence of O_2 **ın it**
- All of our cells are able to use it and **some tissues are even strictly dependent** ɔ not
  - **erythrocytes** – because they do not have mitochondria they therefore d use Krebs cycle or the respiratory chain of their
  - **cells of CNS** - however, during long-term starvation they adapt and 50% consumption is covered by ketone bodies





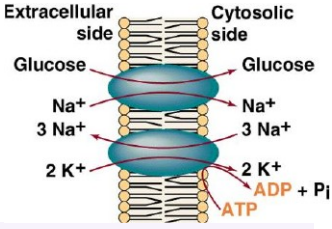
- all saccharides absorbed in the GIT can be transformed to glucose, and every saccharide in the body can be synthesized from it – a universal energy substrate
- Glc-6-P is the hub: → glycolysis (pyruvate), → glycogen, → pentose cycle

Glycaemia = blood glucose concentration

- **normal fasting value is 3,3-5,6 mmol/l**
  - while after a meal it can be up to 7,1 mmol/l even in a healthy person
- regulation of blood glucose
  - **insulin** – lowers glycaemia- glucose from blood enters cells
    - cells use up glucose through glycolysis, pentose formation or glycogen storage
  - **glucagon, adrenalin, growth hormone, cortisol** – increase glycaemia – glucose goes from liver to blood
    - in hepatocytes, glucose is formed de novo by gluconeogenesis or by degradation of glycogen storage
- sources of glucose - exogenous (food), glycogen breakdown and gluconeogenesis (production of glucose from other metabolites)

Glucose transport across membranes

- two different mechanisms
  - secondary active transport using SGLT-1,2 (sodium-glucose transporter)
    - glucose enters the cell by symport with sodium
    - secondary active because the cell must expend energy to get rid of the sodium that came with glucose
    - glucose is absorbed in the intestines and the proximal kidney tubule in this way
    - stoichiometry: **SGLT-1 = 1 Glc / 2 Na^{+}, SGLT-2 = 1 Glc / 1 Na^{+}**
  - facilitated diffusion through GLUT 1-7 transporters

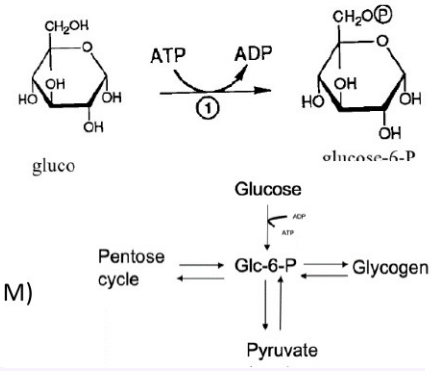


    - the slides list **GLUT 1–14** (GLUT 1–4 are the important ones)
      - used in the transfer of glucose between blood and cells
      - GLUT are channels in the cytoplasmic membrane that can open and allow glucose to pass freely across the membrane

- GLUT 1 - erythrocytes, blood-brain barrier
- GLUT 2 - liver, kidney, pancreatic β-cells, enterocytes
- GLUT 3 - brain
- GLUT 4 - adipose tissue, skeletal muscle, heart
  - **Insulin in the blood increases the amount of GLUT 4 transporters**
- GLUT-1 also in **fetal tissues**
- different K_M for glucose: **GLUT-2 ≈ 20 mM** (low affinity), **GLUT-1 ≈ 1 mM**, **GLUT-4 ≈ 5 mM**

Glucose phosphorylating and dephosphorylating enzymes

- as soon as glucose enters the cell, it is activated by enzymes and ATP through the phosphorylation from Glc to Glc-6-P **- irreversible reaction**
- Activation of glucose is the first step in its metabolism, while ensuring that glucose does not flush out of the cell (phosphate cannot cross the membrane)



M)

- phosphorylation turns the neutral glucose molecule into an **anion** – this activates it for further metabolism
- two isoenzymes phosphorylate glucose
  - **glucokinase** - in hepatocytes and pancreatic β-cells
    - active at higher glycaemia (K_M = 10 mM)
    - β- cells respond to higher blood glucose levels via insulin secretion
    - = **hexokinase IV**, the **"glucose sensor"**; slides give K_M ≈ **8 mM**
    - after a meal there is a high glucose concentration in the portal blood – the liver must take up glucose
  - **hexokinase** - everywhere except hepatocytes and pancreatic β-cells
    - active at much lower concentrations than glucokinase (KM = 0.1 m
    - = hexokinase I, II and III; highly active at low glycaemia
    - is inhibited by its product (Glc-6-P)

Dephosphorisation of glucose

- converts Glc-6-P back to glucose by cleavage of inorganic phosphate
- is present only in the liver, kidneys and enterocytes
- is in smooth ER - Glc-6-P gets here via translocase
  - o is in this cell compartment so that newly formed glucose does not immediately phosphorylate in the cytosol

2.11.2 Glycolysis

- catabolic reaction - conversion of glucose to 2 molecules of pyruvate (or lactate if we do not have enough oxygen)
- occurs in the cytoplasm of all cells with inorganic phosphate
- has two functions
  - energy production under anaerobic conditions - during glycolysis in addition to pyruvate, ATP is produced directly
  - source of acetylCoA - it can be used for everything possible
  - glycolysis is the only energy-producing pathway that also runs under anaerobic conditions – **erythrocytes, working muscle**
  - its acetyl-CoA is mainly a source for **fatty acid synthesis**

Course of glycolysis

- can be divided into 3 phases
  - **First phase - glucose will be converted to fructose-1,6-bisphosphate (Fru-1,6-PP) with the investment of two ATPs**
  - **1. step – glucose activation** - glucokinase or hexokinase enzyme, 1 ATP is consumed
    2. step - isomerization - enzyme isomerase converts Glc-6-P to Fru-6-P
    3. step - phosphorylation - 6-phosphofructo-1-kinase enzyme phosphorylates Fru-6-P to Fru-1,6-PP using 1 ATP
  - **Second phase - Fru-6-P breaks down into two identical three-carbon monosaccharides**
    4. step - cleavage - the enzyme aldolase A divides Fru-1,6-PP into glyceraldehyde-3-phosphate (Gra-3-P) and dihydroxyacetone phosphate (DHAP)
    DHAP is produced much more than Gra-3-P

5. step - isomerization - Triose phosphate isomerase enzyme converts DHAP to Gra-3-P

- **Third phase - Gra-3-P is converted to pyruvate, from which the cell obtains 4 ATP and 1 NADH**
  - since we now have two Gra-3-P molecules, the following reactions will run twice each

    6. step - phosphorylation - enzyme glyceraldehyde phosphate dehydrogenase phosphorylates Gra-3-P to 1,3-bisphosphoglycerate (1,3-PP-Gly), inorganic phosphate (Pi) enters the reaction and NAD is reduced to NADH

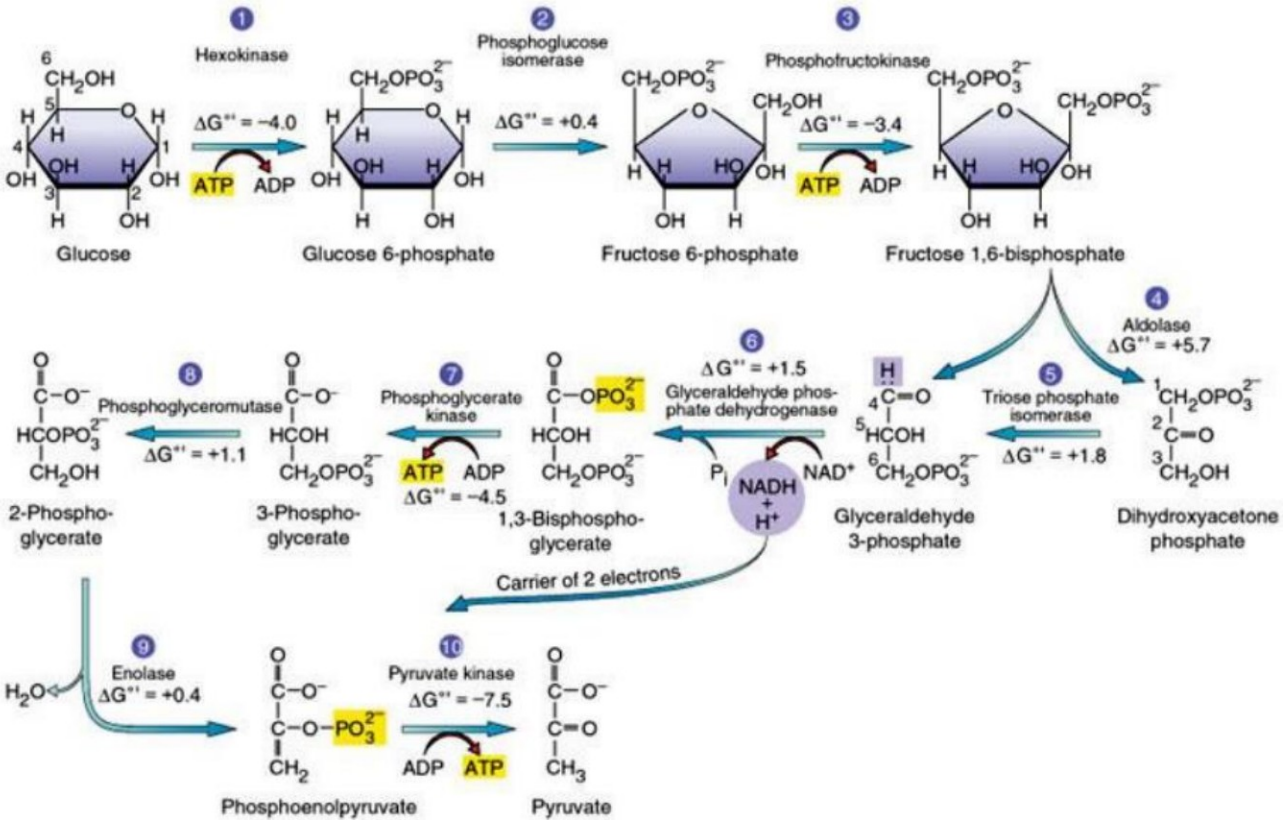
    7. step - dephosphorylation - phosphoglycerate kinase enzyme cleaves one phosphate from 1,3-PP-Gly to form 3-phosphoglycerate (3-P-Gly) and one ATP

    8. step - isomerization - phosphoglyceromutase enzyme converts 3-P-Gly to 2-P-Gly

    9. step - dehydration - enzyme enolase from 2-P-Gly cleaves water, phosphoenolpyruvate (PEP) is formed

    10. step - dephosphorylation - the pyruvate kinase enzyme cleaves the remaining phosphate, producing pyruvate and one ATP

- total 1 NADH and 2 ATP are extracted from glycolysis - two ATPs were invested in the first stage and two ATPs were obtained from each glyceraldehyde-3-phosphate in the third stage = -2 + 2 * 2 = +2



Metabolic fate of pyruvate

- branch point of glycolysis
- fate of pyruvate depends on oxidative and redox state of the cell (sufficient O_2 and NAD)
  - aerobic conditions - pyruvate enters the MIT matrix, where it is converted to acetylCoA
  - **anaerobic conditions**- pyruvate is converted to lactate (via enzyme lactate dehydrogenase) and released into the blood
    - this happens because under aerobic conditions NADH is reduced in mitochondria, becomes NAD and can again help with glycolysis
    - under anaerobic conditions, however, NADH accumulates until finally there is no NAD and glycolysis stops
    - when pyruvate is converted to lactate, NADH is converted to NAD and can immediately return to glycolysis
  - **aerobic glycolysis produces much more ATP than anaerobic glycolysis**
    - Pyruvate + NADH + H^+ <-> Lactate + NAD^+
    - there are no mitochondria in erythrocytes and so anaerobic glycolysis takes place there even under aerobic conditions

Regulation of glycolysis

- the regulatory points are 3 enzymes
  - **glucokinase/hexokinase** – activates glucose to Glc-6-P through using ATP
  - **6-fosfofrukto-1-kináza (PFK-1)** - phosphorylatesFru-6-P to Fru-1,6-PP through using ATP
    - the main regulatory enzyme
    - allosteric enzyme - the activator is Fru-2,6-PP (this molecule is produced by insulin)

        - is inhibited
            - if the cell has enough energy = high concentration of ATP or citrate in the cytoplasm, acidic pH
            - in the presence of counter-regulatory hormones
    - **pyruvate kinase** - dephosphorylates PEP to form pyruvate and ATP
        - all three regulatory enzymes catalyse **irreversible** reactions
- PFK-1 details (slides): **high ATP/AMP** ratio inhibits it; inhibited by **citrate and acidic pH (protons)**; **fructose-2,6-bisphosphate** (made from Fru-6-P by **phosphofructokinase-2**) is its activator; **activated by insulin**, inhibited by counter-regulatory hormones (glucagon etc.)

2.11.3 Glycogenosis

- **apart of the regulatory points, gluconeogenesis is the same as glycolysis, but reverse**
    - glycolysis regulatory points are irreversible - gluconeogenesis bypasses them (bypass 1, 2 and 3)
- gluconeogenesis precursors - pyruvate, lactate, glycerol, oxaloacetate, propionate and glucogenic AMK (Ala, Gln...)
- takes place in the kidneys, liver and a little in enterocytes
- **after depletion of liver glycogen it is the only source of glucose** for the tissues that need it – allows survival of longer starvation; activated in the morning after overnight fasting
- slide list of precursors: C3 and C4 compounds – lactate, pyruvate, glycerol, amino acids (Ala, Gln...), propionate (short-chain FA)
- glucose can only be released by cells that have **Glc-6-phosphatase** – hepatocytes, kidney tubule cells, enterocytes
- begins in mitochondria and then moves to the cytoplasm
- energetically consuming
    - 2 Pyruvates + 4 ATP + 2 GTP + 2 NADH + 2 H^+ + 4 H_2O → glucose + 4 ADP + 2 GDP + 6 Pi + 2 NAD

Bypass 1

- bypasses pyruvate kinase catalysed reaction (10th step of glycolysis)
- transport of pyruvate to mitochondria -> carboxylation of pyruvate (enzyme pyruvate carboxylase, cofactor biotin, consumption of one ATP) -> formation of oxaloacetate (OAA) -> transfer of OAA to cytosol -> conversion of OAA to PEP by PEP carboxykinase (consumption of GTP)
- OAA leaves the mitochondria by **transamination to aspartate** or **reduction to malate** (this also transfers NADH to the cytosol)
- OAA → PEP: **PEP carboxykinase (GTP-dependent)**
    - PEPCK location is species-specific: in **humans 50/50 cytosol/mitochondria**, in mouse/rat almost only cytosol; PEP has a membrane transporter

Bypass 2

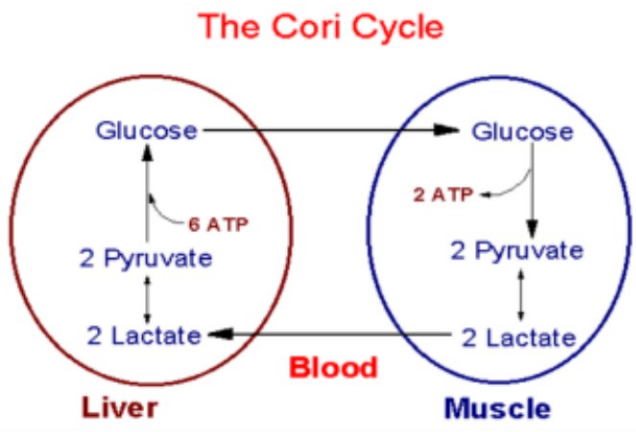
- bypasses PFK-1 catalysed reaction (3rd step of glycolysis)
- the enzyme fructose-1,6-bisphosphatase is used
- simply phosphate cleavage, no ATP formed, only inorganic phosphates

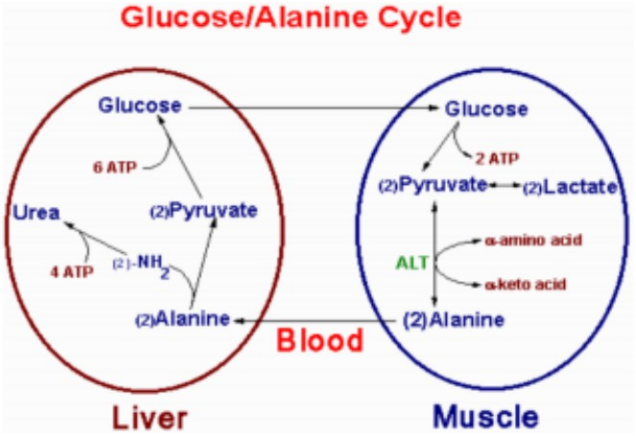
Bypass 3

- bypasses the reaction catalysed by hexokinase (1st step of glycolysis)
- enzyme Glc-6-phosphatase (not found in skeletal muscle)
- Pi just breaks off again

Substrates for gluconeogenesis

- Cori cycle
    - Method by which erythrocytes and muscles get rid of lactate - they send it to the liver to turn it back into glucose
- alanine cycle
    - the way energy is supplied to the muscles while also removing toxic ammonia
    - Ammonia is linked to pyruvate by transamination, resulting in alanine that travels to the liver, where ammonia is detached and processed into urea
    - pyruvate is converted to glucose by gluconeogenesis and sent back to the blood
- amino acids
    - all AA except Leu and Lys are able to degrade directly to pyruvate or Krebs cycle intermediates
- glycerol
    - glycerol phosphorylation to glycerol-3-P - enzyme glycerol kinase
    - dehydrogenation of glycerol-3-P to DHAP - enzyme glycerol phosphate dehydrogenase





Regulation of gluconeogenesis

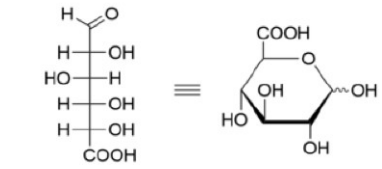
- active in glucose deficiency (starvation) and in pathological conditions (sepsis, polytrauma, burns, tumors ...)
- gluconeogenesis is activated by stress hormones (counter-hormone hormones), inhibited by insulin
- major regulatory enzymes
  - **pyruvate carboxylase** - activated by AcetylCoA
  - **PEP carboxykinase, Fru-1,6- bisphosphatase and Glc-6- phosphatase**- regulated in the same way as glycolysis, except vice versa
  - **Fru-1,6-bisphosphatase**: activated by **glucagon and citrate**, inhibited by **AMP and Fru-2,6-bisP**

2.12 PENTOSE PHOSPHATE CYCLE, METABOLISM OF FRUCTOSE AND GALACTOSE

Mgr. Vladimíra Kvasnicová

Use of monosaccharides in the body

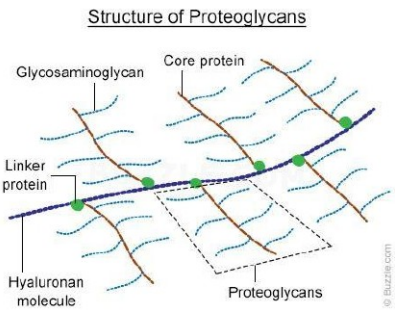
- glucose - energy production (glycolysis)
  - energy supply - glycogenogenesis / fatty acid synthesis
  - conversion to other monosaccharides - pentose cycle (to ribose)
  - conversion to glucuronic acid by oxidation
- fructose - conversion to glucose
- galactose - conversion to glucose / lactose
  - synthesis of glycoproteins and proteoglycans



D-glucuronic acid

Glucuronic acid

- it is glucose with oxidized 6. carbon
- was first isolated in urine, hence its name from Urine
- **biosynthesis proceeds by activation of glucose to UDP-glucose, followed by oxidation by enzyme UDP-glucose dehydrogenase to UDP-glucuronic acid**
- attaches to foreign hydrophobic substances in the liver to increase their solubility and to get out of the body via urine
- it further forms glycosaminoglycans, proteoglycans and proteoglycan aggregates
- **glycosaminoglycans (GAG)**
  - These are long chains formed by alternating two molecules - uronic acid and hexosamine
    - uronic acids are acids containing both aldehyde and carboxylic acid (glucuronic)
    - hexosamines are hexoses with one OH group replaced by an amino group
  - hyaluronic acid, chondroin sulphate, keratan sulphate, heparan sulphate, dermatan sulphate
- **proteoglycans (PG)** - proteins (core proteins) to which all GAGs are perpendicularly bound (protein 5%, sugars 95%)
  - fill the extracellular space and determine some properties - resistance to pressure, return of tissue to its original shape, joint lubrication, articular cartilage hydration, water retention, bone component
- **proteoglycan aggregate (PGA)**
  - hyaluronic acid to which proteoglycans are bound perpendicularly through binding proteins



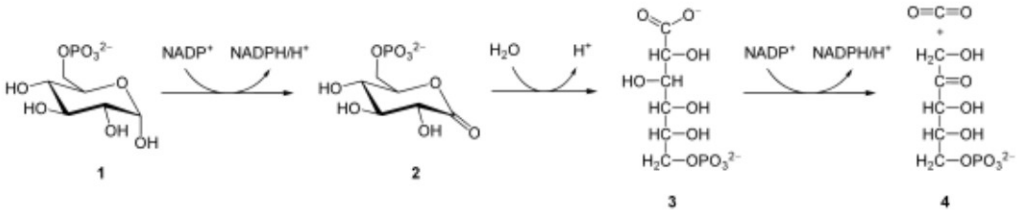
2.12.1 Pentose cycle

- alternative oxidative cleavage of glucose in the cell by NADP
- no ATP, NADH or $FADH_2$is produced
- the product of the pentose cycle is CO_2, ribose-5-phosphate and 2 NADPH - the pentose cycle is the main source of NADPH
- also called **hexose monophosphate shunt** – direct oxidation of glucose to CO_2 without the Krebs cycle and ETC
- located in the **cytosol**, mainly in hepatocytes, adipocytes, testes, adrenal cells, erythrocytes and the lactating mammary gland (enzymes present in every tissue)
- **functions of NADPH**: 1. reducing equivalents for syntheses (**FA, steroids**), 2. **antioxidant defence** (glutathione), 3. **biotransformation** reactions
- **main functions of the pathway**: 1. NADPH production – **the most important source of NADPH**, 2. ribose-5-P production (nucleic acid synthesis), 3. reciprocal conversion of monosaccharides (e.g. for glycoprotein synthesis)
- no ATP is formed – NADPH cannot be oxidized in the ETC
- the pentose cycle can be divided into 2 phases - oxidation (irreversible) and monosaccharide mutual conversion (reversible)
- cycle is regulated via NADPH - high NADPH / NADP ratio inhibits pentose cycle (NADPH is enough = no need to produce another)
- enzymes for the pentose cycle are also present in erythrocytes, because NAPDH is used in defence against oxygen radicals, which are much in erythrocyte - hexokinase (Fru -> Fru-6-P) and fructokinase (Fru -> Fru-1-P)

Oxidation phase

- **at this stage, Glc-6-P is converted to ribulose-5-phosphate (Ru-5-P) and CO_2 to form 2 NADPH**
- **dehydrogenation** - Glc-6-P -> 6-phosphogluconolactate, the enzyme glc-6-P dehydrogenase, results in NADPH
- **hydrolysis** 6-phosphogluconolactate + water -> 6-phosphogluconate, enzyme 6-phosphogluconolactonase
- decarboxylation - 6-fosfoglukonát 6-phosphogluconate -> Ru-5-P + CO2, enzyme 6-phosphogluconate dehydrogenase, NADHP is formed

- overall: **Glc-6-P + 2 NADP^{+} → CO_{2} + 2 NADPH + ribulose-5-P**
- **glucose-6-P dehydrogenase** – irreversible, the most important regulatory step; the rate of the pathway depends on the availability of **NADP^{+}** (excess NADPH slows the PPP)



Mutual transformations of monosaccharides

- **as several possible purposes**
  - **nucleotide synthesis**
    - if the cell carries out a pentose cycle to obtain ribose-5-phosphate for the construction of nuclides, then ribulose-5-P, which is formed in the oxidation phase, simply isomerizes to ribose-5-phosphate and thus ends
  - **synthesis of NADPH**
    - p if the purpose is to get NADPH for some other reaction, this phase is more difficult
    - first wait until the oxidation phase produces 3 Ru-5-P
    - then two 6C carbohydrates (Fru-6-P) and one 3C carbohydrate (glyceraldehyde-3-P) are formed from a series of 2C and 3C residues from three 5C carbohydrates (Ru-5-P)
    - Fru-6-P is then recycled to Glc-6-P and glyceraldehyde-3-P is sent for glycolysis / gluconeogenesis
  - **energy synthesis**
    - proceeds in the same way as NADPH synthesis, except that at the end Fru-6-P does not recycle but phosphorylates to Fru-1,6-PP and sends it to glycolysis
- **Carriers 2C and 3C residues ! the cleaved particle with keto group is always transferred from, ketose to aldose**
  - one Ru-5-P is converted to Ribose-5-phosphate, two Ru-5-P is converted to Xylulose-5-P
  - the enzyme transketolase transfers 2C residues
  - the transaldolase enzyme transfers 3C residues
  - the keto group is always transferred (from xylulose to sedoheptulose to fructose, and then from xylulose to erythrose)

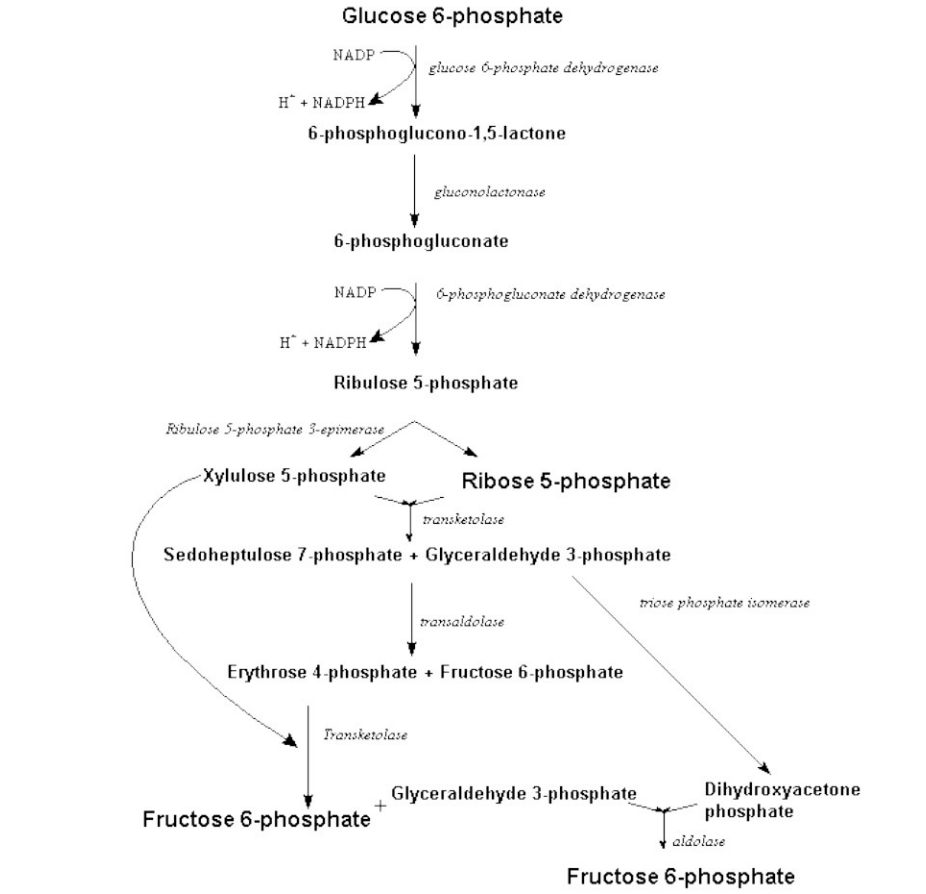
- basic scheme of the non-oxidative phase (freely reversible): **3 C5 → 2 C6 + C3** (3 ribulose-5-P → 2 fructose-6-P + glyceraldehyde-3-P)
  - C5 + C5 ⇌ C3 + C7 (xylulose-5-P + ribose-5-P ⇌ glyceraldehyde-3-P + sedoheptulose-7-P)
  - C3 + C7 ⇌ C6 + C4 (→ fructose-6-P + erythrose-4-P)
  - C4 + C5 ⇌ C3 + C6 (erythrose-4-P + xylulose-5-P ⇌ glyceraldehyde-3-P + fructose-6-P) – prevents accumulation of erythrose-4-P, which cannot enter glycolysis
- **transketolase** needs **thiamine diphosphate (vit. B1)**
- 2C and 3C units come from ketoses and are accepted by aldoses – the ketose becomes a shorter aldose, the aldose a longer ketose
- Glc-6-P formed from the products can enter the PPP again → "pentose **cycle**"
- maximal NADPH production: 6 Glc-6-P → 6 CO_2 + 6 ribulose-5-P + 12 NADPH + H^+; 6 ribulose-5-P → (non-oxidative phase + gluconeogenesis) → 5 Glc-6-P
- when the cell needs ribose but not NADPH, the non-oxidative phase runs **in reverse** – glyceraldehyde-3-P and fructose-6-P are drained from glycolysis to form ribose-5-P

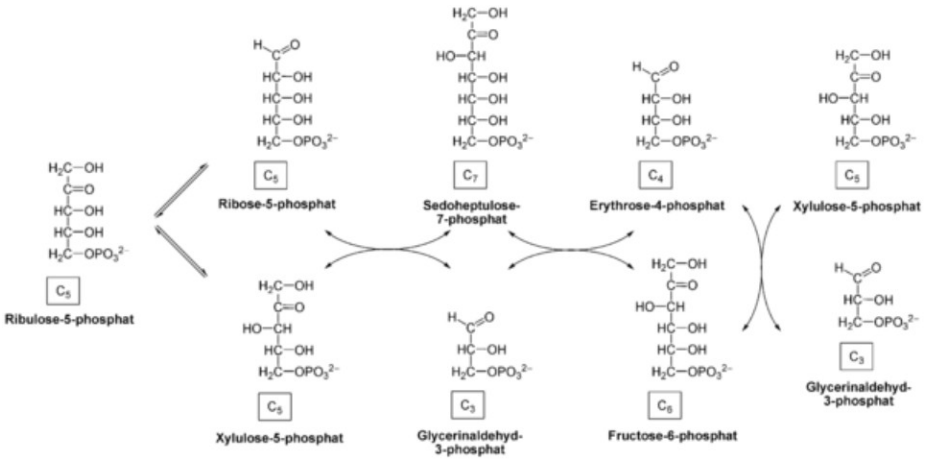
Regulation of the PPP

- by availability of **NADP^{+}** – unused NADPH accumulates → $NADP^+$ falls → glucose-6-P DH and 6-phosphogluconate DH slow down
- synthesis of the key enzymes is **induced by insulin and prolactin** (in lactation)

Glucose-6-phosphate dehydrogenase deficiency

- **the most widespread enzyme defect** – about **400 million** people (Africa, Mediterranean, Middle East, Asia)
- consequence: **haemolytic anaemia** (failure of antioxidant systems in erythrocytes)

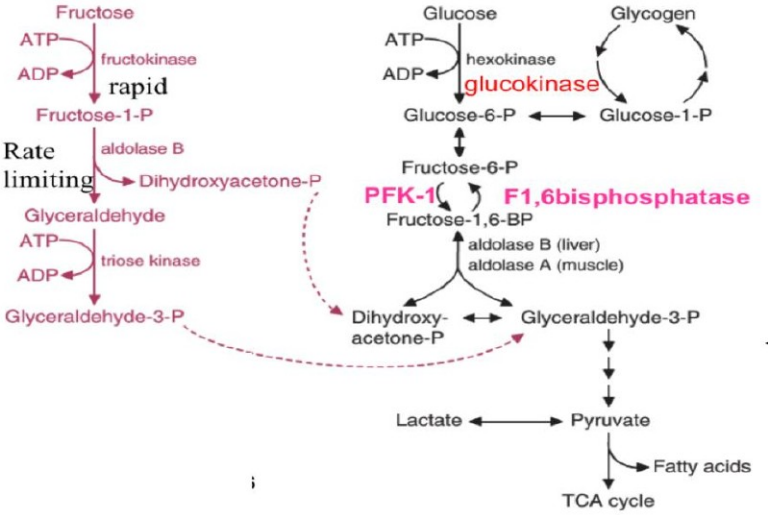


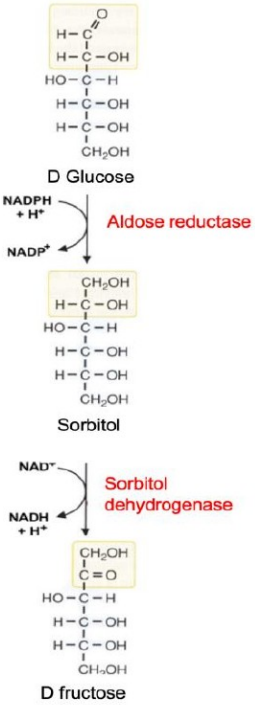


2.12.2 Fructose metabolism

- source of fructose in food - sucrose (disaccharide, glucose-fructose) from fruit
- fructose is activated by fructokinase (on Fru-1-P) or hexokinase (on Fru-6-P)
- sucrose is cleaved in the GIT by **sucrase**; fructose enters enterocytes by facilitated diffusion via **GLUT-5**; a minor part becomes glucose in the enterocytes, most goes to the portal blood
- **liver**: fructose + ATP → fructose-1-P (**fructokinase**); Fru-1-P is not a glycolytic intermediate – it is split by **aldolase B** (≠ aldolase A of glycolysis) to DHAP + D-glyceraldehyde
  - DHAP → glyceraldehyde-3-P (triose phosphate isomerase) → glycolysis
  - glyceraldehyde → glyceraldehyde-3-P (specific kinase) → glycolysis, **or** → glycerol → glycerol-3-P → **TAG**
- **fructose bypasses PFK-1** (the slowest reaction) → it is metabolised faster than glucose and can increase **lipogenesis** in the liver (excess FA, glycerol-3-P, TAG)
- **other tissues** (e.g. muscle): fructose + ATP → fructose-6-P by **hexokinase** – direct glycolytic intermediate, but hexokinase has a high K_M (low affinity) for fructose
  - Defective fructokinase disease is essential fructosuria and is essentially asymptomatic

- there is an aldolase B in the liver that can cleave Fru-1-P into glyceraldehyde (which is immediately phosphorylated to glyceraldehyde-3-P) and DHAP, thereby forming glycolysis intermediates while skipping the major limiting factor of glycolysis (a reaction catalyzed by phosphofructokinase 1) - **fructose is thus metabolised much faster in the liver than glucose**
  - defective aldolase B disease is hereditary fructose intolerance and is very dangerous
    - accumulation of Fru-1-P and disturbed saccharide metabolism
- fructose does not increase insulin production, its entry into cells is independent of insulin (using GLUT 2 and 5 transporters)



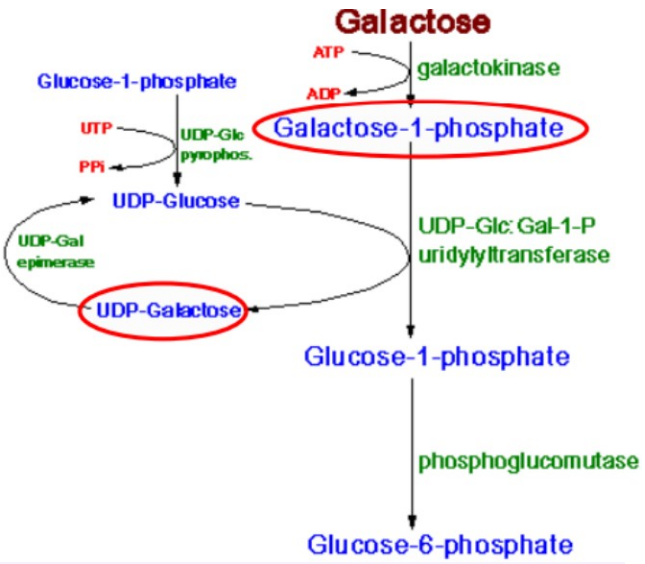


Sorbitol (glucitol)

- a sugar alcohol produced by reducing the carbonyl group of fructose or glucose
- artificial sweetener E420
- glucose is naturally metabolized by the enzyme aldose reductase to sorbitol (NADPH is needed)
  - important mainly in the liver, retina, lens, peripheral nerves and kidneys - sorbitol helps to maintain water
  - there is a problem in patients with hyperglycaemia - changing osmolarity causes cataracts, peripheral neuropathy, renal and retinal damage and vascular problems
- sorbitol is further oxidized to fructose by the enzyme sorbitol dehydrogenase (NAD needed)
  - important in liver and sperm (sperms get energy from fructose)

2.12.3 Galactose metabolism

- dietary source - lactose (disaccharide, galactose-glucose)
- galactose = **4-epimer of glucose**; lactose is cleaved by **lactase**; glucose and galactose enter enterocytes by **SGLT-1**
  - non-insulin-dependent entry into cells
  - it is mainly metabolised to glucose in the liver
    - **phosphorylation**- galactose + ATP -> Gal-1-P + ADP, enzyme galactokinase
    - **transfer UDP** - Gal-1-P + UDP-Glc -> UDP-Gal + Glc-1-P, enzym uridyltransferáza
  - **isomerization**- UDP-Gal -> UDP-Glc, enzyme epimerase
  - galactokinase or uridyltransferase defect = galactosemia



- (slides: **hexose-1-phosphate uridyltransferase** defect = classic galactosemia) – galactose accumulates in the liver,

erythrocytes, gut, eye lens, kidney, heart and brain
- galactose is reduced to **galactitol (dulcitol)** → **cataract**
- therapy – **absolute exclusion of milk products**
- **CAVE: not to be confused with lactase deficiency (lactose intolerance)**
- use - glycoproteins, glycolipids, GAG, breast milk lactose

- Glc ⇌ Gal interconversion happens only at the level of **UDP-derivatives**: UDP-glucose ⇌ UDP-galactose (4-epimerase); UDP-Gal is a macroergic compound
- lactose synthesis only in the lactating mammary gland – UDP-Gal + Glc (**galactosyltransferase**; lactation promoted by prolactin)

2.13 LIPID BREAKDOWN, KETONE BODIES

MUDr. Josef Fontana

Energy Importance of Triacylglycerols (TAG)

- lipids are a very large group of substances and can be divided into many groups
  - Lipids with energetic importance are called **triacylglycerol = TAG** (we are not discussing phospholipids etc.)
- store much more chemical energy than sugars and proteins - fats are hydrophobic, i.e. they do not bind water and are not diluted by it (1g TAG is 100% fat, 1g glycogen is glucose + water is bound to it)
  - 1g TAG has 6 times more energy than 1g glycogen
  - via complete oxidation of 1g of FA, 38 kJ is obtained, while from 1g of carbohydrates or proteins only 17 KJ
- an average 70kg male has 400,000 kJ in TAG, 100,000 kJ in protein (muscle), 2,500 kJ in liver glycogen and 170 kJ in glucose
  - supplies of glycogen and glucose are sufficient for one day, TAG for weeks
- the total weight of TAG in a healthy person is around 15% of the body weight
  - the main site of TAG accumulation is the cytoplasm of adipocytes (fat cells)

2.13.1 Lipid breakdown

Lipids as a source of energy

- **hormone-sensitive lipase (HSL)** releases fatty acids from stores
  - HSL performs complete lipolysis - 1 TAG -> 1 glycerol + 3 FA
  - is inhibited by insulin and activated by counter-regulatory hormones (glucagon, adrenaline, noradrenaline)
  - HSL catalyses the **rate-limiting step** of lipolysis (together with other lipases)
- lipid utilization takes place in three steps
  - **lipid mobilization** - hydrolysis of TAG to FA and glycerol and their transport by blood
  - **activation of FA in** cytosol and their transport to MIT matrix
  - **β-oxidation** - degradation of FA to acetyl-CoA, which enters the Krebs cycle
    - at the same time, reduced cofactors are produced, which are used directly in the respiratory chain

Mobilization of lipids

- TAG is broken down into glycerol and FA and released from the cell into the blood
  - FA longer than 12C need to have a carrier in plasma (because they are hydrophobic) - they bind to albumin
  - glycerol is used in the liver (glycolysis / gluconeogenesis /...)
- conversion of glycerol to glycolysis intermediates
  - **Glycerol is the only component of TAG that can be converted to glucose through gluconeogenesis**
    - first phosphorylation on glycerol-3-P using ATP (enzyme glycerol kinase)
    - then oxidation of glycerol-3-P to dihydroxyacetone phosphate using NAD + (enzyme glycerol-3-P dehydrogenase)
- entrance of FA into cells
  - Short chain FA (<12C, do not need albumin) - they penetrate the membrane by simple diffusion
  - Longer-chain FA's use different transport systems in the membrane - this is facilitated diffusion
    - FATP - fatty acid transport protein
    - FAT/CD36 - fatty acid translocase

FA activation

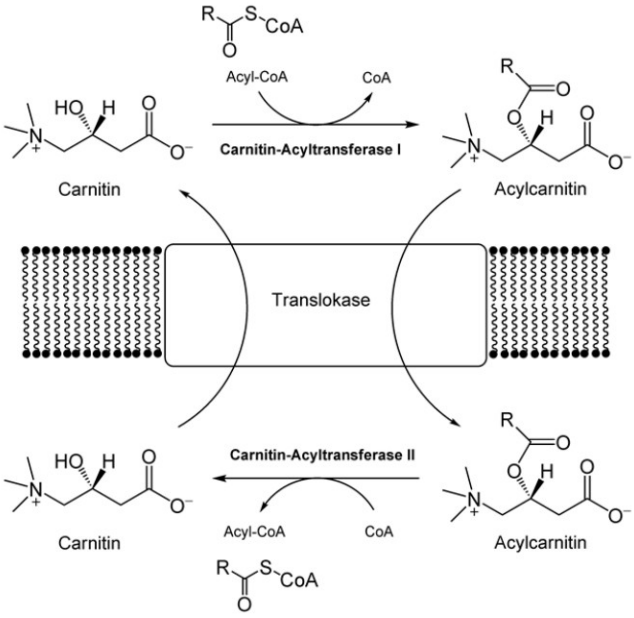
- involvement of FA in metabolism
- in cytosol FA is activated by CoA to acylCoA (enzyme acyl-CoA synthetase)
  - FA + ATP + CoA -> acylCoA + AMP + 2Pi
  - activation takes place in the cytosol and maintains a constant concentration gradient of FA into the cell
  - the resulting bond is between the acid and the sulphur atom CoA = thioester bond

Oxidation of fatty acids

- β-oxidation occurs in the matrix of mitochondria and is the most common fate of activated FA (the so-called majority pathway)
- ER membranes contain enzymes that catalyse ω- (omega) and α- (alpha) oxidation
  - These oxidation pathways are called minority pathways, as they normally only consume about 5% of FA, but in pathological situations their importance massively increases
  - The Greek letters designate the carbon atom on which the reaction takes place
  - take place in HER hepatocytes and tubular kidney cells on FA with 10-12C - dicarboxylic acids are formed
- short and medium FAs (below 12C) enter the mitochondria by simple diffusion
- the long chain (12-18C) must be activated and transferred using the carnitine chain
- very long (20C or more) enter the peroxisome where they are shortened

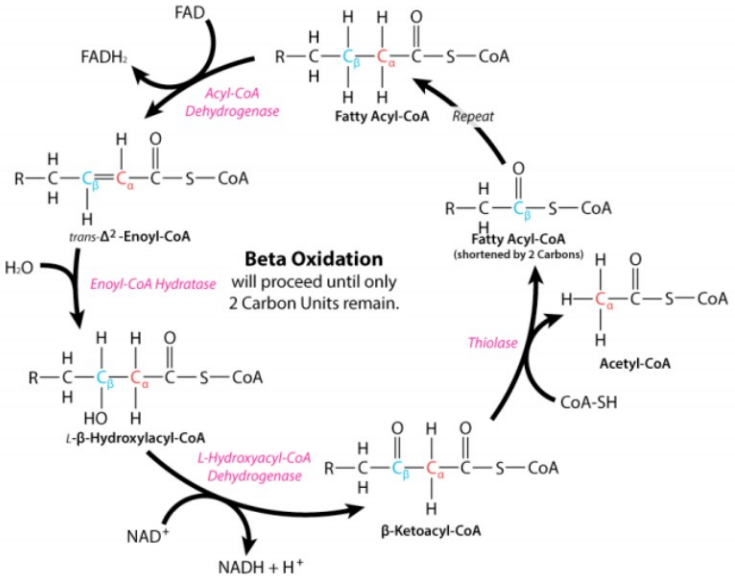
Entrance of FA into mitochodnrial matrix

- acyl-CoA passes through the outer membrane easily, inner membrane causes a little bit more trouble – carnitine and its translocases come into play here
  1) FA leaves CoA and attaches to carnitine
  2) **carnitinacyltransferase I (CAT I)** – on cytosolic side of mitochondrial membrane
     - transfers acyl from CoA to carnitine, resulting in acylcarnitine
  3) **acylcarnitine translocase** - in the inner mitochondrial membrane
     - Acylcarnitine is exchanged, via antiport, for carnitine
  4) **Carnitinacyltransferase II (CAT II)** - in matrix mitochondria
     - transfers acyl from acylcarnitine back to CoA, produces acylCoA
- free carnitine leaves matrix, via antiport, for acylcarnitine



β-oxidation of fatty acids

- a cyclical process, repeating itself
- at each turn, the carbon chain is shortened by two carbons, which they release as acetyl-CoA
- one cycle has four steps
  - **dehydrogenation- acyl-CoA dehydrogenase enzyme**
    - acyl-CoA + FAD -> 2-trans-enoyl-CoA + $FADH_2$
      - formation of trans double bond between 2nd and 3rd carbon
      - different dehydrogenases for short (4-6C), middle (6-10C) and long (12-18C) FA
      - the prosthetic group of acyl-CoA dehydrogenase is **FAD**; the reaction is stereospecific (only trans-enoyl-CoA is formed)
  - **hydration- enoyl-CoA hydratase enzyme**
    - 2-trans-enoyl-CoA -> L-3-hydroxyacyl-CoA
      - Hydration of the double bond that occurred in the previous step
      - OH group is formed on 3rd carbon
  - **dehydrogenation L-3-hydroxyacyl-CoA dehydrogenase enzyme**
    - L-3-hydroxyacyl-CoA + NAD -> 3-ketoacyl-CoA + NADH
      - Oxidation of the hydroxyl group on the 3 carbon to a keto group
  - **thiolysis- β-ketothiolase enzyme**
    - 3-ketoacyl-CoA + CoA -> AcylCoA + AcetylCoA
      - the SH group of HS-CoA attacks the β-keto carbon = **thiolytic cleavage**
- one turn of β-oxidation: **acyl-CoA + FAD + NAD^{+** + HS-CoA → acyl-CoA (2 C shorter) + $FADH_2$ + NADH + H^+ + AcCoA}
  - most FA have an even number of C – the last turn converts **butyryl-CoA to 2 AcCoA**
    - AcylCoA, which is formed here is two carbons shorter than AcylCoA, which entered the cycle
    - when cleaving 3-ketoacyl-CoA (has 4C), it is divided into 2 AcetylCoA = end of β-oxidation
- resulting reduced cofactors (NADH, FADH2) are used directly in the respiratory chain
- equation of the yielf of FA β-oxidation
  - FA long X carbons - X/2-1 turns of β-oxidation, X/2-1 $FADH_2$, X/2-1 NADH and X/2 AcetylCoA
    - example - stearic acid (18C) - 8 turns of β-oxidation, 8 FADH2, 8 NADH and 9 AcetylCoA



Regulation of β-oxidation

- works at the level of CAT I when FA enters MIT - is inhibited by malonyl-CoA (intermediate of FA synthesis)
  - so called cross regulation- an intermediate of one metabolic pathway inhibits another metabolic pathway
  - the principle of this regulation - in the synthesis of FA arises as an intermediate malonyl-CoA, which inhibits ß-oxidation = it prevents the opposite processes in one cell (degradation x synthesis)
  - principle: FA synthesis takes place in the cytosol, where CAT I also faces; malonyl-CoA is made in the **first reaction of FA synthesis**

Animals cannot convert FA to glucose

- FA are a rich source of energy for gluconeogenesis, but we do not get glucose from them (except for FAs with odd number of C)
- AcetylCoA cannot be converted to pyruvate or oxalacetate - pyruvate dehydrogenase reaction is irreversible and both carbons from AcetylCoA are cleaved off as CO_2 during the Krebs cycle
- lants have another 2 enzymes in glyoxylate cycle - they can convert AcetylCoA to oxaloacetate

FA with an odd number of C

- their β-oxidation is the same as in FA with even number of C, only two acetylCoA remain at the end, but one acetylCoA and one propionylCoA
  - **PropionylCoA** is converted to **succinylCoA**, which is involved in the Krebs cycle
    - uccinylCoA can be converted to oxaloacetate from which glucoseogenesis can synthesize glucose
- FA with an odd number of C is very little in the body

Degradation of unsaturated fatty acids

- most unsaturated FA have a cis double bond configuration
- enoyl-CoA-hydratase requires only trans isomers -> it is necessary to convert cis isomer to trans by isomerase
- For the oxidation of unsaturated FAs, two additional enzymes are needed - isomerase and reductase

Oxidation of long chained FAs

- takes place on peroxisomes (contain catalase)
  - here is only a shortening -> then moving to mitochondria
- the pathway is induced by a high fat diet and hypolipiemic drugs – clofibrate
- oxidation ends with octanoyl-CoA, which is removed from peroxisomes by binding to carnitine
  - (one slide states that peroxisomal oxidation ends at "stearoyl-CoA" – the shortened acyl-CoA is then exported bound to carnitine for mitochondrial β-oxidation)
- the first stage is catalysed by flavoprotein dehydrogenase - electron transfer to O_2 -> H_2O_2
  - is not attached to the respiratory chain -> $FADH_2$ from the first step of β-oxidation is not reoxidised in the respiratory chain, but in a reaction with O_2
    - $FADH_2 + O_2$ -> $FAD + H_2O_2$
  - peroxisomal catalase decomposes H_2O_2
    - $2\ H_2O_2$ -> $2\ H_2O + O_2$
- reactions do not lead to the formation of ATP

2.13.2 Ketone bodies

- there are 3 ketone compounds - acetoacetate, ß-hydroxybutyrate and acetone
  - the first are the most essential, acetone is a more of a waste product and is useless
- can be completely degraded to CO_2 and water
- derived from AcetylCoA - ketones are essentially a blood-transportable version of acetyl
- formed only in liver mitochondria

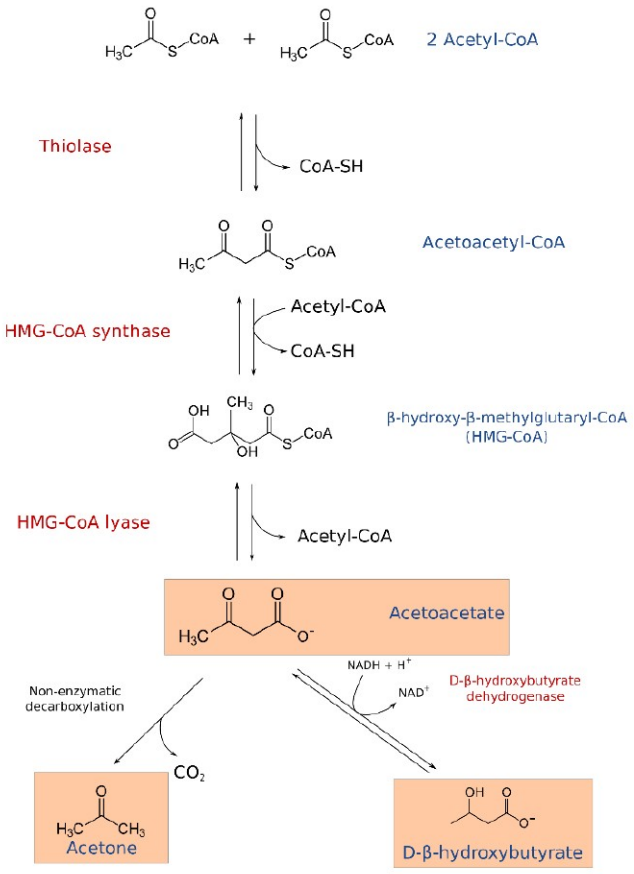
- ketone bodies = the **water-soluble form of FA**
- OAA (needed for AcCoA entry into the Krebs cycle) is produced by **pyruvate carboxylation** – in starvation OAA is consumed by gluconeogenesis

Function of ketones

- they replace glucose as the main energy substrate during starvation - glucose runs out, so the liver begins to produce ketone bodies and distributes them across the body to other tissues
- ketones can nourish all organs except liver (no enzyme) and erythrocytes (no mitochondria)
- AcetylCoA entry into KC depends on the availability of oxaloacetate - during fasting oxaloacetate is consumed for glucose synthesis
  - carbohydrate deficiency -> oxaloacetate deficiency -> KC slows down -> high AcetylCoA -> ketogenesis
  - carbohydrate deficiency -> low glycemia -> activated HSL -> TAG breakdown -> high FA -> β-oxidation -> high AcetylCoA -> ketogenesis

Synthesis of ketone bodies

- **condensation- enzyme ketothiolase**
  - 2 AcetylCoA -> acetoacetyl-CoA + CoA
  - the enzyme is the same enzyme that catalyses the last ß-oxidation reaction, but here it works the other way around
- **condensation- enzyme 3-hydroxy-3metylglutaryl-CoA synthase (HMG-CoA synthase)**
  - acetoacetyl-CoA + AcetylCoA -> 3-hydroxy-3metylglutaryl-CoA (HMG-CoA)
  - New acetyl is not connected to the end, but to the 3rd carbon in acetoacetyl-CoA
- **breakdown - enzymeHMG-CoA lyase**
  - HMG-CoA -> acetoacetate + AcetylCoA
  - produces acetoacetate, which is the first ketone
- **bonus - reversible hydrogenation - β-hydroxybutyrate dehydrogenase enzyme**
  - acetoacetate + NADH -> β-hydroxybutyrate + NAD
  - β- hydroxybutyrate carries more energy than acetoacetate (by 1 NADH)
- in massive ketone formation β-hydroxybutyrate is quantitatively the most important ketone in the blood
- acetoacetate spontaneously decarboxylates to acetone - technically also ketone, but also kind of useless
- in the uncontrolled formation of ketones, ketoacidosis occurs because they are acids and lower the pH
  - diabetic ketoacidosis - a condition typical of type 1 diabetes mellitus

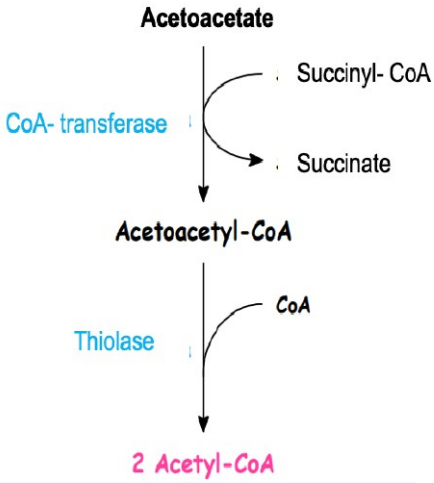


Ketone body activation

- the liver produces them but does not use them – does not have CoA-transferase
  - It would be unnecessary to synthesize ketone bodies if they were to be degraded immediately
- Once formed in the MIT matrix, ketone bodies enter the plasma
- one of the Krebs cycle intermediates - succinyl-CoA - is used to activate ketone bodies
- acetoacetate is activated by transfer of CoA from succinylCoA (using CoA-transferase) - succinate and acetoacetyl-CoA are formed
- Acetoacetyl-CoA is then cleaved by β-ketothiolase to 2 acetyl-CoA
  - **the CoA-transferase is not in the liver!** – activation and use is **extrahepatic**; the resulting AcCoA enters the Krebs cycle
- β-hydroxybutyrate will first give its two extra hydrogens to the nearest NAD, thereby forming NADH and acetoacetate

Role of acetoacetate

- cardiac muscle and kidney cortex prefer acetoacetate over glucose
- eventually the brain adapts to acetoacetate during starvation (up to 50% of energy can be taken from ketone bodies)
- it also has a regulatory role - high levels of acetoacetate in the blood means the presence of a large amount of acetyl-CoA, which is a signal to reduce lipolysis (this means that the body has enough energy at that time)



Regulation of ketogenesis in 4 steps

- HSL (hormone sensitive lipase) - lipolysis in adipose tissue
- CAT I – FA into MIT
- targeting of acetyl-CoA from β-oxidation to ketogenesis and not to KC - little oxaloacetate + much acetyl-CoA = ketogenesis
    - HMG-CoA synthase – regulatory enzyme, 2nd enzyme of ketone body synthesis

Fatty acid oxidation disorders

- mitochondrial FA oxidation is an important energy source, not only in fasting (complete oxidation + ketone body production in the liver)
- mutations at different levels of FA degradation – **defects of FA transport into mitochondria** (carnitine system) and **defects of β-oxidation enzymes**
- **common symptoms**
    - **acute** – **hypoketotic hypoglycaemia during fasting** (lethargy, convulsions, coma), **sudden infant death syndrome (SIDS)**
    - **chronic** – hepatopathy and (cardio)myopathy (accumulation of toxic metabolites)
- what goes wrong: starvation starts lipolysis → plasma FA rise, but β-oxidation is blocked →
    - 1. plasma FA increased, ketone bodies rise much less (normal or ↓)
    - 2. accumulated FA are metabolised by **ω-oxidation into dicarboxylic acids** → **dicarboxylic aciduria** (its pattern depends on the defect; **C8–C10** in MCAD)
    - 3. lack of plasma glucose
- onset typically **7th–12th month** (children start to sleep longer) or with the first serious infection causing anorexia/starvation (depletion of liver glycogen)
    - **CAVE: children look healthy until they decompensate**
- diagnosis: FFA, 3-hydroxybutyrate and acetoacetate (e.g. during controlled fasting), urinary organic acids (dicarboxylic aciduria), **acyl-carnitine profile** (GC-MS)
- treatment: i.v. glucose and electrolytes, **avoid fasting**, sometimes carnitine supplementation

MCAD deficiency

- deficiency of acyl-CoA dehydrogenase for **medium-chain FA** – **the most common defect** (1 : 6 000–10 000)
- most patients have the same mutation (**A985G** – lysine replaced by glutamic acid) → quick molecular-genetic testing
- dicarboxylic aciduria + **octanoylcarnitine and hexanoylcarnitine**

2.14 ENERGY STORAGE IN CELLS

MUDr. Josef Fontana

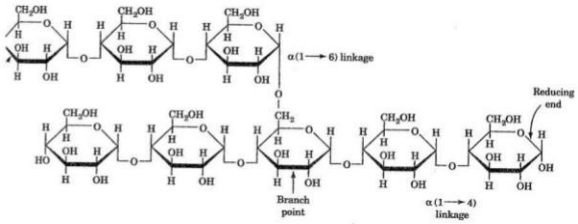
Overview of material stored in the body

- There is storage for fat (**TAG**) and saccharides (**glycogen**) but none for proteins (muscle breakdown is the last resort)
- **TAG** - convenient storage (because unlike glycogen it does not bind water)
  - with complete oxidation of 1g of MK, **38 kJ** is obtained, whereas from 1g of carbohydrates or proteins only **17 kJ**
- 70kg man has **400.000 kJ in TAG, 100.000 kJ in protei**n (muscle), **2.500 kJ in liver glycogen** and **170 kJ in glucose**
  - glycogen and glucose storages are sufficient for one day, TAG for weeks

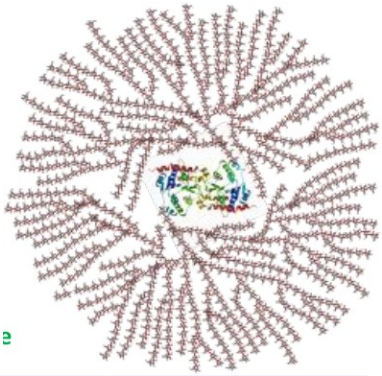
2.14.1 Glycogen metabolism

- carbohydrate storage in animals, in the cytosol (explains its violet colour), in almost all cells
- in the liver (80-100g), skeletal muscle (300g) and in small quantities in each cell
- women have more glycogen - vaginal mucosa
  - **Liver glycogen -** used to maintain stable blood glucose
  - **Muscle glycogen** – used during short-term strenuous muscle work
  - **vaginal mucosa** - glycogen -> lactate -> H^+ production -> acidic environment -> defence against microorganisms
- PAS reactions in histological preparations brightly colours glycogen violet

Structure of glycogen



- branched homopolymer
- Most glucose bound by α-1,4-O-glycoside bonds
- branching from the strain of glycogen is by α-1,6 bond
- two groups of enzymes - one cleaves α-1,4 bonds, the other α-1,6 bonds
- **glycogen has 2 ends** - elongation and shortening takes place at non-reducing ends containing terminal glucose (enzymes work with this end) - **branching glycogen will provide more non-reducing ends**
- reducing end has a **semi-acetyl hydroxyl** – it is bound by the protein **glycogenin**, which forms the anchor at the beginning of the glycogen strain



Glycogenesis

- **all glycogen metabolism occurs in the cytosol**
- **1) phosphorylation**- enzyme **glucokinase** in liver and hexokinase in muscle
  - glucose + ATP -> Glc-6-P + ADP
- **2) isomerization**- enzyme **glucose phosphate isomerase**
  - Glc-6-P -> Glc-1-P (= Cori ester)
  - **the slides name the enzyme phosphoglucomutase** (Glc-6-P ⇌ Glc-1-P)
- **3 coupling of UDP** - enzyme **UDP-glucose pyrophosphorylase**
  - Glc-1-P + UTP -> UDP-Glc -> formation of a macroergic compound
  - slides: enzyme **Glc-1-P uridylyltransferase**; UDP-Glc = activated glucose (bond on C1)
- **4 connection of UDP-Glc to non-reducing end of glycogen**- enzyme **glycogen synthas**
  - UDP is released and α-1,4 bond is formed
- the chain of linked glucose grows until it reaches a certain length and branches
- **branching enzyme** - **branching enzyme - amylo-(1,4-1,6)-translocase**
  - catalyses glycogen branching - it forms α-1,6 bonds
  - removes an oligosaccharide of about **7 Glc residues** from the growing chain and attaches it to the OH group on **C6** of a glucose
  - new branches extended again by glycogen synthase

Regulation of glycogenesis

- **regulation of glycogen synthase** - phosphorylation (phosphorylated is inactive, dephosphorylated is active)
  - activated by **insulin** (trying to lower free glucose levels in blood), inhibited by **glucagon** (trying to get more free glucose into bloodi) and adrenaline
- **phosphorylation of glycogen synthase is done by proteinkinase and dephosphorylation by phosphoprotein phosphatase**
- **protein kinases** - protein kinase A (PKA) is cAMP dependent - it needs cAMP to work
  - calmodulin dependent protein kinase (CAMK) - is active after protein binding calmodulin

        - calmodulin itself is bound to CAMK only when four Ca^2 atoms bind to calmodulin
- binding of glucagon (or adrenaline) to the membrane receptor activates adenylate cyclase, which starts to form cAMP, which activates PKA and inactivates glycogen synthase
- intracellular Ca^2 increases in muscle during contraction - its binding to calmodulin activates calmodulin, which activates CAMK, which inactivates glycogen synthase
- insulin activates phosphodiesterase, which cleaves cAMP to AMP and inactivates PKA
    - insulin also activates phosphoprotein phosphatases, which dephosphorylate glycogen synthase

Glycogenolysis

- degradation of the glycogen molecule
- again, in cytosol
- **1. phosphorolytic cleavage** - cleavage of α-1,4 bond and formation of Glc-1-P (= Cori ester)
    - Free inorganic P is used - linked to C1 glucose
    - enzyme **glycogen phosphorylase**- cleaves α-1,4 bond – attaches phosphate to it
    - ends 4 glucose molecules before branching
- **2. isomerization** Glc-1-P to Glc-6-P – enzyme **phosphoglucomutase** (glucose phosphate isomerase)
- **3. Cleavage of branch – removal of α-1,6 bond**
    - glucose cleavage from glycogen ends up in the 4th glucose before the branching point, because **glycogen phosphorylase** is a large enzyme and no longer physically gets closer
    - enzyme **gluconotransferase** (transglycosidase) takes 3 glucose from that small branch and moves it to the backbone
    - one glucose attached by an α-1,6 bond left by the debranching enzyme (amylo-α1-6-glucosidase))
    - linear chain is formed – **glycogen phosphorylase** continues

Regulation of glycogenolysis

- glycogen phosphorylase is active phosphorylated - it is phosphorylated by **phosphorylase kinase**
- **insulin** inhibits **phosphorylase kinas** (insulin does not want to have more free glucose in the blood → wants to prevent glycogen breakdown) and **counter-regulatory hormones** activate it
- again, signalling via PKA and CAMK
    - in muscle, **Ca^{2+}/calmodulin-dependent protein kinase** activates glycogenolysis during contraction (opposite effect to glycogen synthase)

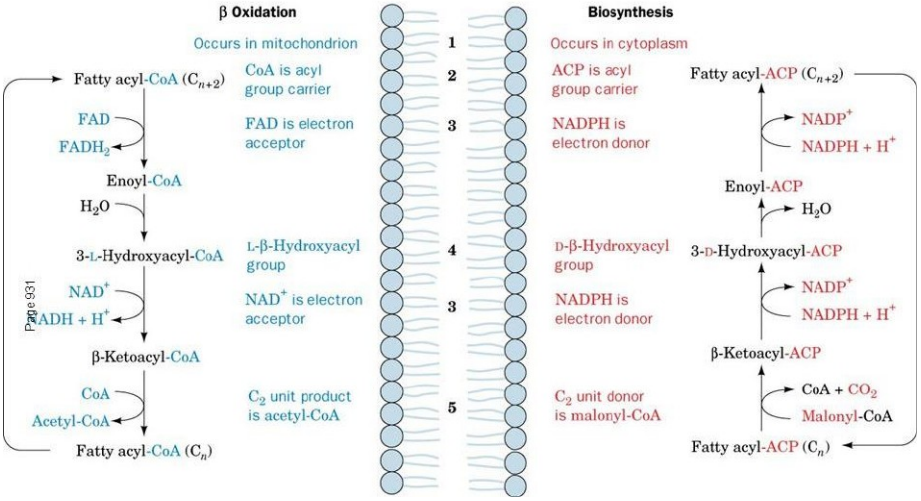
Glycogen storage diseases (GSD)

- inborn metabolic disorders caused by defects of enzymes of glycogen synthesis or breakdown → glycogen **accumulation** or **abnormal structure** in various tissues
- most are **autosomal recessive**, except some forms of **type IX (X-linked)**
- by affected organ: **hepatic** – types I, III, VI, IX, 0; **muscular** – types V, VII; **generalized** – types II, IV
- typical laboratory findings: **hypoglycaemia, hyperlactacidaemia, metabolic acidosis, hyperlipidaemia**
- main types
    - **I – von Gierke** (hepatorenal) – **glucose-6-phosphatase**; liver, kidneys – hypoglycaemia, lactic acidosis, hyperlipidaemia, hepatomegaly, slow growth
    - **II – Pompe** (generalized) – **lysosomal acid α-glucosidase**; muscle, liver – hypotonia, cardiomyopathy, respiratory failure; glycogen accumulates in **lysosomes**
    - **III – Cori** – **debranching enzyme**; liver, muscle – hypoglycaemia, muscle weakness, hepatomegaly
    - **IV – Andersen** – **branching enzyme**; liver, muscle – liver failure, muscle weakness
    - **V – McArdle** – **muscle phosphorylase**; skeletal muscle – muscle cramps, **myoglobinuria after exercise**

2.14.2 Synthesis of Fatty acids

Comparison of fatty acid synthesis vs degradation

- FA **synthesis in the cytosol**, **degradation in the mitochondrial matrix**
- intermediates of synthesis are bound to **ACP**, intermediates of degradation to **CoA**
- synthesis enzymes form one big multienzyme complex (FA synthase); degradation enzymes are free in the matrix
- the chain is extended by 2 C from AcCoA, but the activated substrate is **malonyl~CoA**
- reducing cofactor of synthesis = **NADPH**; oxidising cofactors of degradation = **FAD and NAD^{+}**
- synthesis on FA synthase ends with **palmitate (C16)**; elongation and desaturation are done by other enzymes



Formation of malonyl-CoA

- initial substance for the synthesis of FA - acetyl-CoA
- **carboxylation to malonyl-CoA**
  - acetyl-CoA + ATP + HCO_3 -> malonyl-CoA + ADP + Pi + H^+
- enzyme **acetyl-CoA carboxylase**, biotin cofactor (or vitamin H / vitamin B7)
  - **regulatory enzyme of the whole pathway** - also affects β-oxidation of FA
- CO_2 removed during condensation with increasing FA

Mammalian fatty acid synthase

- multienzyme complex for FA synthesis
- has several active sites
- it is a homodimer of 2 identical subunits (260 kDa) - attached 2 identical synthases - they exchange an intermediate
  - (slides give 272 kDa per subunit, the Czech slide 260 kDa); the three domains are connected by moving regions
    - domain 2 = reduction unit: **ACP, β-ketoacyl reductase, dehydratase, enoyl reductase**
  - **each subunit has 3 functional domains** - several sites with functional enzymatic activity
    - **domain1** - has transferases which prepare substrates and condensation enzyme **β-ketoacyl synthase**
    - **domain2** - carriers the main enzymes that take care of FA synthesis
    - **domain3** - here the whole synthesis ends, where palmitate cleaves off
- ACP (acyl carrier protein) is part of the condensation enzyme and serves as a carrier of intermediates
- the condensation enzyme (CE) contains a thiol group (SH) to which FA can bind

Individual steps of FA synthesis

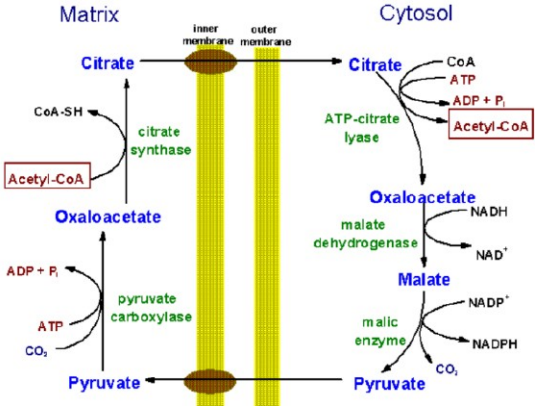
- use of NADPH from the pentose cycle
- **synthesis of malonylCoA** - carboxylation- enzyme **acetylCoA carboxylase**
  - acetylCoA + ATP + HCO_3^- -> malonylCoA + ADP + Pi + H^+
- **binding of acetyl- to the condensation enzyme (CE)** - enzyme **acetyltransacylase**
  - acetylCoA + CE -> acetyl-CE + CoA
- **binding of malonyl-CoA na ACP** (Acyl Carrier Protein) - enzyme **malonyltransacylase**
  - malonyl-CoA + ACP -> malonyl-ACP + CoA
- **condensation reaction** - connection malonyl-ACP a acetyl-CE - **enzyme 3-ketoacyl-ACP-synthase**
  - acetyl-CE + malonyl-ACP -> acetoacetyl-ACP + CE + CO_2
- **reduction** - enzyme **β- ketoacyl reductase**
  - acetoacetyl-ACP + NADPH -> D-3-hydroxybutyryl-ACP + H_2O
- **dehydration** - enzyme **3- hydroxyacyldehydratase**
  - D-3-hydroxybutyryl-ACP -> crotonyl-ACP + H_2O
- **redukce** - enzyme **enoyl reductase**
  - Crotonyl-ACP + NADPH -> butyryl-ACP + NADP
- Now from acetyl (2C) to butyryl (4C) - FA chain extending
- another malonyl binds to the ACP of the second subunit and butyryl binds to the CE of the second subunit
  - During condensation, 1 CO2 is disconnected and a 6C chain is connected to the ACP
- palmitate (16C) is the end product
- enzyme **thioesterase** cleaves palmitate from ACP by hydrolyzing the thioester bond
  - the thioester bond is with **phosphopantetheine** (the prosthetic group of ACP); after each turn the subunits swap

roles (FA synthase works as a dimer)

- all carbon atoms of FA synthesis are derived from Acetyl-CoA because C from HCO_3^- is cleaved off in the form of CO_2

Requirements for palmitate formation

- 8 acetyl-CoA, 14 NADPH a 7 ATP
- acetyl-CoA is transported from the mitochondria matrix using citrate
- 8 NADPH is obtained by transporting citrate to the cytoplasm and the remaining 6 in the pentose cycle



Regulation of FA synthesis

- sufficient substrates (carbohydrates / AMK) and energy are needed
  - the main regulatory enzyme **acetyl-CoA- carboxylase** (active in dephosphorylated form)
  - activated by – **insulin and citrate** – insulin and citrate - insulin activates glycolysis -> Acetyl-CoA, which is formed from pyruvate after glycolysis, is required for FA synthesis
  - inhibited by - **glucagon, adrenalin, palmitoyl-CoA** (if there is enough palmitoyl-CoA, there is no need to synthesize others**) and AMP** (in the absence of energy)
  - palmitoyl-CoA inhibition = **feedback inhibition**

Elongation and desaturation of FA

- elongation, enzymes **elongase**, if we need over 16C long FA
- formation of unsaturated FA is desaturation, enzymes **desaturase**
- both occur on the SER membrane on the side facing the cytosol

Desaturation

- s mammals lack enzymes catalysing the double bond entry after C9 FA
- new double bonds are always introduced between an existing double bond and a carboxyl group
- we cannot synthesize **linoleate** (18:2 cis D^9, D^{12}) and **linolenate** (18:3 cis D^9, D^{12}, D^{15}) - both essential

2.14.3 Synthesis of TAG

- final form of FA storage
- starting with DHAP (**dihydroxyacetone phosphate**, intermediate of glycolysis)
  - **reduction**- enzyme **DHAP dehydrogenase**
    - DHAP + NADH -> glycerol-3-P + NAD
  - **addition of the first two FAs** - enzyme **acyltransferase**
    - glycerol-3-P + acylCoA -> monoacylglycerol-3-P + CoA
    - monoacylglycerol-3-P + acylCoA -> phosphate acid = diacylglycerol phosphate
  - **hydrolytic separation of phosphate from phosphate acid** - enzyme **phosphatidic acid phosphatase**
    - phosphatidic acid + H_2O -> 1,2-diacylglycerol + Pi
  - **connection of the last FA** – again the enzyme **acyltransferase**
    - 1,2-diacylglycerol + acylCoA -> TAG + CoA

2.15 MUSCLE TISSUE

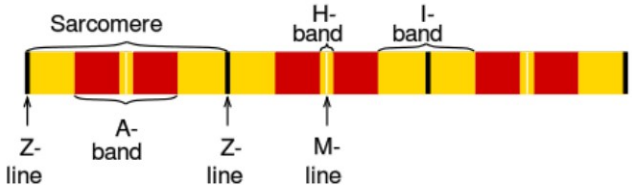
MUDr. Eva Maňáková, Ph.D.

- tissue characterized by **excitability**, **contraction** and **relaxation** - due to myofilaments (actin and myosin II)
- Mechanocytes - cells capable of movement, have a high amount of actin and myosin
- types of muscles - smooth, striated skeletal and striated heart

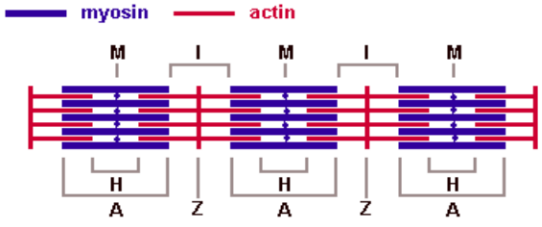
Terminology

- **sarco** Greek "sarx" = meat
  - sarcolema - cytoplasmic membrane of muscle cells
  - sarcoplasma - cytoplasma of muscle cells
  - sarcomer - interval between two Z lines in muscle
  - sarcoma - a tumour derived from muscle cells
  - sarcolemma (slides) = plasma membrane **+ basal lamina** of the muscle fibre; sarcoma = malignant tumour of **mesenchymal** origin
- **myo** - from Greek "mys, myos" = muscle
  - myocyte - muscle cell
  - myofilaments - actin and myosin
  - myofibril - a thin fibre inside muscle cells; it is a sarcomere chain attached at both ends to the sarcolemma
  - myotoma - a block of embryonic cells from which muscles develop
  - myocardium - muscle of the heart
- fibre
  - **muscle fibber** skeletal muscle cell (these cells are formed by combining a lot of immature muscle cells into one huge muscle cell)
  - **myofibril** - basic contractile unit inside muscle cells, is formed by layers of sarcomeres, in one cell there are thousands of myofibrils
  - **myofilaments** - individual actin and myosin filaments are only seen on an electron microscope

Sarcomere

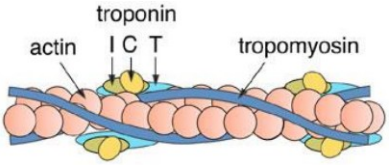


- skeletal and cardiac muscle
- five strips - A, I, M, H a Z
  - **A band** indicates the presence of myosin
  - **I band** indicates the absence of myosin, the presence of actin
  - **M band** indicates the centre of myosin fibers
  - **H band** indicates gaps between actin fibers
  - **Z band** indicates anchoring of actin fibers
- **H and I strips shorten during contraction**
- the sarcomere is the section between two Z strips
- actin fiber consists of **G-actin** and regulatory proteins
  - actin polymerizes and forms the F-actin fiber to which the regulatory proteins **tropomyosin and troponin I, C and T bind**



Bands and lines in the contractile apparatus of skeletal muscle

  - **myosin** molecule = head (binding sites for **actin and ATP**) + tail; myosin molecules form the **thick filaments**
  - troponin = binding site for Ca^{2+}; tropomyosin covers the myosin-binding site on G-actin
  - anchoring: thick filaments by **titin**, thin filaments by **α-actinin** (in the Z line)
- other proteins necessary for muscle condition
  - **α- actinin**- in the Z band
  - **desmin, plectin** - secures the connection between the myofibril and the surface membrane
    - desmin occurs only in muscle, so it can be used as a marker
  - **nebulin** - template for f-actin
  - **titin** - keeps myosin in sarcomere



Contraction

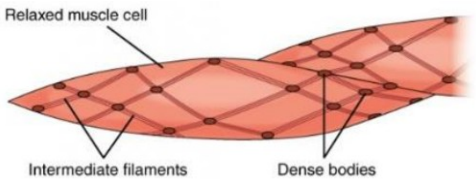
- contraction looks similar in skeletal and cardiac muscle, but slightly different in smooth muscle
- **skeletal and cardiac muscle has large reserves of Ca^{2+}** and is therefore less dependent on plasma calcium
  - Ca^{2+} is released from the sarcoplasmic reticulum (that is a form of modified SER) into the cytoplasm where it binds to troponin C and activates myosin phosphatase - triggers myosin dephosphorylation (one of the contraction steps)
- smooth muscle contraction

- Ca^{2+} binds to the calmodulin, which then activates myosin light chain phosphorylating kinase
- Phosphorylation of light chain is required in smooth muscle for its binding to actin
  - in the dephosphorylated state, myosin is rolled up

Excitation–contraction coupling in skeletal muscle (slides)

- **motor end plate** – axon terminal + muscle cell surface with **junctional folds**; ACh binds the **ACh receptor** (non-selective cation channel) → cation influx → depolarization → action potential
- the action potential spreads along the **T-tubules** to the **terminal cisternae** → Ca^{2+} channels open → Ca^{2+} released into the cytosol
- **cross-bridge cycle**
  - Ca^{2+} binds to troponin → tropomyosin shifts → actin binding site for myosin exposed
  - myosin binds to actin → release of **P_{i}** from myosin
  - myosin head rotates towards the centre of the sarcomere (power stroke) → actin filament slides → release of **ADP**
  - **ATP binds** to myosin → myosin detaches from actin
  - ATP hydrolysis to ADP + P_i → myosin head returns to its previous ("cocked") state
- smooth muscle: Ca^{2+} comes from the **extracellular space** and the sarcoplasmic reticulum → binds calmodulin → activates **myosin (light chain) kinase** → phosphorylated myosin binds actin

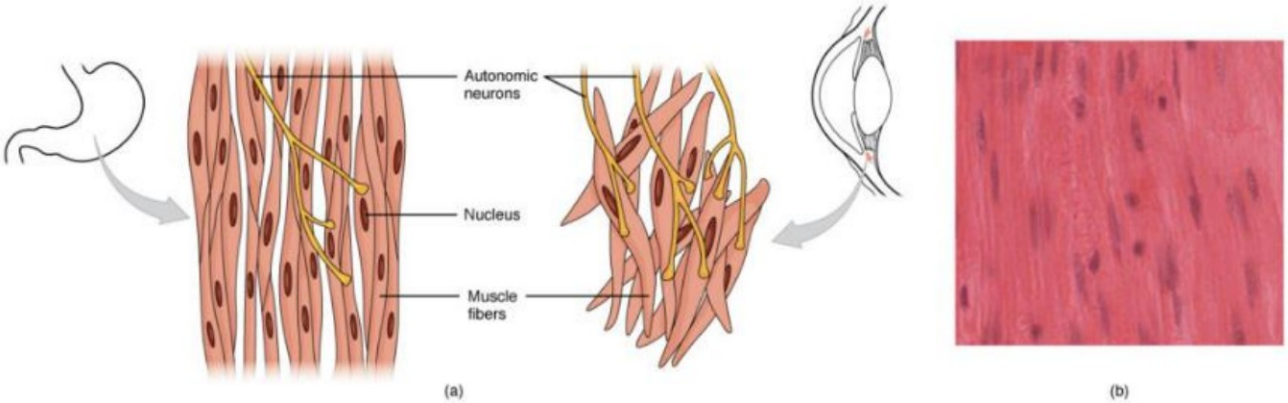
2.15.1 Smooth muscle



- contraction is not controllable by will - it is ensured by autonomous nerve
- consists of thin, spindle likecells
  - nucleus is centrally placed, long and narrow
  - are full of actin, myosin and intermediate filaments (desmin and vimentin)
  - **dense bodies** (desmin) similar to Z lines; they are thickened sites where the filaments connect to each other or to the plasma membrane

- size: length ~20 µm (up to **500 µm in the pregnant uterus**), width 5–10 µm; 1 nucleus, no striations, **no sarcomeres** – actin/myosin bundles run diagonally
- surface invaginations = **caveolae**; sarcoplasmic reticulum rudimentary (small Ca^{2+} store); **no T-tubules**
- each cell is covered by a basal lamina + endomysium with **reticular fibres**; cells are interconnected by dense bodies and gap junctions
- innervation: branching autonomic axons with **varicosities**; neurotransmitters **acetylcholine, noradrenaline**; the stimulus spreads through gap junctions
- smooth muscle cells are **synthetically active** (elastin, collagen – e.g. aorta wall) and **able to proliferate** (mitosis)

- smooth muscle cells have cellular connections via hemidesmosomes and nex
  - they transfer substances by pinocytic invagination
- **occurrence** - walls of hollow organs (thickness is about 150-200µm), blood vessels (15-20µm), skin, prostate eye
- smooth muscle contraction is induced by the mediator being spilled into the environment, transmitted through the nexes, automatically or stretched

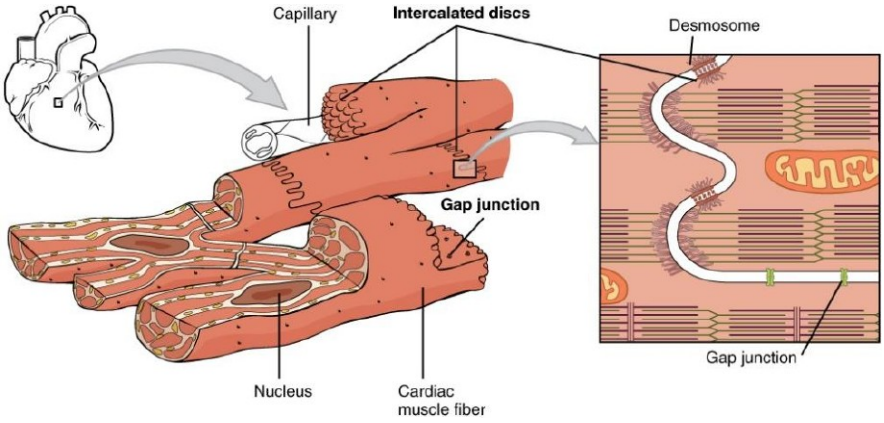


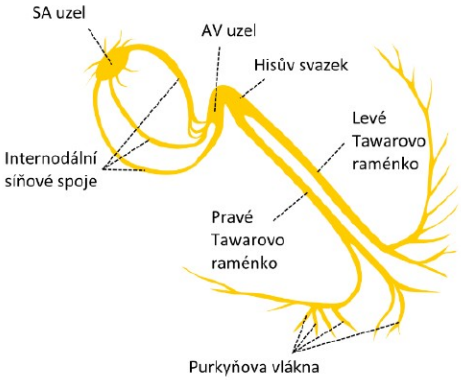
2.15.2 Cardiac muscle

- the basic building unit is a cylindrical cell - **cardiomyocyte (85-100µm)**
  - lengthened cells, nucleus in the middle of the cell, a lot of mitochondria, a large supply of glycogen and lipids
  - slides: branched cell, diameter **10–20 µm**, length **50–100 µm**, 1 nucleus; cells in the ventricles bigger than in the atria; surrounded by basal membrane, endomysium and abundant capillaries
  - energy: **fatty acids** (lipid droplets) and **glycogen** granules
  - contain a lot of myoglobin - similar to haemoglobin but have only one binding site; is only in muscle cells
  - **diads** - at the Z line level; it is formed by T-tubule (invagination of sarcolemma) and cistern of sarcoplasmic ER
  - sarcoplasmic reticulum less abundant than in skeletal muscle; **T-tubules big** (differ between atrium and ventricle);

diad = T-tubule + **small** terminal cisterna

- the cardiomyocyte junction is called an **intercalated dis** containing several intercellular junctions
  - **fascie adherens** - anchoring actin filaments
  - **desmosomes** - anchorage of intermediate filaments
  - **nexes** - they ensure the transfer of information between cells and serve to coordinate contraction
  - transversal part of the disc = mechanical (fascia adherens, desmosomes); **longitudinal part = gap junctions** → rapid impulse conduction and contraction
- cardiomyocytes contain **natriuretic peptides A (atrial) and B (brain)**, by which the heart responds to increased tension in the atrial wall
  - natriuretic peptides have a vasodilating effect and increase the secretion of sodium and urine (thus reducing heart strain)
- **cardiac conduction system** - an independent system that maintains the regularity and autonomy of heart beat
  - conduction system: sinoatrial (SA) node, internodal atrial connections, atrioventricular (AV) node, His bundle, Tawar arms, Purkinje fibres
    - **Purkinje fibres** - contain much less myofibrils and mitochondria, lack of intercalated discs, have only nexes and local adhesive bonds; they contain a lot of glycogen
- cardiomyocytes have a very low ability to regenerate - in the case of cell death, a ligament is formed which is not capable of conducting electrical excitement and cannot even contract
  - this ligament can rupture under high pressure and blood starts to escape from the heart to the pericardium cavity, where it accumulates and prevents heartbeat, which very quickly leads to death
  - adults have only a small number of cardiac stem cells → defects (infarct) are repaired by **fibroblast proliferation → connective-tissue scar** with limited function

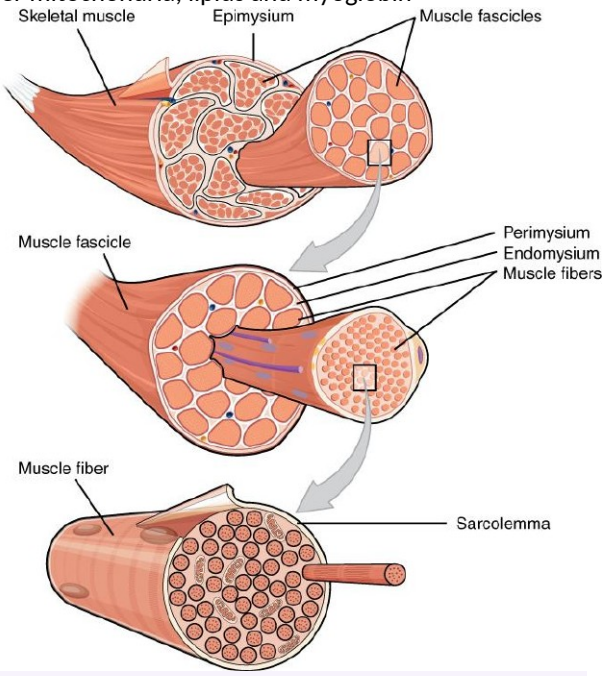




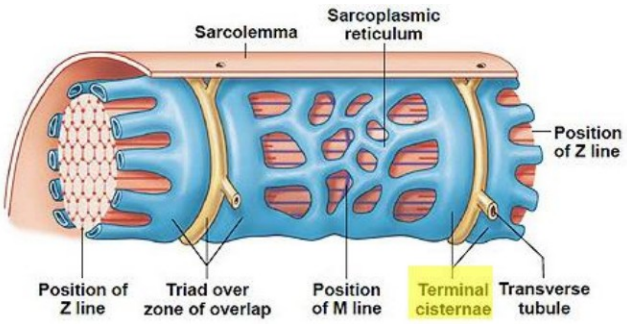
2.15.3 Skeletal muscle

- the basic unit of skeletal muscle is the fiber- **multinucleated syncytium** – (fusion of a large number of cells in one) with a diameter of 60-100µm
- the skeletal fiber nuclei are oval and are located just below the sarcolemma
- muscle development - mesenchymal cells develop into myoblasts (which migrate and divide), which gradually begin to connect to myotubules and to the final muscle fibers
  - development (slides): mesoderm → mesenchymal stem cells → **myoblasts** → fuse into **myotubes** (nuclei in the centre) → more myofilaments, nuclei pushed aside to the periphery, cells grow → myofibrils, attachment to motor neurons
  - some myoblasts stay undifferentiated → **satellite cells** (stem cells of skeletal muscle, located under the basal lamina)
  - skeletal muscles are covered by fascia (a string) on the surface, which is a cover of dense collagen ligament

- **Satellites cells** (belong to stem cells) serve for muscle growth and repair
- they are able to divide and fuse to prolong or repair an already existing muscle fiber
  - they are able to divide and fuse to prolong or repair an already existing muscle fiber
  - their abilities are not unlimited, in case of too much damage the muscle dies and is replaced by connective tissue
- the sarcolemma in the skeletal muscle clings in and forms tubulous invaginations - so-called T-tubules
- **sarcoplasmic reticulum (SER)** - highly developed, modified ER full calcium forming a network around each myofibril
  - together with invaginations of sarcolemma form so-called **triads** (SER-T tubules) at the A and I strip level
  - sarcoplasmic reticulum: in its membrane **voltage-gated Ca^{2+} channels**; it widens into **terminal cisternae at the A/I border, twice per sarcomere**
  - **triad = T-tubule + 2 terminal cisternae**
- Skeletal fibers differ in structure, function and metabolism
  - **White fibre** - larger; more actin, myosin and glycogen; fewer mitochondria, lipids and myoglobin
    - predominantly anaerobic metabolism - rapid contraction of muscles, but quickly tired
    - suitable for sprints
  - **red fibre** smaller; multiple mitochondria, lipids and myoglobin; less actin, myosin and glycogen
    - mainly aerobic metabolism - slow muscle contraction, but lasts for a very long time
    - suitable for marathons



  - slide classification
    - **slow oxidative (red) – type I** – slow contraction for a long period; many mitochondria, capillaries, myoglobin; ATP from oxidative phosphorylation
    - **fast glycolytic – type IIb** – rapid short-term contraction; ATP from anaerobic glycolysis; fewer mitochondria, capillaries and myoglobin
    - **fast oxidative glycolytic – type IIa** – features of both
    - most muscles are a combination of all three types, according to the prevailing type of movement
- tendon - collagen connective tissue that clamps the muscle into the bone
- connective tissue coverage
  - **endomysium** - envelops one muscle fiber
  - **perimysium** - envelops several bundles of fibers
  - **epimysium** - envelops the entire muscle
  - fascicle = bundle of fibres wrapped by perimysium; each muscle fibre is covered by a **basal lamina**



Attachment of myofibrils to the cytoskeleton

- **protein dystrophin** - inside stabilizes plasma membrane and connects it with actin (in Z bands)
  - There may be many mutations in the dystrophin gene - mutations that damage the function of dystrophin (or its auxiliary proteins) give rise to various diseases collectively called muscular dystrophy
  - Duchenne muscular dystrophy - X-linked genetic disease where part of the dystrophin gene is deleted

        - Gradually laterally striated muscle breaks ip, which eventually leads to death around the age of 20 due to respiratory failure

2.15.4 Muscle innervation

- **Motor fibres** – they are the axons of spindle like cells that give impulses for muscle contraction
    - The place where motor fibres end and the beginning of muscle fibre begins (place where the information is passed on) is called the (**neuro**) **motor disc**,
    - So that the signal can be passed on, the axon has to send out a mediator – **acetylcholine**
    - each muscle fibre is innervated by one axon
    - slides: **motor unit** = 1 axon + all muscle fibres it innervates
        - ocular muscles – 1 axon : 1–several fibres; large muscles – 1 axon : 100 and more fibres
        - intensity of contraction is proportional to the number of active motor units
    - the basal lamina of the synaptic cleft directly follows the basal lamina of the muscle fiber
- **sensitive fibres** - divert information about muscle tension and orientation from muscle receptors
    - information about pain comes from free nerve endings that are arranged around the muscle fibres
- **muscle spindle** – mechanoreceptor detecting muscle **stretch**; thin **intrafusal fibres** with sensory and motor nerve fibres, covered by connective tissue

Regeneration of muscle tissue (summary)

- **skeletal** – satellite cells: quiescent → activated → proliferation → differentiation → regeneration of fibres
- **cardiac** – limited (see above), scar formation
- **smooth** – smooth muscle cells have proliferative potential and can compensate for injured tissue; smooth muscle progenitors exist (e.g. in arteries and veins)

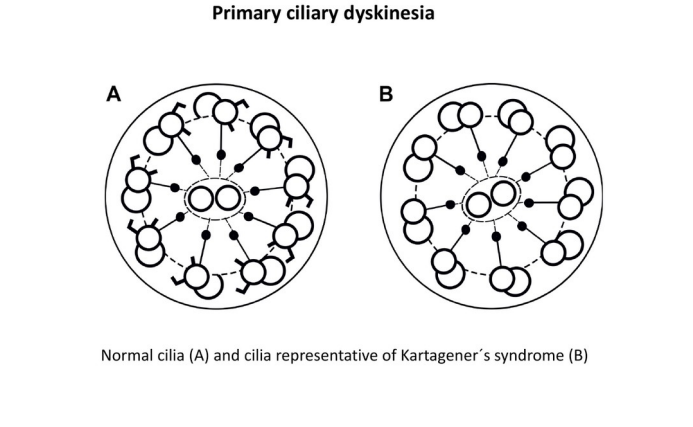
2.16 CELL MOTILITY AND MOLECULAR MOTORS

prof. RNDr. Jan Kovář, Ph.D.

- Cellular movements are usually based on the interaction of motor proteins (molecular motors) with cytoskeletal structures – proteins move along cytoskeletal structures while using up ATP

Significance in medicine – Kartagener syndrome

- example of a molecular-motor disease: defective **dynein** → **primary ciliary dyskinesia (Kartagener syndrome)**
  - cilia lack functional dynein arms → immotile cilia (impaired mucociliary clearance – recurrent respiratory infections, infertility)
  - often with **situs inversus** – e.g. **dextrocardia** on the chest X-ray



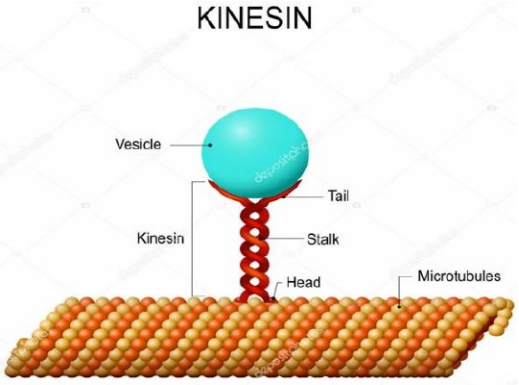
[slides] Normal cilia (A) vs. cilia in Kartagener syndrome (B)

2.16.1 Molecular motors

- kinesins (interact with microtubules), dynein (interacts with microtubules) and myosin (interact with microfilaments)

Kinesins

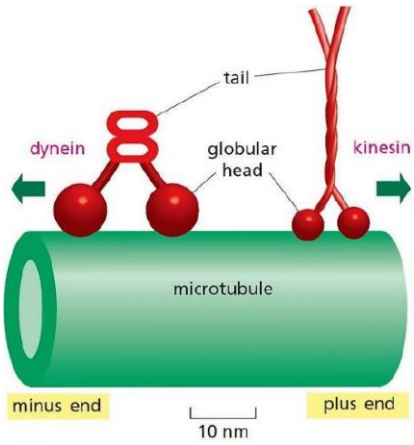
- structure - 2 globular ATP binding heads (which interact with microtubules), stalk and tail (made of light chains which bind cargo)
- movement of kinesin on microtubules mimics the walk of a human – one leg forwards, anchors and the other leg steps forward
  - except in kinesin the legs are called heads
  - movement uses up ATP
- **function - transport of material into + end of microtubules** (from centre to periphery)
- occurrence – mitotic spindle



  - and **intracellular transport of organelles**

Dynein

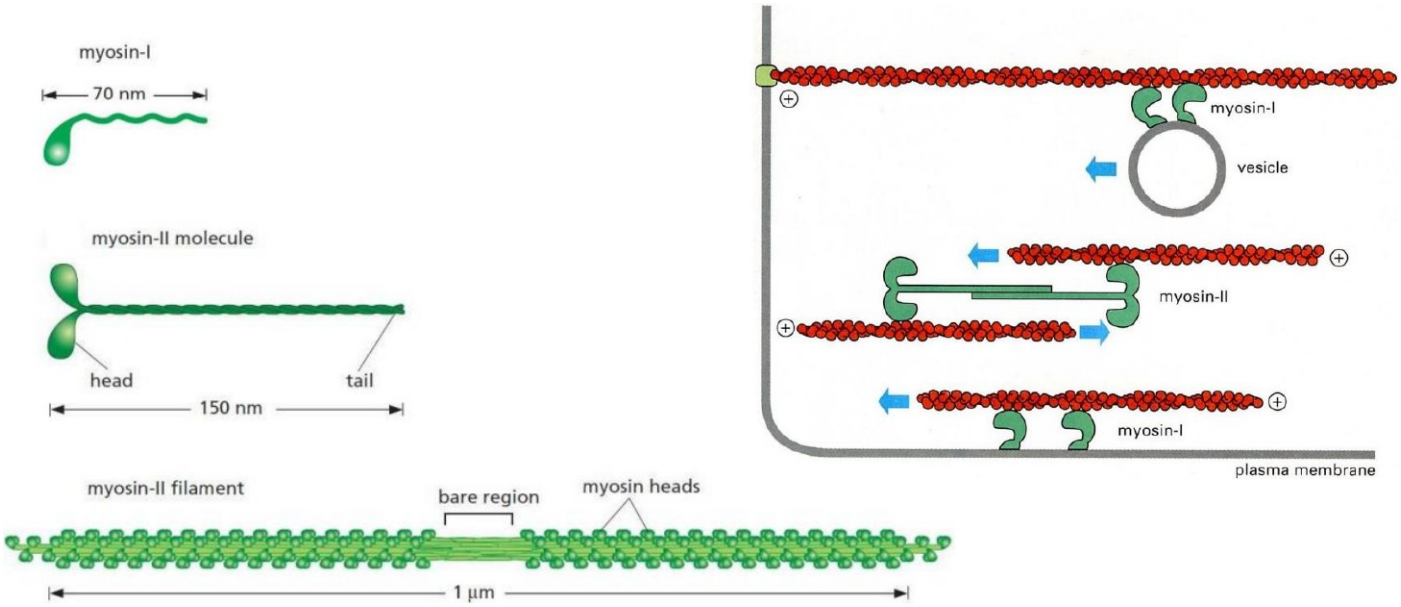
- structure - 2 globular ATP binding heads (which interact with microtubules) and stalk (which binds cargo)
  - head formed from 7 domains
- movement of dynein is quite chaotic and can be described as the walk of a drunk sailor – movement forward follows is accompanied with wobbling from side to side
- **function - transport of material to – end of microtubule** (from periphery to centre)
- occurrence – mitotic spindle, flagella, cilia



  - and intracellular transport of organelles

Myosin

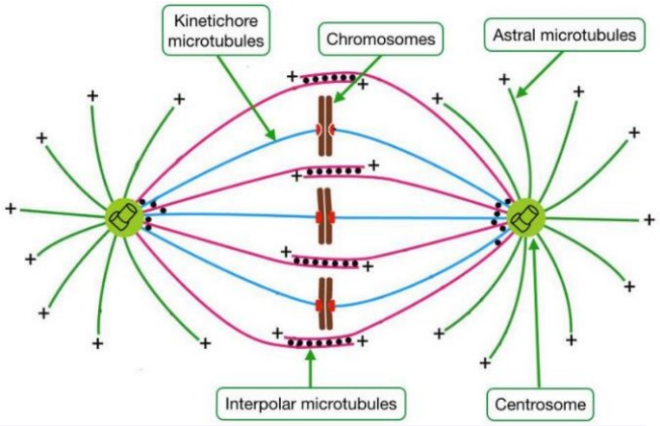
- structure - globular ATP binding head (which interacts with actin microfilaments) and tail (which may bind cargo or bind to other myosins and form myosin filaments)
- types of myosin
    - **myosin-I** – found in nearly all types cells, monomer (1 head + 1 tail)
        - transport of vesicles inside cell
    - **myosin-II** – in muscles, dimers (2 heads + 1 tail in helix)
        - movement of whole cell – contractile ring, muscle movement
- **function – movement to + end of actin microfilaments**
    - transport of vesicle, movement fo plasmatic membranes and muscle movement



2.16.2 Individual sites of motor protein function

Mitotic spindle

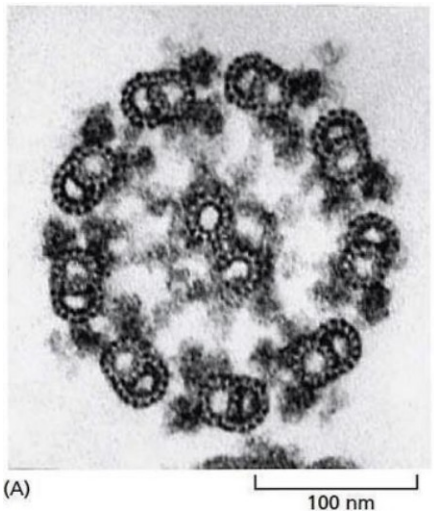
- 3 types of microtubules
    - **kinetochore**- reach from centrosomes to kinetochores (sites on centrosomes where microtubules anchor)
    - **polar** – bind with polar microtubules of opposing centrosome and push centrosomes apart
    - **astral** – reach out into all sides from centrosome, bind to surrounding structures and anchor the centrosome

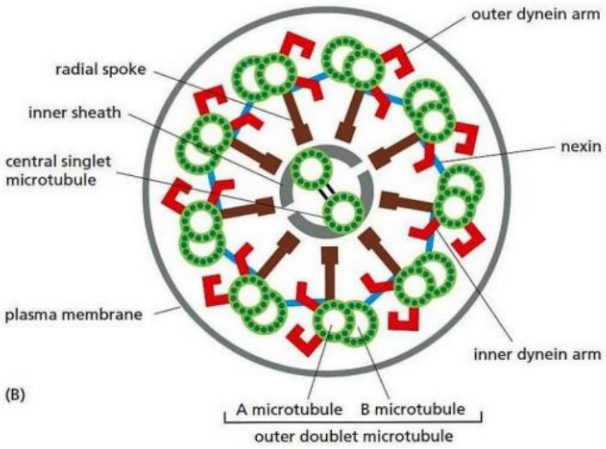


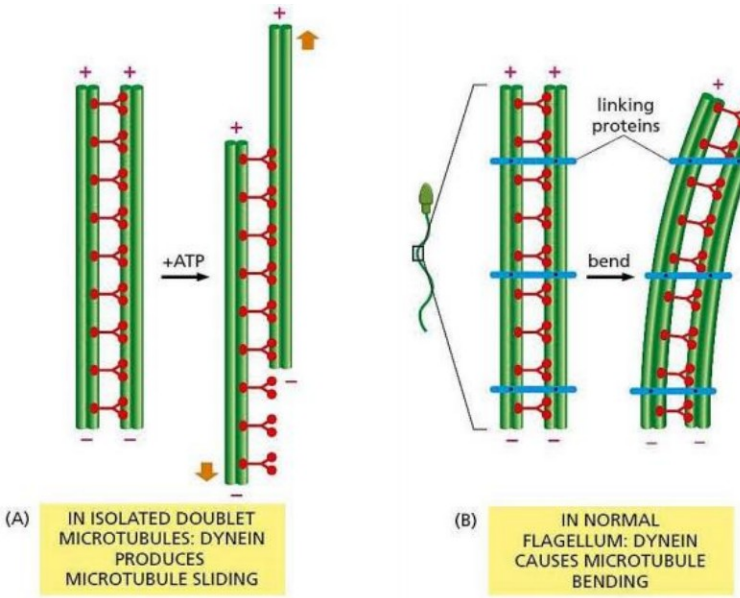
- the movements of the mitotic spindle are driven by **kinesins and dyneins**

Flagella and cilia

- **flagella** – allows for cellular movement
- **cilia** – allows for cell to manipulate/move its surrounding
- flagella and cilia appear the same on the inside - 9 doublets of microtubules with a central pair of microtubules
    - anchored by - end in basal body
- both structures move via the help of dynein – **microtubule pairs are connected by dynein**, which is anchored to one microtubule and "walks" towards the other
    - when microtubules are not tightly connected – one microtubule moves
    - when microtubules and tightly connected – both microtubules bend towards the same direction





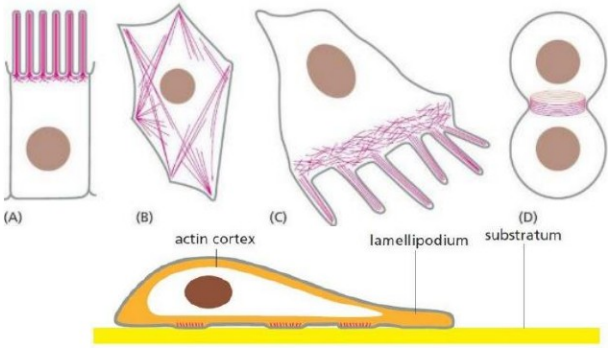


Intracellular transport of organelles

- transport of organelles (along microtubules) is mediated by **kinesin and dynein**
- transport of vesicles (along actin microfilaments) is mediated by **myosin I**

Contractile ring and contractile bundles (actin filaments)

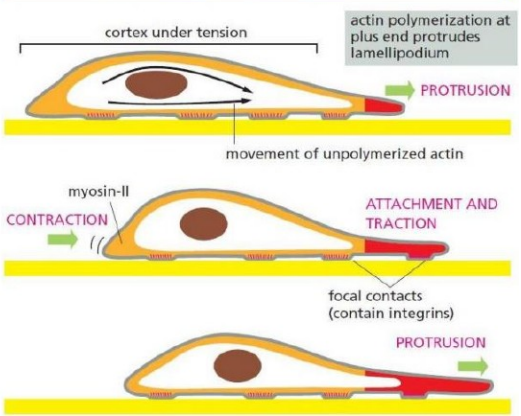
- **D – contractile ring** - during cytokinesis at the equatorial line "strangle" the cell
- **B - contractile bundles** - „muscle "of cells, attached to the plasmatic membrane, can change shape of cell
- Myosin II helps with contraction



Amoeboid movement of cell

- Used by for example by macrophages
- Lamellipodia, filopodia, pseudopodia – projections of plasmatic membrane filled with actin filaments
- growth of actin filaments and myosin II → growth of actin filam elongation of membrane → attachment of growth/projection to surface through focal adhesions → butt contraction (using myos
- for amoeboid movement, you always need a stable surface

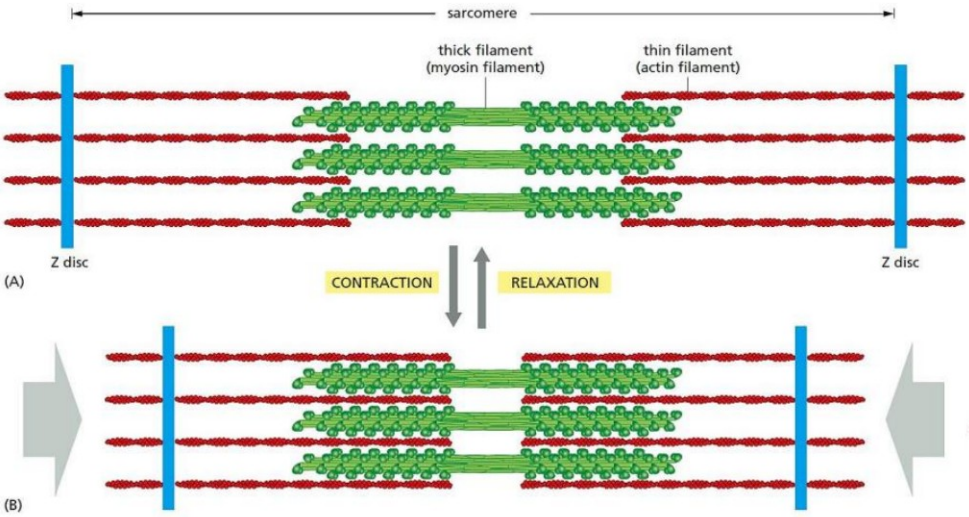
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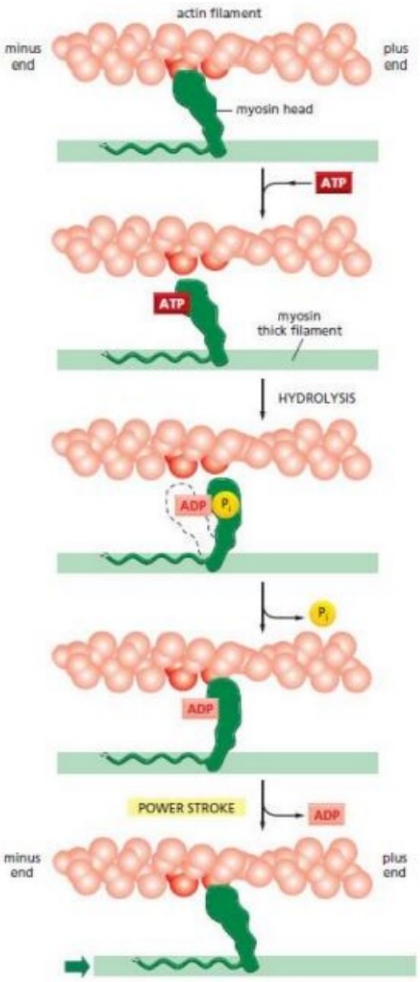


Muscle cells

- structure – mainly myofibrils (actin microfilaments + myosin II)

- **muscle contraction** - myosin II drags actin filaments towards itself
    - resting sate – myosin bound to actin
    - binding of ATP onto myosin causes actin to move away
    - breakdown of ATP to ADP and Pi releases energy and myosin moves gently backwards
    - in its new location, myosin weakly binds to actin (onto a different monomer than before)
    - Pi is released from myosin and the bound between myosin –actin become stronger
    - in the last step, ADP is released which causes the movement of the whole actin fibre





2.17 METABOLISM OF AMINO ACIDS

MUDr. Josef Fontana

NOTE, this topic is long and divided into two lectures, but there is only one presentation and one set of statements

2.17.1 Proteins

- Lack long-term stability, they constantly degrade and rebuild
- Protein daily turnover varies by tissue type- skeletal muscle 10%, liver 40%, small intestinal mucosa 80%
- Recommended daily intake (for 70kg male) – 100g
    - Oxidation of 100g of protein will cover 10-20% of the bodies energy needs

Metabolism of amino acids

- Sources of AAs – diet, degradation of body of body proteins, synthesis de novo
- Protein turnover is strictly regulated
- AA pool – amino acids, which are freely available in the body to be used, about 100g
    - Highest level of AA in the body is **glutamine** and **alanine** – transport ammonia in blood
- **Use of AA** – proteosynthesis, degradation (to energy/ glucose/ FA), production of nitrogenous substances
    - Excess AA cannot be stored (there is no storage protein) so it is burned as energy

Nitrogen balance

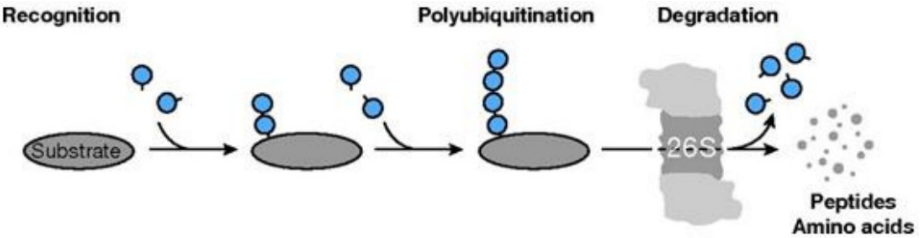
- Expresses the balance between the intake of nitrogen in the diet and loss of nitrogen (mostly through urine)
- in adulthood, we have a balanced nitrogen equilibrium - N accepted = N excluded
- Higher protein intake triggers catabolism of excess AA and urea or NH3 excretion
- **Positive nitrogen balance**
    - Protein intake exceeds losses
    - for example, during recovery from illness (we replenish our losses), in periods of growth or by administration of proteoanabolic hormones (insulin, testosterone, growth hormone)
- **negative nitrogen balance**
    - losses of nitrogen exceed its intake
    - e.g during starvation, severe illnesses or administration of catabolic hormones (glucagon, cortisol)
    - 1g of nitrogen comes from approximately 6.25g of proteins

Degradation of cellular proteins

- cellular proteins have different half-lives
    - ornithine decarboxylase - lasts 11 minutes
    - haemoglobin - lasts 120 days, just like erythrocyte
    - Y-Crystalline (eye lens protein) - lasts a lifetime

Regulation of protein turnover

- **protein ubiquitin** (= ubiquitin)
    - serves as a label – when several ubiquitins are bound to a protein it is labelled for destruction- so called **polyubiquitination**
- the degradation of polyubiquitinated proteins itself takes place in proteasomes - a large compound of proteolytic enzymes



    - Nobel Prize in Chemistry 2004 – **Ciechanover, Hershko and Rose** – "for the discovery of ubiquitin-mediated protein degradation"

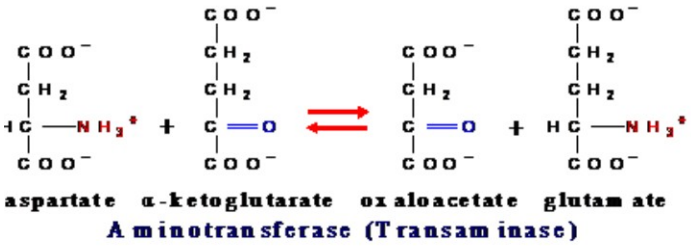
Essential and non-essential amino acids

- **essential amino acids**
    - **branched** - Val, Leu, Ile
    - **aromatic** - Phe, Trp
    - **basic** - Lys
    - **contain sulphur** - Met

    - **with a hydroxyl group** - Thr
- **conditionally essential** - Arg, His
- **non-essential amino acids** - Gly, Ala, Ser, Pro, Cys, Tyr, Asn, Gln, Asp, Glu

2.17.2 Important amino acid reactions

- **decarboxylation – amino acid -> biogenic amines**
    - COO- cleaves from AAs during decarboxylation
    - histidine -> histamine
- **transamination – amino acid -> α-keto-acid**
    - alanine -> pyruvate
    - runs simultaneously with transamination in the opposite direction - $AMK_1 + KK_2 \rightarrow KK_1 + AMK_2$
        - alanine + α-ketoglutarate -> pyruvate + glutamate
- **oxidative deamination – amino acid -> α-keto-acid**
    - glutamate -> α-ketoglutarate
    - NH group is released in the form of ammonia
- **Formation of peptide bonds - formation of peptides and proteins**



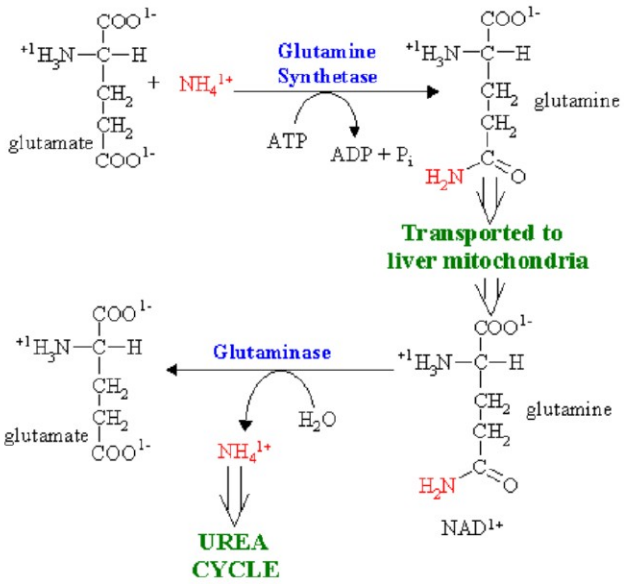
Transamination

- freely reversible reactions
- exchange between the amino group on the amino acid with a keto group on the α-keto acid
    - the amino acid becomes an α-keto acid and α-keto acid becomes an amino acid
- **enzyme - transaminase (aminotransferases)**
    - **ALT – alanine aminotransferases**
    - **AST – aspartate aminotransferases**
- Cofactor is **pyridoxal pyrophosphate** (derivative of vit. B6)

- each transaminase is **specific for one pair** of amino acid and the corresponding α-keto acid; ALT and AST are **liver enzymes**

Conversion of glutamate - glutamine

- conversion of the carboxyl group of glutamate (in the side chain) to the amide group of glutamine
    - $Glu + NH_3 + ATP \rightarrow Gln + ADP + Pi$
- enzyme glutamine synthetase (in cytosol)
- **glutamine is the most transporter of nitrogen (ammonia) in the blood**
- glutamine enters the liver and the mitochondria, where glutaminase converts glutamine to glutamate
    - $Gln + H_2O \rightarrow Glu + NH_4^+$
- This results in nitrogen getting out of the periphery directly into the liver mitochondria, where it is converted into urea and eliminated from the body = **detoxification function**



Oxidative deamination of glutamate

- **glutamate dehydrogenase** enzyme
- occurs in mitochondria, mainly liver
- glutamate was most likely formed by transamination of α-ketoglutarate
- it is the removal of NH_2 group from glutamate and formation of α-ketoglutarate from glutamate
    - $Glu + NAD \rightarrow$ α-ketoglutarate + NADH
- - the ammonia goes back into the ornithine cycle
- - it is mostly linked to glutaminase, which produces glutamate from glutamine

Fate of nitrogen from amino acids

- **extrahepatically** attempt to bind ammonia to carriers and send them to the liver for urea formation
    - transamination most often to pyruvate, alanine or α-ketoglutarate is formed, glutamate is formed
    - amidation - $Glu + NH_3 \rightarrow Gln$
- **in the liver –** what occurred extrahepatically + oxidative deamination of glutamate

Urea cycle (ornithine cycle)

Ammonium ion

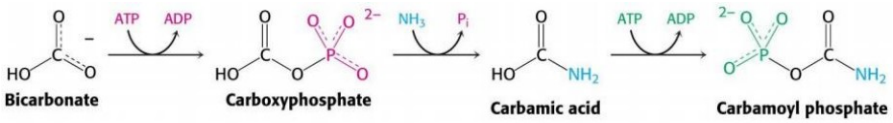
- most terrestrial vertebrates convert ammonia into urea
- concentration of ammonia in blood is 35µmol/l
- **is toxic for the brain – is non polar and therefore freely crosses the blood-brain barrier**
  - **merges with α-ketoglutarate in the brain, forms glutamate and blocks Krebs cycle**

Urea cycle

- urea forms from NH_3, CO_2 and NH_2 groups, that have been donated by aspartate
- occurs mainly in the liver, but urea is excreted by the kidneys
- urea cycle begins in the mitochondrial matrix, the rest in the cytosol

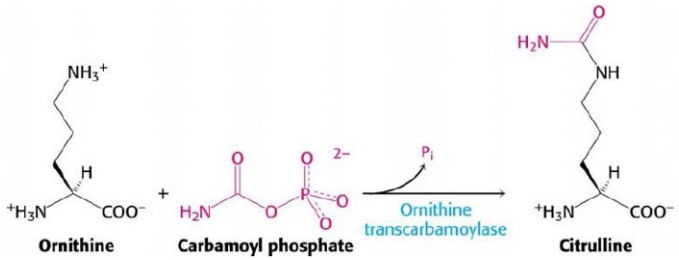
1) formation of carbamoyl phosphate – in mitochondria

- ammonia is carboxylated with ATP to carbamoyl phosphate
- **carbomoyl phosphate synthetase I enzyme**
- free ammonia is combined with HCO_3
- regulatory reaction of urea synthesis
- consumptions of 2 ATP



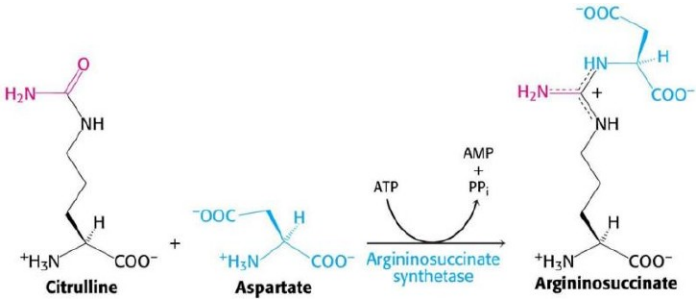
2) formation of citrulline

- carbamoylphosphate enters the ornithine cycle (just like Acetyl CoA into the Krebs cycle)
- carbamoylphosphate donates its carbamoyl group ($CONH_2$) onto ornithine to form citrulline
- **ornithine transcarbamoylase enzyme**
- ornithine is an amino acid, which is carried from cytosol into the matrix specifically for this reaction
- **the resulting citrulline is immediately transported from the mitochondrial matrix to cytosol, where further reactions take place**



3) formation of arginosuccinate

- in the cytosol citrulline fuses with aspartate with the help of ATP and the formation water
- citrulline + aspartate -> arginosuccinate + H_2O
- **arginosuccinate synthetase enzyme**
- part of the reaction is the breakdown of ATP to AMP + PPi, which means the use of 1 molecule of ATP, with the energy equivalency of 2 ATP

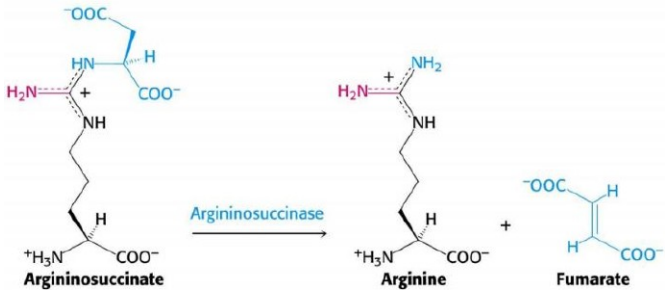


4) breakdown of arginosuccinate

- in the next step arginosuccinate is broken down to arginine and fumarate
- **argininosuccinase enzyme**
- arginine continues in the urea cycle
- fumarate returns into the Krebs cycle

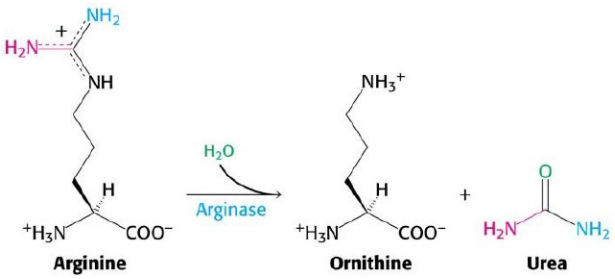
5) formation of urea

- arginine undergoes hydrolysis -> formation of urea and ornithine
- **arginase enzyme**
- urea is excreted from the cell and from the body
- ornithine is carried into the matrix and the cycle continues



Regulation of the ornithine cycle

- **regulatory enzyme carbamoylphosphate synthetase I**
  - activation of N-acetylglutamate which is formed by the reaction of acetyl-CoA and Glu
  - enzyme N-acetylglutamate synthetase activates arginine
- urea cycle is a proton production reaction, therefore during acidosis (excess of H^+) the reaction is stopped



- during high levels of protein in diet there is an increased transcription of all enzymes of the urea cycle

Urea cycle disorders

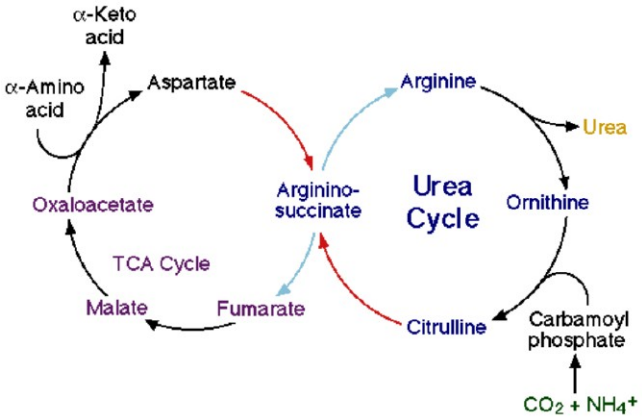
- congenital diseases caused by a defect of one of the urea cycle enzymes – total frequency about **1 : 30 000**
- **accumulation of ammonia** (+ intermediates before the block, lack of intermediates after it) → toxicity → **CNS damage**
  - early manifestation after birth: brain oedema, convulsions, vomiting, even coma
  - long-term: disorders of psychomotor development, hypotonia, anorexia
  - **glutamine** formation and concentration increases (glutamine synthetase)
- treatment
  - stop catabolism (AA degradation) – give **glucose and insulin**
  - eliminate excess ammonia – **haemodialysis, sodium benzoate, phenylbutyrate**
  - substitute the missing AAs in some defects; reduce dietary protein; **liver transplantation**

Citrullinemia

- **type 1** – **argininosuccinate synthase** defect
- **type 2** – defect of the mitochondrial **aspartate/glutamate transporter** → lack of aspartate in mitochondria
- low **arginine**, high **citrulline, glutamine and orotic acid**; early and late (chronic) forms

Relation between urea and Krebs cycle

- it is the active exchange of intermediates
- occurs through aspartate and fumarate
- each degraded amino acid donates its amino group onto α-ketoglutarate, which forms glutamate
- enzyme AST (aspartate aminotransferase) switches the amino group from glutamate to oxaloacetate, to form aspartate
- aspartate enters the urea cycle, fumarate exits
- fumarate is then recycled to oxaloacetate in the Krebs cycle, waiting for the next amino group



2.17.3 Individual metabolic pathways of amino acids

Utilization of carbon skeleton of amino acids

- carbon skeleton of each amino acid is metabolized by its own specific pathway
- eventually, however, 7 intermediates are formed

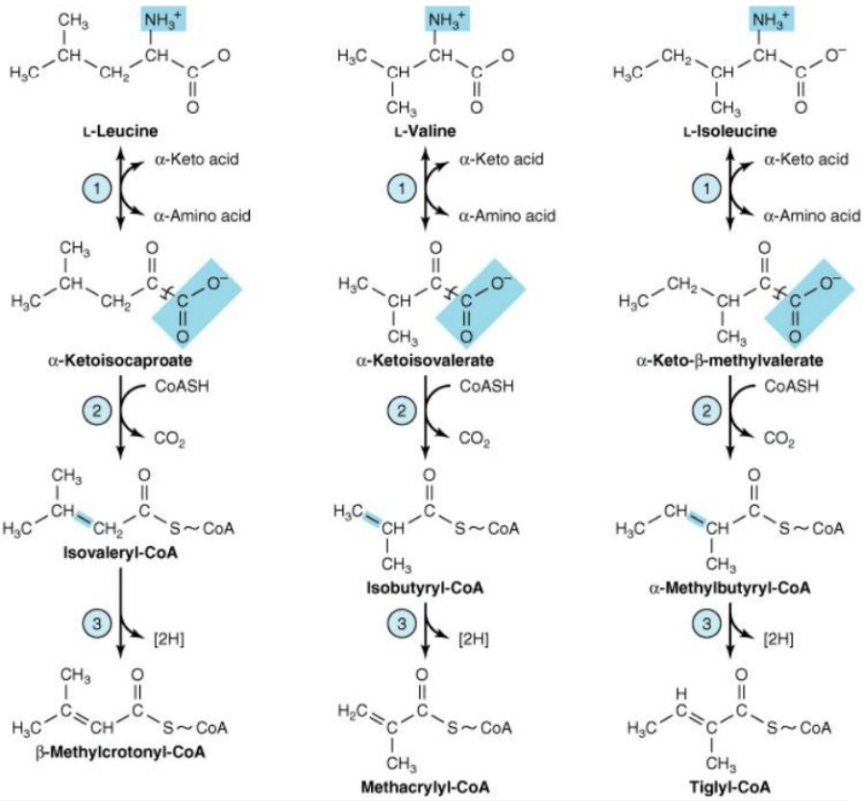
  KETOGENIC
  - **acetoacetyl-CoA - Lys**, **Leu**, **Phe**, **Tyr**, **Trp**
  - **acetyl-CoA - Ile**, **Leu**

  GLUCOGENIC
  - **fumarate - Phe**, **Tyr**
  - **oxaloacetate - Asp**, **Asn**
  - **succinyl-CoA - Val**, **Ile**, **Met**, **Thr**
  - **α-ketoglutarate - Glu**, **Gln**, **Pro**, **Arg**, **His**
  - **pyruvate - Gly**, **Ala**, **Ser**, **Thr**, **Cys**, **Trp**
- amino acids that break down into acetyl-CoA or acetoacetyl-CoA are so called ketogenic (because glucose can't be synthesized from them, but ketone bodies can) and others are so called glycogenic (because glucose can be synthesized from them
- division of amino acids
  - **ketogenic - lysine a leucine** (begin with L)
  - **glycogenic - serine, threonine, cysteine, methionine, aspartate, glutamate, asparagine, glutamine, glycine, alanine, valine, proline, histidine, arginine**
  - **ketogenic and glycogenic - isoleucine, phenylalanine, tyrosine a tryptophan** (aromatic AA)
  - some are quite easy to derive/remember
    - 4C amino acids - **aspartate and asparagine** transaminate to oxaloacetate
    - 5C amino acids - **glutamine and glutamate** transaminate to α-ketoglutarate
    - **alanine** transaminates to pyruvate
    - **lysine a leucine** (both branched AAs begin with L) are ketogenic
    - **glycine, serine a cysteine** – all small amino acids as well as alanine, turn into pyruvate

Degradation of branched amino acids

- valine, leucine, isoleucine
- it occurs not only in the liver but also, to a significant extent, in skeletal muscles and the heart
- used in those with hepatic failure and athletes to provide quick energy
  1) transamination - specific transaminase
  - greater activity in skeletal muscle and heart, less activity in liver
  - products are 2-keto acids, which are washed out of the muscle into blood and taken up by the liver

  2) decarboxylation
  3) dehydrogenation
- product is acyl-CoA



Leucinosis – maple syrup urine disease (MSUD)

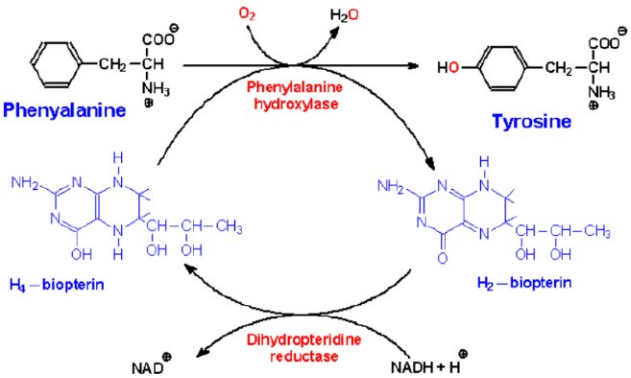
- very rare **AR** disease (1 : 185 000), classified as an **organic aciduria**; part of **newborn screening** – symptoms usually in the newborn period
- deficiency of **branched-chain α-keto acid dehydrogenase** (step 2) → affects all branched AAs
- accumulation of branched-chain AAs and their acids – toxic; worsens with increased BCAA load (starvation, common illness, stress)
- typical odour of urine and body fluids – **"maple syrup"** (caramel, "Maggi")
- progresses to hyperammonaemia, **ketoacidosis**, convulsions, vomiting, coma
- lifelong diet with protein (BCAA) restriction, proactive management of risk situations

Amino acid formation

- essential - Phe, Trp, Val, Leu, Ile, Met, Thr, Lys
- conditionally essential - Arg, His
- non-essential
  - oxaloacetate -> Asp, Asn
  - 2-ketoglutarate -> Glu, Gln, Pro, (Arg)
  - pyruvate -> Ala
  - 3-phosphoglycerate -> Ser, Cys, Gly
  - Phe -> Tyr

Syntehsis of tyrosine from phenylalanine

- Phe and Tyr differ by one OH group
- The enzyme phenylalanine hydroxylase and cofactor biopterin (H_4) can bind OH to Phe and produce Tyrosine
- Enzyme dihydropteridine reductase recycle biopterin
- from Tyr, for example, melanin and thyroid hormones are synthesized



Phenylketonuria

- **AR disease due to the absence or decreased activity of enzyme phenylalanine hydroxylase**
- The body begins to accumulate and alternatively breaks down Phe
- Alternative degradation of Phe may produce (inter alia) phenylethylamine, which damages the brain
- Phenylketonuria is resolved by strict withdrawal of phenylalanine from the diet and artificial administration of tyrosine

- alternative Phe degradation: **phenylpyruvate** (in urine – the "phenylketone") → phenyllactate and phenylacetate
- PKU is part of **newborn screening**

2.17.4 Significant amino acid derivatives

Biogenic amines

- Group of amines from by the decarboxylation of amino acids
- **(Phe ->) Tyr -> catecholamine's** (adrenaline, noradrenaline, dopamine; hormones and neurotransmitters)
- **Trp -> serotonin** (5-hydroxytryptamine; neurotransmitter)
- **His -> histamine** (immune system signalling agent, neurotransmitter)
- **Ser -> ethanolamine** -> choline -> acetylcholine (neurotransmitter in neuromuscular plates)
- **Cys -> cysteamine** (component of Coenzyme A)
- **Asp -> β-alanine** (component of Coenzyme A)
- **Glu -> γ-aminobutyrate** (GABA; neurotransmitter)

Nitric oxide (NO)

- Small molecule, short half-life in our body
- Precursor is arginine – produced No and citrulline
- enzyme NO-synthase - isoenzyme, has several forms
  - **NOS I - brain** - neurotransmission (helps neurotransmission of other transmitters)
  - **NOS II - macrophage** – killing of bacteria
  - **NOS III - endothelial** - NO causes vascular dilation – vasodilation (relaxes smooth muscle)
- Clinical significance of NO – nitrates in the treatment of angina pectoris (restriction of blood flow to the heart, pre-infraction)
  - Hypotension accompanying septic shock

Thyroid hormones

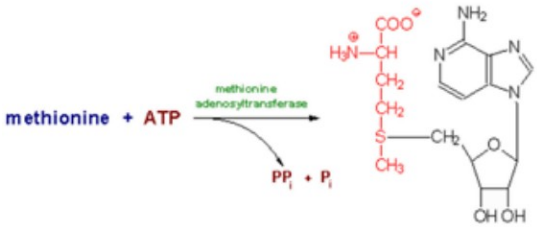
- thyroxine (T4) a triiodothyronine (T3) – from tyrosine, they are not biogenic amines (they still have COO^-)

Melanin

- tyrosine derived pigment (produced by its oxidation and polymerization)
- we recognize two types
  - occult – melanocytes in skin
  - neuromelanin – mainly in the substantia nigra of the middle brain
  - (the slides call the first type **oculocutaneous** melanin – skin melanocytes)

formation of activated methionine (SAM = S-adenosylmethionine)

- SAM is a very common methyl donor in methylation reactions
- **Formed by the fusion of methionine and ATP**, during which ATP loses all its phosphates
  - enzyme methionine adenosyltransferase
- after methyl donation, SAM is regenerated with N^5-metyl-THF



Hyperhomocysteinemia

- increased blood **homocysteine** = **risk factor for cardiovascular diseases**
- often caused by **vitamin B9 and B12 deficiency** (both needed to convert homocysteine back to methionine)
- congenital enzyme defects: **MTHFR** (methylenetetrahydrofolate reductase – 5,10-methylene-THF is not reduced to 5-methyl-THF) or **cystathionine β-synthase (CBS**, cofactor = vit. B6 derivative) → congenital **homocystinuria**

Creatine synthesis

- provides a very fast energy reserve for muscle tissue
- arginine + glycine -> guanidinoacetate + ornithine
  - enzyme glycinamide transferase
  - occurs in the kidneys
- guanidinoacetate + SAM -> creatine + S-adenosyl-homocysteine (recycled back to SAM)
  - enzyme guinidinoacetate methyltransferase
  - takes place in the liver
- creatine -> creatinine + H_2O

Overview – conversion of AA into specialised products

- histidine - histamine

- tryptophan - serotonin, melatonin
- phenylalanine, tyrosine - adrenalin a noradrenalin, thyroid hormones
- glutamate - GABA
- glycine - heme, purine, bile acid conjugation, creatine
- ornithine and arginine - creatine, polyamines (spermidine, spermine)
- glycine, glutamine, aspartate - purines and pyrimidines
- arginine - NO
- cysteine - taurine
- serine –phospholipid base

2.17.5 Organ specifics of amino acid metabolism

Blood

- concentration of **amino acids in plasma - 2,3-4,0 mM**
  - **glutamine** (**0,6 mmol/l** has the highest amount; main form of transport of ammonia) and **alanine** (**0,3 mmol/l**; main component of glucose-alanine cycle)
- **ammonia - 6-35 µmol/l**, when it exceeds 50 µmol/l its toxicity starts to appear and damages the CNS
- **urea - 2,5-8,3 mmol/l**
- **creatinine - 50-120 µmol/l**

Liver

- major organ of amino acid metabolism
  - removal of amino groups from amino acids
  - ammonia detoxification - 95% is excreted in the urea cycle, glutamine is produced from the rest and sent to the kidneys
  - conversion of the C-skeleton to glucose, fatty acids or ketone bodies
  - formation of non-essential amino acids

Intestine - enterocytes

- all the amino acids we take in the diet must pass into the blood through enterocytes
- enterocytes regulate the entry of individual amino acids into the body
- **glutamine is the basic energy substrate of rapidly dividing cells** (e.g. enterocytes or immune cells)
  - amino group of glutamines enters the purine formation, and oxidation of the C-skeleton yields energy
  - enterocytes change the spectrum of absorbed AAs – e.g. more **proline and citrulline** (made from Glu/Gln) in the portal blood
  - during starvation and stress, the main source of glutamine is skeletal muscle

Skeletal muscle

- main 'reservoir' of proteins – used during stress and/or starvation
- even muscle cells alter the spectrum of amino acids released into blood
  - what they gain by proteolysis of their own proteins does not correspond to what they secrete into the blood
- branched amino acids can be transaminated in muscle – their α-keto analogues are released into the blood (or oxidized to gain energy), the amino groups are transferred to glutamine or alanine – they go back to the blood

Kidneys

- are the major site of N-catabolite excretion (urea, ammonia, creatinine, uric acid etc.)
  - = nitrogen catabolites begin to accumulate in during kidney damage
- tubular cells are able to convert glutamine to glutamate into α-ketoglutarate - ammonia is released
- glycogenesis also occurs in the kidneys - about 10 - 15%
  - (this means **gluconeogenesis** – the kidney is the second gluconeogenic organ)
- conversion of citrulline -> arginine

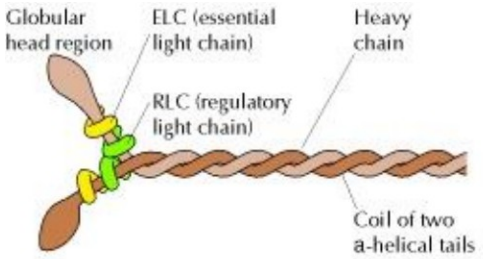
Starvation states

- **marasmus** – starvation due to insufficient energy and protein intake
  - proportional reduction of both fat and non-fatty matter
- **kwashiorkor** – inadequate intake of protein with adequate intake of energy and vitamins
  - retarded growth, loss of pigmentation on skin and hair, ascites, mental apathy

2.18 MUSCLE METABOLISM, MUSCLE CONTRACTION

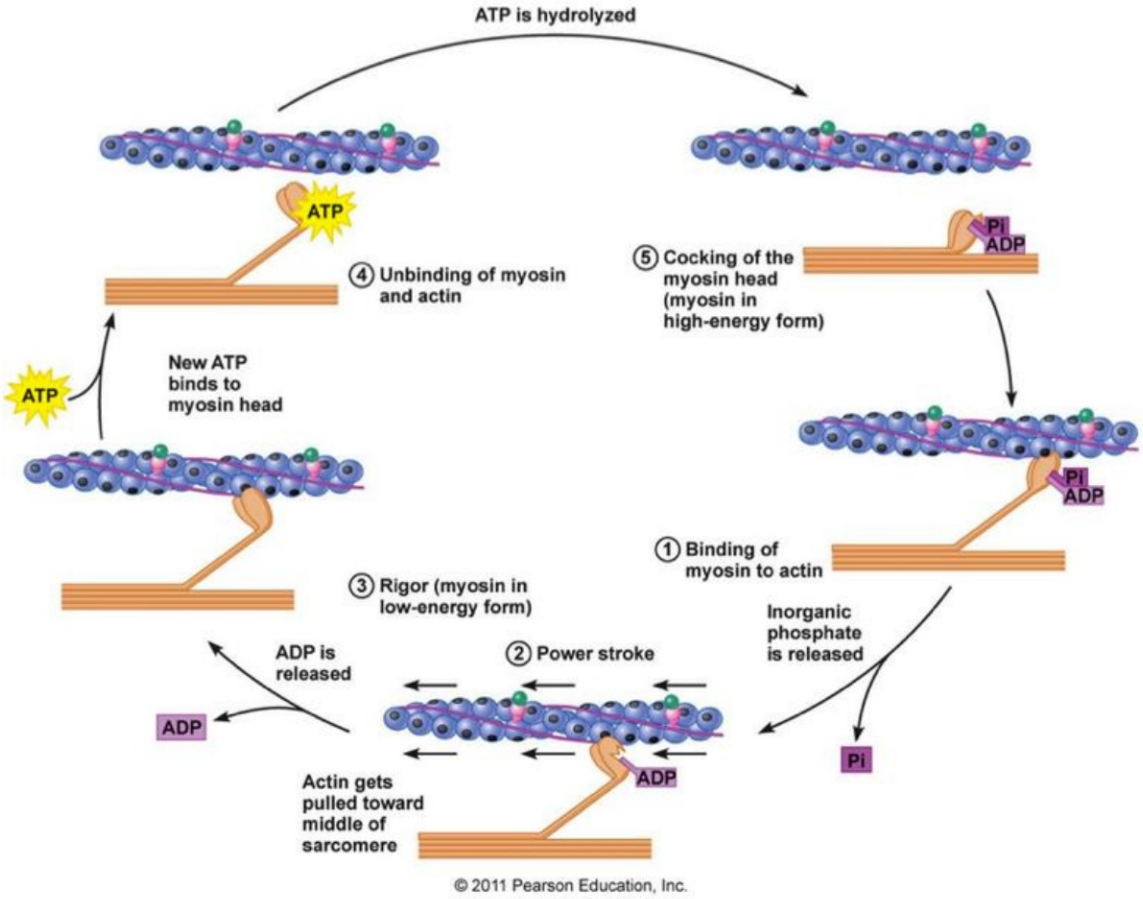
doc. MUDr. Jan Trnka, Ph.D.

- muscle contraction is a mechanism for converting potential energy into mechanical energy
  - two contractile proteins – **actin a myosin** (myosin type II) are responsible for this
- **myosin II**
  - creates dimers, which then stack on top of each other to form one long filament
  - one monomer consists of 1 heavy chain (globular head and long fibrillary tail) and 2 light chains (adjacent to the globular head and have an important regulatory function)



2.18.1 Muscle contraction

- universal stimulus to muscle contraction in all type of muscle tissue is- **increased calcium concentration in the cytosol**
- muscle contraction is a cyclic process, at its end/beginning **myosin is strongly** (ionically) **bound to actin**
  - at this stage, the muscle is contracted
- **release of contraction – binding ATP** to myosin releases a strong bond that previously held actin and myosin together
  - we need ATP to relief the muscle, which is the basis of rigor mortis – after death there is no ATP = no muscle relaxation
- **hydrolysis of ATP** - myosin hydrolyses ATP to ADP and phosphate, and both hydrolysis products remain bound to myosin
  - during hydrolysis, the myosin head's conformation changes - head moves backwards
  - this is the moment when energy is transformed form chemical to mechanical
- **again, the binding of myosin onto actin** – a step strictly regulated by the calcium concentration in the cell
  - myosin head reconnects to actin
    - but this time to another location, because in the previous step the head moved
  - at the same time the inorganic phosphate is released form myosin (so that only ADP remains on myosin)
- **moving the head back to its original state** – once myosin head with ADP is attached to actin, it moves forward
  - however, because it is bound to actin, it moves with actin
  - this is the phase we see – in this, the arm/leg moves j
- **removal of ADP from the myosin head**
- all of these steps take place several thousand times during, muscle movement – one cycle is not the entire muscle movement



Skeletal muscle

- increased calcium concentration in skeletal muscle occurs during **membrane depolarization**
    - stimulus for membrane depolarization comes from neuromuscular plate - binding of acetylcholine to membrane ion channels opens them and releases sodium ions into the cell, thereby depolarization of membrane - **acetylcholine channels would not be enough, therefore thet are supported by voltage-controlled sodium channels**
    - depolarization gradually spread across the membrane and **activates voltage- controlled calcium channels**
        - these channels are called **dihydropyridine receptors**
    - dihydropyridine receptors are mechanically linked to calcium channels in the sarcoplasmic reticulum through which calcium leaks into the cytosol
        - calcium channels in the sarcoplasmic reticulum are called **ryanodine receptors**
    - all the calcium required for muscle contraction comes from sarcoplasmic reticulum
- **calcium sensor** - a protein that sits on the actin-myosin complex and prevents the binding of myosin to actin without sufficient calcium in its surroundings
    - it is called **troponinotropomyosin complex** - it is composed of 3 troponin proteins and 1 tropomyosin
        - tropomyosin is a protein that periodically blocks binding sites on actin (= blocks myosin binding)
        - troponins are globular proteins whose function is to monitor Ca2 + levels
            - troponins are divided into troponins C, I and T
        - once troponins detect enough calcium, the entire troponinotropomyosin complex will change conformation, move away and unblock the calcium binding sites
- **contraction arrest** - breakdown of acetylcholine in the neuromuscular plate and membrane repolarization
    - Ca^{2+} pump in the sarcoplasmic reticulum membrane (SERCA) returns calcium to SER
        - SERCA is automatically activated at high cytoplasmic Ca^{2+} concentration

Heart muscle

- in rough outlines, depolarization is similar to skeletal muscle (although it has a different stimulus)
    - does not acquire a stimulus to contract the neuromuscular plate, such as skeletal muscle, but has its own system that controls the heart beat - the so-called cardiac conduction system (more in the 2nd year)
- however, there is a big difference in the mechanism of calcium channel opening in the sarcoplasmic reticulum
    - **dihydropyridine a ryanodine receptors are not linked** - instead, ryanodine receptors open in response to Ca^{2+} molecules that have entered the cytosol through open dihydropyridine receptors
    - the heart muscle thus **requires calcium from the extracellular environment**
    - dihydropyridine receptors therefore allow a few calcium ions to enter the cytosol, which then open up the ryanodine receptors
- dihydropyridines - drugs called calcium blockers
    - affect the heart contraction (make it more difficult) without affecting the skeletal muscles - i.e. drugs against hypertension and arrhythmia

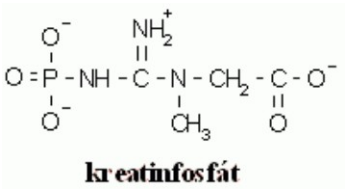
Smooth muscle

- contraction is regulated through hormonal signalling
- different structure - smooth muscle has single cells with one nucleus, that are connected together
- the main difference is the duration of the contraction - the contraction has a much longer onset and a much longer duration
- for smooth muscle, much of the calcium required to contract comes from outside
    - thus, calcium channel blockers are very effective in blocking smooth muscle contraction
- **smooth muscle has no tropinotropomyosin complex**
    - instead the calcium sensor is **myosin light chain kinase - MLCK**
    - MLCK is activated by the calmodulin-calcium complex
- Smooth muscle needs to **phosphorylate one myosin** to contract (without it no contraction will occur)
    - phosphorylation is catalysed by MLCK, which is activated by the calmodulin-calcium complex, which is the calcium regulating factor - when there is no calcium, there is no calmodulin-calcium complex, no MLCK is active, no phosphorylation, no muscle contraction
- **muscle relaxation is regulated by myosin light chain phosphatase**
    - cuts off phosphate groups as soon as the calcium level is reduced

2.18.2 Energy for muscle

- **skeletal muscle** - at rest gains most of the energy from fatty acids, with increasing level of effort carbohydrate utilization increases
- **has two type of fibres that differ in their substrate orientation**
    - **white fibre** - glycolytic (primary substrate is glucose), very fast pulling ability, can work well even without oxygen, produce lactate

        - **red fibre** - rely on aerobic metabolism (mitochondria, oxygen, fatty acids)
- **cardiac muscle** - can work only aerobically
    - It primarily processes fatty acids, but can also metabolize glucose and lactate
- **smooth muscle** - not very energy intensive
- **creatine** - a small molecule composed of three pieces of amino acids (arginine, glycine, methionine), which act as an ATP buffer
    - is able to be phosphorylated from creatine to creatine phosphate so far from equilibrium that it has the character of ATP
        - **ATP + Cr <-> ADP + Cr-P is a reversible reaction with no energy loss**
    - Cr-P can be thought of as a form of ATP - it is possible to make Cr-P stocks, which are converted into ATP and then into energy
    - creatine and creatine phosphate are spontaneously converted to a degradation product called **creatinine**
        - **creatinine** acts as a marker of kidney function (more creatinine in blood and less in urine means trouble)

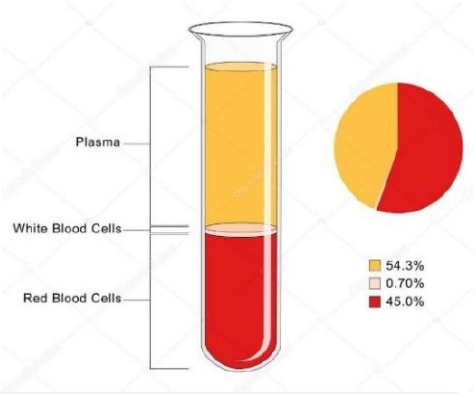


- **creatine kinase** - an enzyme that cleaves phosphate from ATP and passes it on to creatine, creating creatine phosphate
    - it can catalyse the same vice versa, without any loss of energy
- energy to contract
    - ATP, which is stored in the muscles, is sufficient for about 5 seconds
    - Cr-P reserves are consumed for a further 15 seconds
    - then energy needs to be met by the metabolism of sugars or fatty acids

2.19 BLOOD - MORPHOLOGY OF BLOOD ELEMENTS

MUDr. Eva Maňáková, Ph.D.

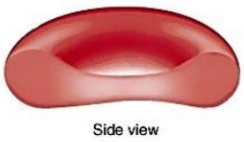
Blood



- blood = a **special type of connective tissue**: cells (formed elements) + ECM (plasma)
- functions: distribution of O_2 and CO_2, transport of nutrients, **regulation of pH, temperature, glucose and cholesterol**, protection, haemostasis
- reference counts (slides, per litre): erythrocytes 4.3–5.3 × 10^{12}, leukocytes 4–9 × 10^{9}, thrombocytes 150–300 × 10^{9}
  - **Volume** - around 5,5l
    - **haematocrit** - percentage of erythrocytes
      - 35-45 in women, 40-50 in men
    - leukocytes and thrombocytes forming a white, cloudy layer are found in the space between plasma and erythrocytes
      - this layer is about 1% of blood volume, it is made of leukocytes and thrombocytes and it is called **buffy coat**
    - rest of blood is **plasma** water, inorganic salts and organic compounds (proteins - albumins, globulins...)
  - **serum** - liquid separated from clotted blood.
    - composition similar to blood plasma, but unlike plasma it does not contain fibrinogen and other blood clotting factors
  - **Blood smear** – examination of peripheral venous blood
    - We determine the differential white blood cell count and information about the blood cell morphology
    - Nowadays we often machine differentials and evaluation
    - however, some abnormal cells are not recognized by the machine, so if a disease that could affects blood cells is suspected, the blood count is examined manually under a microscope - manual differential
    - **Blood smear staining is standardly performed according to Pappenheim**

2.19.1 Erythrocytes

- nuclear-free biconcave cells
- **size** - 7,5 x 2,6 µm (only 0,8 µm in the centre)
  - biconcave shape means larger surface area and better gas exchange
- contain a 33% haemoglobin solution
- **number** - 3,9 - 5,5 million in 1µm
  - the number is dependent on gender, race, geography and many other factors, so it is necessary to determine (statistically calculate) reference values in each country
- **important protein - spectrin** - together with actin form the characteristic shape of erythrocyte


Side view


Top view

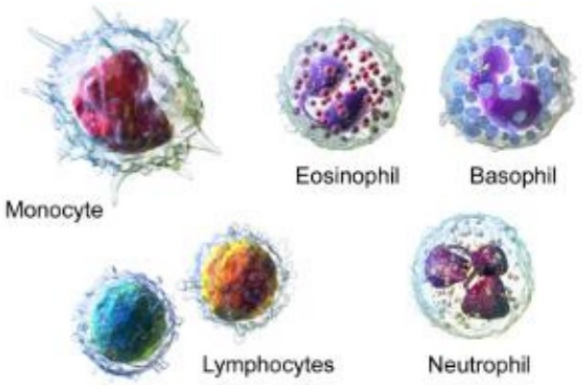
- erythrocyte cytoplasm is **eosinophilic** (haemoglobin); blood group systems by surface antigens – mainly **AB0 and Rh**
- a network of spectrin and actin is bound to the membrane via ankyrin proteins
- erythrocytes do not have mitochondria (so they wouldn't consume the oxygen they carry)
  - are reliant on **anaerobic metabolism**, which means glucose - its entry into the cell is facilitated by insulin non-dependent diffusion
  - a small portion of glucose enters the pentose cycle, by which the RBC gains NADPH, which the cell needs to protect against free radicals and keep iron in Fe^{2+} form
- **lifespan in circulation is 120 days**

- gradually become less elastic, deforming
- they are destroyed in the spleen by macrophages
- 0.5-1.5% of red blood cells in the blood may be reticulocytes, which are immature erythrocytes that mature over time
- erythrocytes carry oligosaccharides on their membranes that determine the AB0 system
- **normal values**
  - **men 18 - 110 let haematocrit 39-51 4,19-5,75 * 10^{12} erythrocytes per litre**
  - **women 18 - 110 let haematocrit 33-47 3,54-5,18 * 10^{12} erythrocytes per litre**
- **average values**
  - **men 18 - 110 let haematocrit 45 5.1 * 10^{12} erythrocytes per litre**
  - **women 18 - 110 let haematocrit 40 4,5 * 10^{12} erythrocytes per litre**

Clinical application

- **anaemia** - decreased haemoglobin concentration in the blood, often associated with decreased haematocrit and increased bleeding
- **polycythaemia**- increased erythrocyte count and haemoglobin concentration, increasing blood viscosity and risk of thrombosis
  - caused by either adaptation or hemoblastosis (tumour growth from erythrocyte precursors)
- **anisocytosis**- higher size variability
- **macrocytes**- larger erythrocytes, often means a lack of Vitamin B12
- **microcytes**- smaller erythrocytes (Fe deficiency)
- slides: macrocytes **> 9 µm**, microcytes **< 6 µm**
- **sickle cell anaemia** - point mutation in the haemoglobin β-chain gene

2.19.2 Leukocytes

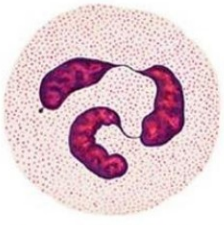


- **6.000 - 10.000 in 1µl**
- wice as many at birth than in adulthood
- granulocyte and agranulocyte differential count
  - not counting the from the whole blood volume (that would-be madness), but only by smearing a small blood sample
  - **granulocytes**
    - **neutrophils 60 - 70%**
    - **eosinophils 2 - 4%**
    - **basophils 0 - 1%**
  - **Agranulocytes**
    - **lymphocytes 20 - 30%**
    - **monocytes 3 - 8%**
  - (the slide gives slightly different ranges: eosinophils 1–3 %, basophils 0–2 %, lymphocytes 20–40 %, monocytes 5–10 %)
- **leukocytes vs. erythrocytes**: counted in thousands; contain a nucleus (segmented in granulocytes); colourless (no haemoglobin); bigger; contain enzymes; **amoeboid**; some are phagocytes; life span only days; function – specific and non-specific immunity

Granulocytes

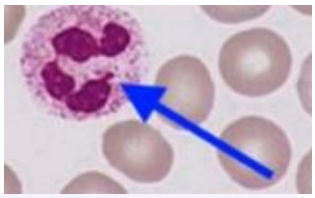
- size 12-15 µm
- **contains two types of granules**
  - **specific** with a specific function for the given type of leukocyte
  - **azurophilic** - lysosomes
- all granulocytes in peripheral blood are terminal stages of development - they no longer diivide and survive in the blood for only a few days
- the last step of granulocyte maturation is nuclear segmentation
- left shift - terminology, means the presence of a greater number of less mature granulocytes in peripheral blood

Neutrophil granulocytes (polymorphonuclear cells)


neutrofilní granulocyt

- **nucleus divided in to 2-3 lobes connected through chromatin bridge**
  - Barr's body may be visible on the neutrophil as it peeks from the lobe (blue arrow)
  - Immature granulocytes do not have a segmented nucleus and are called band neutrophils.
- anaerobic metabolism - energy source glucose
- they are present in the bloodstream for 1 day, in tissues for 1 to 2 days, after which they become apoptotic

- **content of their granules**
  - **specific** - modified lysosomes, contains alkaline phosphatase, collagenases and basic proteins
  - **azurophilic** – many hydrolytic enzymes



  - slides: specific granules – **lactoferrin, lysozyme, collagenase** (small, near the resolution limit of the light microscope); azurophilic granules (0.5 µm) – **myeloperoxidase, cathepsin, DNase**
  - energy from **glycogen** (anaerobic glycolysis); nucleus has on average 2.7 lobes
- in addition to neutrophils present in blood there are 2 other populations – veins and bone marrow
- their aim is to phagocytose the foreign organism
  - **oxidative burst** - enrage of white blood cells, after phagocytosis of pathogens, they utilise alot of oxygen to produce hypochlorite, which destroys pathogens. They are able to form this molecule with the help of enzyme **myeloperoxidase**

Eosinophilic granulocytes

- typically has a two-lobed nucleus
- specific granules
  - crystalline medulla (internum) – main basic protein
  - matrix (externum) - lighter
- lay a role in allergies and parasitic infections - eosinophilia occurs
  - **eosinophilia** - a state of increased eosinophils in the blood
- modulation of inflammation (inactivation of leukotrienes and histamine)



Eosinophilic granulocytes

- size 12–15 µm; the specific granule = modified lysosome for exocytosis; **amoeboid motility**

Basophillic granules

- the core is divided into irregular lobes, but is not visible because it is covered by granules
- contains specific **metachromatic granules, which contains histamines and heparin**



Basophilic granulocytes

- size **10–12 µm – the smallest granulocyte**
  - o these granules are degranulated after binding of certain antigens
  - severe degranulation may lead to the development of anaphylactic shock
- function - regulation of immune response

Agranulocytes

- **do not contain specific granules, only azurophilic granules**
- nucleus is round – kidney shaped
- typical of acute inflammation (they work fast), have a short lifespan
- accumulation of dead agranulocytes produces pus
- includes lymphocytes & monocytes

Lymphocytes

- sorted by size into small (6-8µm), medium (9-18µm) and large (over 18µm)
- **have basophilic cytoplasm and isolated azurophilic granules**
- contain a large spherical nucleus, condensed chromatin, a distinct nucleolus, a thin band of cytoplasm, ribosomes and azurophilic granules
- small B and T lymphocytes - the so-called memory cells, which provide specific immunity - predominate in the blood
  - 65-75 of which are T lymphocytes
  - we do not recognize them in common observation, we need cytogenetic methods
- **they are divided into B-lymphocytes, T-lymphocytes and NK-cells**
  - **B-lymphocytes** differentiate in bone marrow into plasma cells and produce immunoglobulins
    - Provide antibody (humoral) immunity
  - **T-lymphocytes** provide cellular immunity - they differentiate in the thymus and learn to recognize their own antigens



lymfocyt

        - d according to surface CD molecules are further divided into auxiliary, cytotoxic and suppressor
        - **T_H-lymphocytes (helper)** - CD4, allow cooperation of APC (antigen-presenting cells) and B-lymphocytes and stimulate antibody production
        - **T_C- lymphocytes (cytotoxic)** - CD8, they clear common cells, they can induce apoptosis in target cells
        - UP TSUP-lymphocytes
        - **T_{SUP}- lymphocytes (suppressor)** - CD8 control activity of other lymphocytes - induce apoptosis
    - **NK (natural killers)** - cells provide non-specific cellular immunity by inducing apoptosis
        - roughly like macrophages
        - destroy tumour cells and cells infected with viruses
        - developmentally closer to T lymphocytes than B lymphocytes
    - NK cells make up **10–15 %** of peripheral blood lymphocytes

Monocytes

- 12-20µm
- oval, kidney-shaped eccentric nucleus
- have basophilic cytoplasm, azurophilic granules, RER, polyribosomes, mitochondria, Golgi complex
- **circulating monocytes are precursors of macrophages** - after a short period of time in circulation they leave the blood and enter into the connective tissue and differentiate into macrophages (eg Langerhans cells in the skin))
- **they are phagocytes and antigen-presenting cells**- they are able to phagocytose a pathogen, distinguish it and send on its membrane an antigen that tells B and T lymphocytes what pathogen is found inside the monocyte


monocyt

Leukocyte migration

- leukocytes leaving the blood
    - **rolling** of leukocytes over on endothelium due to the presence of selectins (P a E)
    - **adhesion** - leukocytes attach to the endothelium via integrin
    - **diapedesis** (transmigration) - leukocytes pass through the endothelium

2.19.3 Platelets

- they are nuclear-free biconvex particles, dropped from the cytoplasm of megakaryocytes in the bone marrow
- 200.000 - 400.000 per 1µl
- size **2–4 µm**
- break down in the liver and spleen
- the centre is called the granulomere and the lighter peripheral area is the hyalomer
    - **granulomere -** contains mitochondria, glycogen and granules
        - **δ (delta) granule** - dense bodies, containing ATP and serotonin
        - **α (alfa) granule** specific platelet clotting factors (fibrinogen, PDGF, FGF, von Willebrant factor, platelet factor IV)
        - **λ (lambda) granule** - lysosomes
    - **hyalomer** - open system of channels, surface plasmalema is invaginated
        - contains a marginal bundle of microtubules
        - **actin and myosin allow contraction**
- **function- haemostasis**
    - **Contact with extracellular matrix induces thrombocyte adhesion**
    - after injury of a small blood vessel, a platelet plug (primary hemostasis) is formed from aggregated platelets within a few minutes, followed by a mechanically stable blood clot (secondary hemostasis) from a ball of insoluble fibrin fibers
    - fibrin fibres are formed from soluble fibrinogen protein by a chain reaction - coagulation cascade



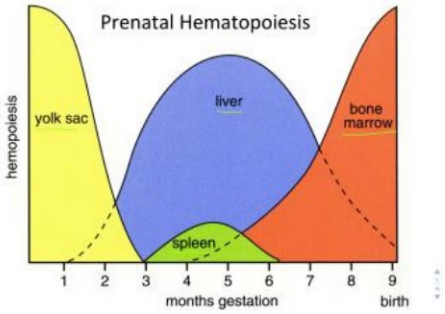
2.20 HEMOPOIESIS

MUDr. Eva Maňáková, Ph.D.

Note: hemopoiesis and hematopoiesis are exactly the same, they are synonyms and freely interchangeable words

- blood cells defend against microbes, carry oxygen and stop bleeding; most have a **short lifespan** → constant renewal is needed
- parts of haemopoiesis: **erythropoiesis, granulopoiesis, lymphopoiesis, thrombocytopoiesis**

Prenatal hemopoesis



- the baby in the uterus also needs blood cells, but at the beginning of the development he/she does not have bone marrow yet, so they have to produce it differently
- **prenatal haemopoiesis occurs in several periods, which overlap partially**
  - **mesoblast period – yolk sac**
    - in the 3rd week of embryonic life blood islands appear in the walls of the yolk sac (in mesenchyme), where primitive erythrocytes begin to develop (they are larger than later generations)
    - from the 16th to the 60th day of development
    - embryonic type of haemoglobin
  - **hepato-lienal period - liver and spleen**
    - around 6 weeks, hematopoiesis begins in the extravasal space of the liver and, to a lesser extent, in the spleen
    - This phase lasts until delivery
    - fetal type of haemoglobin
  - **medullary period - bone marrow**
    - since the 11th week, hematopoiesis is already in the (newly created) bone marrow
    - **the slides give the medullary period from the 20th week**
- before the 3rd week, oxygen and nutrients reach the embryo only by diffusion; blood islands in the yolk sac mesenchyme give rise to **endothelial cells** (periphery) and **primitive erythrocytes** (centre)
- **at the end of the embryonic period, only 50% of the erythrocytes are nuclear-free, in the fetal period around 90%**

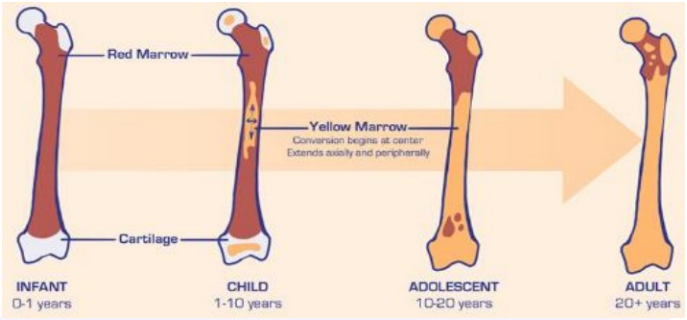
Haemopoietic stem cells

- haemopoiesis is the result of simultaneous proliferation and differentiation from haemopoietic stem cells
- pluripotent stem cells = **hemocytoblasts** - self-renewable, low mitotic activity
- multipotent stem cells come from pluripotent, have 2 strains - **lymphoid a myeloid**
  - unlimited direction of differentiation, but only a slight ability of self-renewal
- unipotent stem cells (progenitor cells) - intended only for one cell type, self-renewal ability, high mitotic activity
- scheme of differentiation: **stem cell → progenitor → precursor (...blast) → mature cell**
- the HSC gives 2 types of multipotent progenitors
  - **myeloid progenitor** → progenitors for erythrocytes, megakaryocytes, granulocytes and monocytes
  - **lymphoid progenitor** → T lymphocytes, B lymphocytes, NK cells
- the HSC "chooses" between self-renewal, proliferation, differentiation (cell line), migration and apoptosis

Bone marrow

- soft tissue formed by reticular connective tissue in the bodies of long bones and in the spaces between the trabeculae
- **reticular connective tissue (or niche)** consists of modified fibroblasts, reticular fibres (collagen III) and an inorganic component (fibronectin, laminin, proteoglycans)
  - **vascular niche** - blood supply, it is a place of maturation of blood cells and the appearance of helper cells
    - sinusoids - they provide blood supply, they are capillaries with discontinuous endothelium
  - **endosteal niche** - at the marrow margins, it contains stem cells, osteoblasts (building bone) and osteoclasts (bone breaking)
- according to the colour of the pulp we distinguish red (haemopoietically active), yellow (inactive) and grey (typical for late age)
- ratio of red and yellow marrow changes during life: **newborn – all marrow red**; in adults yellow marrow increases; after serious blood loss yellow marrow can reactivate to red
- red marrow in adults (from about the 20th year): **vertebrae, hip bone, sternum, ribs, scapula, clavicle, epiphyses of long bones** (femur, humerus)
- sinusoids = discontinuous capillaries with large openings in the wall and basal lamina (bone marrow, spleen, liver) – allow passage of whole cells
- **red bone marrow - red bone marrow's reticular cells influence Hemopoesis by their cytokines**

    - contains in addition to ligament also ropes, mesenchymal cells, fat cells and macrophages, sinusoids
        - **mesenchymal cells -** the least differentiated, they can differentiate into many different cells
        - **adipose cells** - n the red bone marrow, they represent a reserve space with increased need for hematopoiesis
        - **macrophages**- phagocytose e.g apoptotic immature blood cells, erythrocyte nuclei or old red blood cells and participate in the control of haemopoiesis by releasing factors
    - at older age, hematopoiesis occurs only in flat bones
- **white bone marrow**
    - **arises from red bone marrow**- haematopoiesis decreases in the pulp of long bones during growth, reticular connective tissue is replaced by fat cells and red pulp turns yellow
    - is capable of reactivating to red bone marrow
- **cytological examination of bone marrow smear is performed after collection (for example) from sternum and stained similarly as a blood smear**



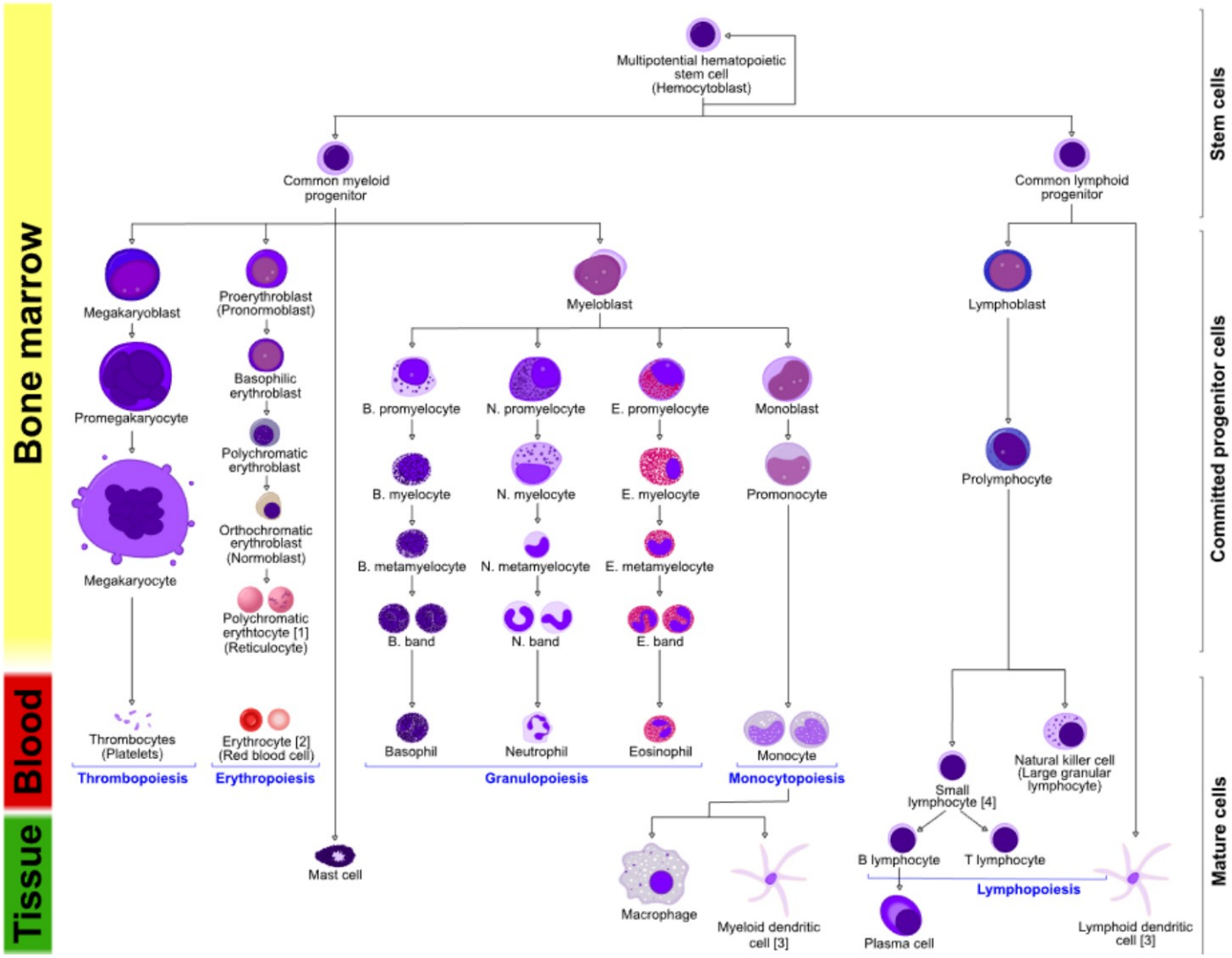
- **aspirate** – fluid portion, better cytological detail; **biopsy** – small solid part of marrow, shows topography
- diagnosis: activity of the marrow, morphology of cells, number and stages of WBC (**leukaemia**), other malignant cells, parasites, bacteria

Regulation of haematopoiesis

- Some cytokines, which mostly originate from bone marrow stromal cells or from kidney and liver, act to maintain the equilibrium between the need for individual cell types and their overproduction.
- **growth factors colony stimulating factors (CSF) - glycoproteins that promote stem cell proliferation**
    - **Erythropoietin (EPO)** - in kidneys, doping substance, promotes the production of red blood cells
    - **thrombopoietin**- from kidneys and liver, promotes the formation of platelets
- the **niche** around the stem cell: macrophages, osteoblasts, mesenchymal stem cells, adipocytes, endothelium, nerve tissue, ECM, adhesive molecules, cytokines
- regulation through cell–cell interactions (HSC – stromal cell) and HSC – ECM interactions

2.20.1 Developmental lines

- hematopoietic stem cells can be defined by containing unique differentiation groups (CDs) on their surface, while others do not contain
    - A typical CD for pluripotent hematopoietic stem cells is the marker CD34
- At the beginning of development, the stem cells migrate from the endostal to the vascular niche, where they mature



Colony-forming units

- in the context of hematopoietic stem cells, colony-forming units (CFUs) are stages where the stem cell is already partially differentiated and only a certain cell line can develop from it

CFU-GEMM - Colony-forming unit-granulocyte-erythrocyte-monocyte-megakaryocyte - it is basically a common myeloid progenitor

CFU-L Colony-forming unit-lymphocyte progenitor lymphocytes CFU-E Colony-forming unit-erythrocyte progenitor erythrocyte CFU-GM Colony-forming unit-granulocyte-macrophage progenitor granulocyte and macrophage CFU-Meg Colony-forming unit-megakaryocyte progenitor megakaryocyte CFU-B Colony-forming unit-basophil progenitor basophilic granulocyte CFU-Eos Colony-forming unit-eosinophil progenitor eosinophilic granulocyte Etc..

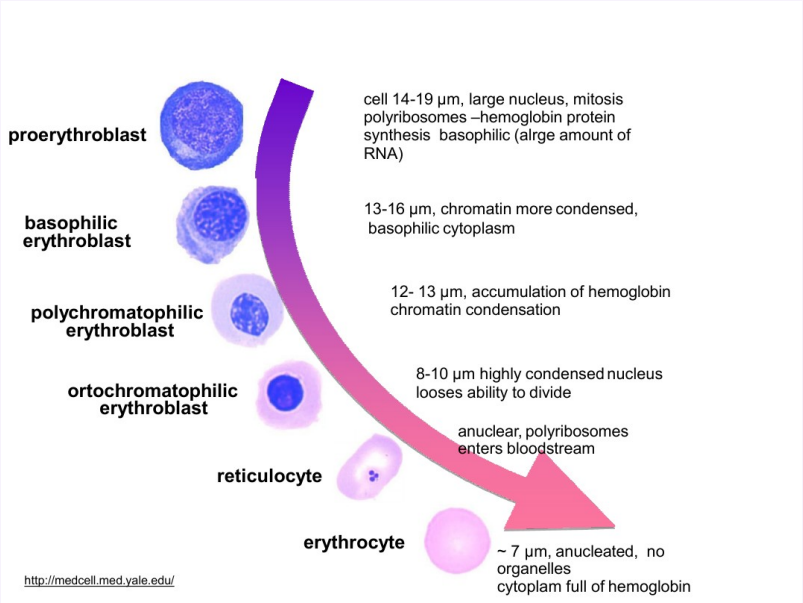
Erythropoiesis

- CFU-E erythropoiesis involves five stages of nuclear cell maturation, including four mitoses
- **differentiation leads to an increase in haemoglobin, a decrease in ribosomes, cell shrinkage, condensation and loss of nucleus**
  - pycnose- increased density and condensation of chromatin of the cell nucleus observable by light microscopy
- lasts about 8 days -> 5 days proliferation + differentiation and 3 days differentiation without further division
- is stimulated by the cytokine **erythropoietin** (glycoprotein), which is produced in the kidney mainly during hypoxia
- for normal erythropoiesis cyanocobalamin (Vitamin B12), folic acid and iron are also required
  - iron deficiency -> erythrocytes light and small because they have haemoglobin deficiency
  - lack of folic acid or B12 -> large erythrocytes because there are few of them and everybody tries to carry as much haemoglobin as possible
- **erythropoiesis occurs in so-called erythropoietic islets - groups of immature erythrocytes around the macrophage**
  - The macrophage provides them with a favourable microenvironment - eg it removes secreted cell nuclei
  - islets are formed during the mesoblastic period of heamopoesis

Developmental stages of erythropoiesis

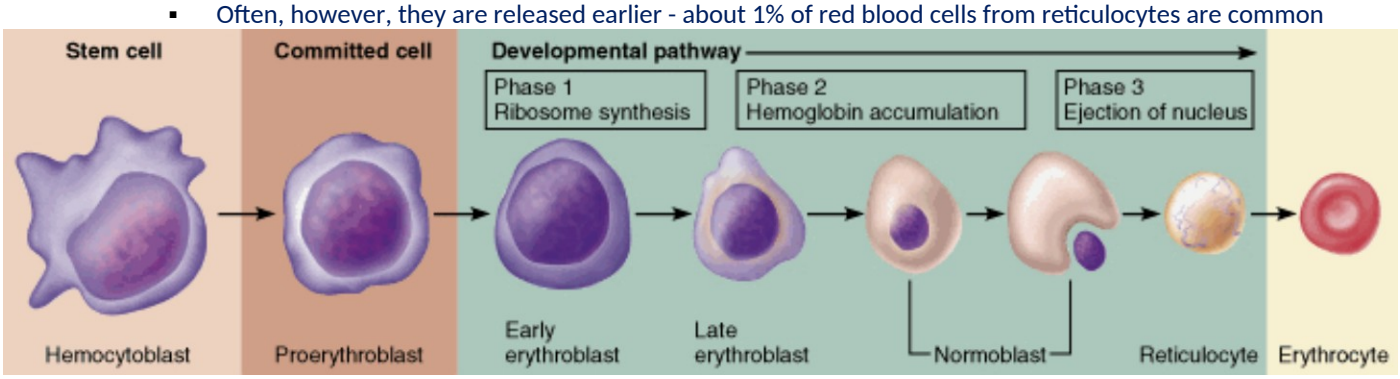
- **proerythroblast**- large oval cell with basophilic cytoplasm ((due to polyribosomes that synthesize haemoglobin)
  - nucleus – loose chromatin, large in comparison to plasma, has a nucleolus
- **basophilic erythroblast** - condensed nucleus, basophilic cytoplasm (polyribosomes)
- **polychromatophilic erythroblast** - decrease in the number of ribosomes, haemoglobin occurs
- **orthochromatophilic erythroblast**- maximum condensed dark nucleus, eosinophilic cytoplasm (due to haemoglobin)
  - ejection of nucleus (modified apoptosis)
- normoblast

- sizes (slides): proerythroblast **14–19 µm** → basophilic erythroblast 13–16 µm → polychromatophilic 12–13 µm → orthochromatophilic 8–10 µm (loses the ability to divide) → reticulocyte → erythrocyte ~7 µm
- erythropoietin stimulates the mRNA for the protein component of haemoglobin



[slides] Stages of erythropoiesis with sizes

- **reticulocyte** - nuclear-free cell with ribosome residues and mitochondria (=substantia reticulo-filamentosa)
  - after three days of maturation, it is released into the circulating blood as mature erythrocyte
    - Often, however, they are released earlier - about 1% of red blood cells from reticulocytes are common



Granulopoiesis

- granulocytes and monocytes are derived from a common progenitor cell CFU-GM
- **promyelocytes have extensive GER and GA, due to the formation of azurophilic granules and later specific granules**
- elements of granulopoiesis are more frequent in bone marrow than elements of erythropoiesis (due to the short lifetime of granules)
- differentiation may include core change (segmentation) and the appearance of specific granules
- the whole development (from myeloblast to mature granulocyte) takes about 11 days
  - (slides: 10–14 days)
- **azurophilic (non-specific) granules** are formed first – lysosomal hydrolases; **specific granules** are smaller and differ by cell type
- neutrophil line: myeloblast (big round nucleus, fine chromatin, no granules) → promyelocyte (azurophilic granules) → myelocyte (nucleus flattens, + specific granules; lines can now be distinguished) → metamyelocyte (**end of proliferation**) → **band** (horseshoe nucleus) → mature segmented neutrophil

Developmental stages of granulopoiesis

- the basophilic, neutrophil and eosinophilic granulocytes have the same number of stages of development and, except for the B / N / E character, they are named the same
- **myeloblast** - the youngest cell, contains no granules, finely dispersed chromatin
- **B/N/E promyelocyte**- basophilic cytoplasm, RER, GA, large violet azurophilic granule (functionally corresponding to lysosomes)

    - Nucleus is eccentric and flattened
- **B/N/E myelocyte** ucleus condensation, specific granules increase, azurophilic granules decrease
- **B/N/E metamyelocyte** - diminished nucleus
    - at this stage, the cells stop dividing
- **B/N/E granulocyte**
- **mast cells** - development proceeds differently
    - he precursor is released into the peripheral blood it travels until it settles in some tissue where it matures
    - has an IgE receptor
- the whole development takes place in the bone marrow, where they are also stored

Monocytopoiesis

- **monoblast** -> promonocyte -> monocyte -> macrophage -> activated macrophage
- **monoblast** is identical to myeloblast
- **promonocyt** large cell, basophilic cytoplasm, large lightly inverted nucleus, abundant GER, large GA
- monocytes form azurophilic granules - lysosomes (peroxidase, acid phosphatase)
- they live in the blood for about 8 hours and then on the periphery for several months as macrophages, which are antigen-presenting cells
- mainly acts in tissues, not in blood; they function as osteoclasts
- sizes: monoblast 10–20 µm, promonocyte 16–20 µm (nucleus invaginated), monocyte 12–15 µm; monocytes circulate for **hours**, then enter tissues (**months**)
- monocytes are also precursors of **dendritic cells** (in tissues in contact with the outer environment – skin, GIT mucosa; phagocytosis, antigen presentation)
- macrophages 10–30 µm, prominent nucleus, lysosomes, irregular surface; some tissue macrophages originate already from the **yolk sac** (microglia, partly Kupffer cells)

Lymphopoiesis

- there are no specific morphological differences between T and B lymphocytes
- the progenitors differentiate in the bone marrow
- lymphocytic progenitor cells give rise to precursor stages of lymphocytes (lymphoblasts, prolymphocytes), which are functionally competent after maturation
    - maturation processes occur in primary lymphatic organs of bone marrow (B-lymphocytes) or thymus (T-lymphocytes)
- is also divided into periphery in secondary lymphatic organs (spleen, lymph node, tonsila
    - **primary lymphoid organs** – red bone marrow, thymus; **secondary** – lymph nodes, spleen, tonsils, intestine (lymphatic tissue)
    - lymphocytes are distinguished by surface **CD molecules**; during development cells and nuclei get smaller – the changes are mainly functional

Developmental stages of lymphocytes

- the precursor cell is a **lymphoblast**, which differentiates into **prolymphocytes**, which no longer divide and mature into **lymphocytes**
- Prolymphocyte can differentiate into NK cell, or naïve B / T-lymphocyte
    - naive lymphocyte - have not yet encountered antigen
        - travel through the blood to peripheral lymphatic organs, where 95% of them disappear by apoptosis
    - activated B-lymphocyte differentiates into plasma cell
- **condition of survival and successful differentiation**
    - generation of surface receptors capable of recognizing and binding foreign antigen
    - not to have affinity for self-tolerance antigens (self-tolerance)

Thrombopoiesis

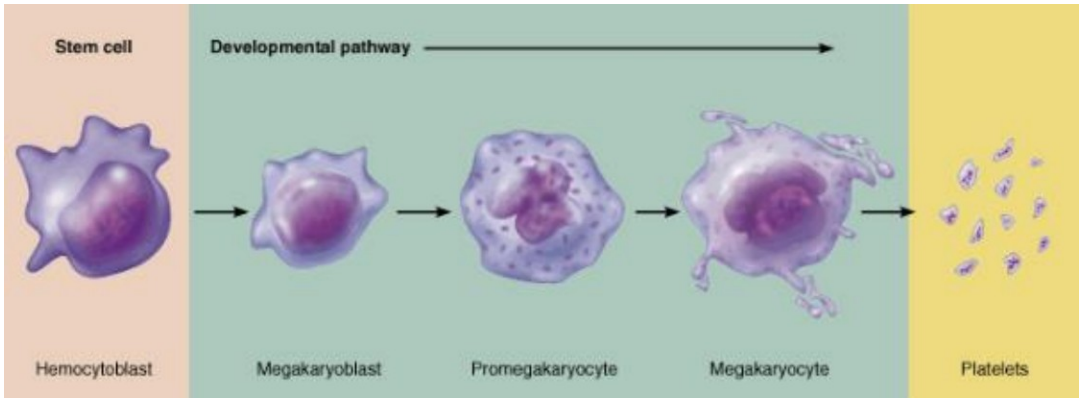
- megakaryocyte development is based on CFU-Meg via immature intermediate stages of megakaryoblast and promegakaryocytes
- huge nucleus size is caused by repeated DNA duplication without nucleus separation (so-called endomitosis)
- cell development, polyploidy (several sets of chromosomes) and platelet cleavage during development
- **thrombopoietin** a cytokine controlling the development of megakaryocytes, produced by osteoblasts of endostal niche, liver and kidney

Megakaryocyte development

- megakaryoblast -> promegakaryocyte -> megakaryocyte
- **megakaryocyte**- huge polyploid cell (up to 64 sets of chromosomes)
    - megakaryoblast 15–45 µm with a round nucleus; megakaryocyte up to **150 µm**
    - irregular lobed nucleus, numerous mitochondria, abundant RER, GA

- formation of granules in the cytoplasm (later platelet granules)
- plasmalema invagination = system of demarcation membranes - invagination delimits the cytoplasm region to platelets
- creates long protuberances into the vessel, gradually constricts them
- after the cytoplasm breaks down into platelets, the cell is destroyed by apoptosis
  - remaining megakaryocyte's nucleus with a narrow margin of the cytoplasm removes macrophages

- Platelets are produced by the fragmentation of megakaryocyte processes, referred to as proplatelets



2.21 HEMOCOAGULATION, BIOCHEMISTRY OF THROMBOCYTES AND LEUKOCYTES

MUDr. Josef Fontana

2.21.1 Haemostasis

- **Stopping bleeding** - it is a protective mechanism to heal damage in the blood circulation
- the fluidity of the blood and the intact and (unwettable) endothelium are prerequisites for continuous circulation
- haemostasis, however, also has negative effects - blood clots can form on the surface of atheroma plaques (atheroma or atherosclerotic plaque is the deposition of fat on the inside of blood vessels), which can even completely clog the vessel
- sepsis or tumors may cause haemostasis

Haemostasis vs. haemocoagulation

- haemostasis is a broader term and encompasses all stages of haemostasis
  1) **reflex vasoconstriction** - blood vessels immediately contract when breached to reduce bleeding rate
  2) **platelet activation and aggregation** - a precursor of blood clotting, enough to cover small defects
  3) **haemocoagulation**- **soluble fibrinogen is converted to fibrous insoluble fibrin** and with platelets forms a network
     - The result is a blood clot on the wound surface
     - **Thrombin catalyzed**
     - This is where the **coagulation cascade** takes place
  4) **blood plasma proteins break down by platelets and fibrin fibres = thrombolysis**



  - slide analogy: platelet clot = **wound bandage**, fibrin clot = **bandage fixation**, thrombolysis = **removal of the bandage**
- coagulation factors are activated by **partial proteolysis**

Platelets - thrombocytes

- they are nuclear-free fragments of megakaryocytes (biconvex disks) without their own proteosynthetic apparatus - everything they have in them is prepared in advance
- blood concentration - 150.000 - 400.000 / µl (spontaneous bleeding occurs when platelets drop to 20.000 - 50.000 / µl)
- the central zone with granules is the granulomere, the peripheral lighter zone is the hyalomer
  - **granulomer** contains mitochondria, glycogen and granules
    - **δ (delta) granules** dense bodies, containing ATP and serotonin
    - (slides: δ granules also contain **ADP and Ca^{2+}**)
    - **α (alfa) granules** - specific platelet clotting factors (fibrinogen, PDGF, FGF, von Willebrant factor, factor IV)
    - **λ (lambda) granules** - lysosomes
    - in general, substances contained in granules are used in vasoconstriction, haemocoagulation and repair
  - **hyalomer**- s formed by membrane invagination and forms a system of channels that are important for the secretion of signaling molecules
    - contains, for example, enzymes for the formation of eicosanoids

Platelet metabolism

- platelets are nuclear-free = no proteosynthesis
- **aerobic metabolism** - through their mitochondria, they process their reserves of glycogen
- the cytoplasm contains
  - microtubules, actin and myosin - a contractile apparatus that allows platelet contraction and granule release
  - phospholipids - necessary for activation of some coagulation factors
  - organelles (MIT, GA) – intense metabolism; energy source **glucose – aerobic glycolysis**; contraction also enables **thrombus retraction**
  - glycoproteins - allow platelets to attach to a damaged vessel wall and prevent attachment to a healthy wall

Role of thrombocytes in haemostasis

- participates in the whole course of haemostasis, but play a crucial role in the so-called primary haemostasis
  1. **Adhesion** - binding to injured subendothelial collagen
     - GP binding (platelet glycoprotein receptor) and vWF (von Willebrand factor)
       - vWF binds collagen and **GP Ib** on the platelet
  2. **Activation** - Platelets activate exposed collagen, thrombin, ADP, PAF and TXA_2
     - positive feedback - activated platelets produce TXA_2, activating additional platelets
     - expression of GP IIb / IIIa receptors, which allow platelets to interact well with each other
       - up to **100 000 GP IIb/IIIa receptors** on one platelet
       - shape change from **disc to amoeboid** shape

- Plate shape change - contractile proteins and cytoskeleton ensure a shape that best covers the wound space

3. Aggregation - through thrombin and other substances it connects through fibrinogen
- primary (reversible) aggregation - necessary for ever-forming gaps in the endothelium and healing of small defect
- secondary (irreversible) aggregation - associated with a release reaction (degranulation)

4. Degranulation (secretion reaction) - secretion of granule content

5. Formation of white (platelet) thrombus- such a temporary haemostatic plug
- the breakdown and fusion of platelets occurs (viscous metamorphosis)

6. Connection with coagulation - platelet phospholipids are expressed on the surface, which promotes coagulation
- the platelet phospholipid expressed on the surface = **PF 3** (platelet factor 3)

7. Wound healing - platelets secrete substances with proliferative effects, eg Platelet Derived Growth Factor (PDGF)

The old and the new coagulation model

- old model is more suitable for in vitro measurements (in laboratories)
  - cascading activation of coagulation factors with internal, external and common part
  - The old model emphasizes the importance of the internal system
- the new (revised) model is valid in vivo
  - from the old model
  - Slightly rearranges and introduces 3 phases - initiation, amplification and propagation
  - The new model emphasizes the importance of the external system and thrombin

Coagulation factors

- Most are produced in the liver
- according to their different function in the process of blood clotting we can divide them into 5 basic groups
  - **1. Serine proteases**, that catalyze partial proteolysis of coagulation factors
  - **2 proteins serving as cofactors of enzymes**
  - **3. Ca^{2+} (factor IV)** - helps to bind some coagulation factors to membrane phospholipids
  - **4. fibrinogen (factor I)** precursor of the fibrin network
  - **5. transglutaminase (factor XIII)** - stabilizes the fibrin network

I	fibrinogen	VIII	antihemophilic
II	prothrombin*[1)]	IX	Christmas*[1)]
III	tissue factor	X	Stuart-Prower*[1)]
IV	calcium ions	XI	PTA[1)]
V	proaccelerin	XII	Hageman[1)]
VII	proconvertin*[1)]	XIII	fibrin-stabilizing

* vitamin K dependent factors
[1)] protease zymogen

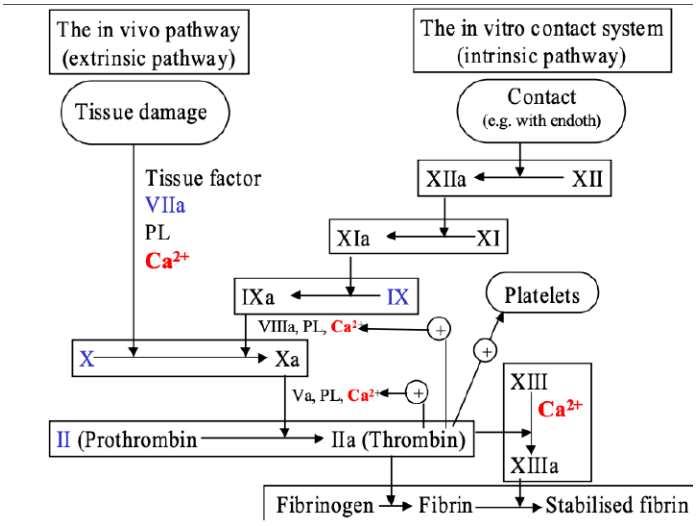
- factors are marked by Roman numerals (factor VI no longer exists – it is Va):
  - **I** fibrinogen, **II** prothrombin, *III tissue factor*, *IV Ca^{2+}*, *V proaccelerin*, *VII proconvertin*, **VIII** antihaemophilic factor, **IX** Christmas factor, *X Stuart-Prower factor*, **XI** PTA, **XII** Hageman factor, **XIII** fibrin-stabilizing factor (* = vitamin K dependent; II, VII, IX–XII are protease zymogens)

Vitamin K dependent factors

- Factors II, VII, IX and X and anticoagulant factors (protein C and S
- are formed in the liver as inactive zymogens, which are only activated post-translationally by vitamin K
- Vitamin K enables posttranslational carboxylation of glutamate residues in γ-carbon to form γ-carboxyglutamates (Gla)
  - **10–12 glutamate residues** are carboxylated in each factor
  - Gla can then bind Ca^{2+} and thus bind coagulation factors to membrane phospholipids
- some blood thinners work as non-functional vitamin K analogues – **e.g. Warfarin**

2.21.2 Haemocoagluation

- haemocoagulation occurs when platelet-mediated primary haemostasis is not sufficient
- **has two phases - activation of factor II** (internal or external, that is one) **and formation and stabilization of insoluble fibrin**
  - **internal and external phases meet at the site of coagulation factor X**



Extrinsic pathway

- initiated by tissue **tromboplastin (factor III; tissue factor)**
  - thromboplastin - a mixture of proteins and phospholipids released from damaged cells or released by activated endothelial cells and leukocytes
- tissue factor activates f. VII to VIIa
  - Tissue factor a f. VIIa together with phospholipids and Ca2 + form so called external tenase, which activates f. X to Xa
  - VIIa can also activate f. IX from the inner path -> connects the outer and inner path
- **the external pathway is important for the onset of blood clotting**
- the internal pathway is important for the amplification of haemocoagulation

Intrinsic pathway)

- **initiated by contact phase** - negatively charged surface (injured exposed collagen and platelets membranes) **activate f. XII to XIIa**
  - XIIa converts precalikrein to kallikrein (in the presence of high molecular weight kininogen - HMWK)
  - Kallikrein enhances the conversion of f. XII to XIIa = positive feedback
- XIIa activates f. XI to XIa
- XIa activates f. IX to IXa (this can also be done by VIIa)
  - IXa is part of the internal tenase complex
- **internal tenase complex**
  - substrate - f. X
  - enzyme - f. IXa
  - cofactors - f. VIIIa, platelet phospholipids and Ca^{2+}
    - f. VIII is activated by thrombin
  - The product of the tenase complex is Xa, which becomes part of the **prothrombinase complex**

Common Path aka Prothrombin Complex

- substrate - prothrombin (f. II)
- enzyme - f. Xa
- enzyme cofactors - f. Va, platelet phospholipids and Ca^{2+}
  - Factor V activated again by thrombin -> positive feedback
- the product of the prothrombinase complex is **thrombin (IIa)**

Thrombin

- **central position in the whole haemostasis** - ensures coagulation, anticoagulation, regulation of fibrinolysis and activation of cellular components
- cleaves fibrinogen into fibrin
- activates
  - platelets and endothelium
  - Neutrophils and monocytes - they express tissue factor on them
  - factor XIII that provides stabilization of the fibrin network
  - factors V, VIII and XI that amplify coagulation
  - and with the help of thrombomodulin, thrombin also activates protein C -> anticoagulation

New model of haemocoagulation

Initiation

- **tissue factor** (TF) **and VIIa** ((so the extrinsic pathway) starts coagulation
  - a small amount of VIIa is permanently in the blood; TF in case of injury or inflammation
  - VIIa autoactivates other molecules f. VII
- TF-VIIa complex activates f. IX and f. X -> IXa and Xa are formed
- **Xa activates other f. VII molecules and generates a small amount of thrombin,** which is not enough to form a fibrin clot, but this small amount can start another phase

Amplification

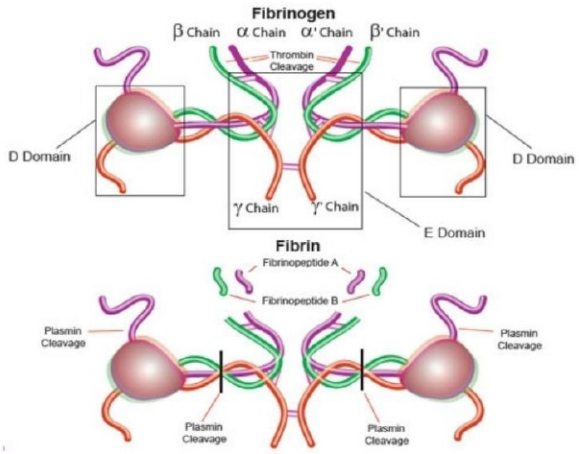
- thrombin activates f. V, VIII and XI -> Va, XIa, VIIIa are formed
- XIa activates other molecules f. IX -> IXa is formed
- thrombin activates platelets and leukocytes that express negatively charged phospholipids on the surface and binds f. IXa, VIIIa and Va
- Thrombin activates endothelium, thereby retuning them to a procoagulant state
- **the result of amplification is**
  - **more thrombin**
  - **the creation of reaction surfaces with bound activated factor molecules for complementing the internal tenase and prothrombinase complexes**

Promotion

- binding of f. X to the internal tenase complex (IXa and VIIIa) -> Xa is formed
- **Xa forms a prothrombinase complex with Va**-> massive thrombin production -> conversion of fibrinogen to fibrin
- unstable fibrin is stabilized by the action of stabilizing factor XIIIa

Fibrinogen

- consists of 3 globular domains (two peripheral D domains and one central E domain)
  - domains are connected to each other by 2 rods (triple helix α, β, γ)
  - there are 2 peptide tails on the central domain - fibrinopeptides A and B (from α, β helix)
    - **Thrombin cleaves both fibrinopeptides**, to form fibrin monomer, which spontaneously polymerizes to form fibrinpolymer



Strengthening of fibrin network

- unpaved mesh is not resistant (only non-covalent bonds hold it)
- the strengthening is catalysed by factor XIIIa
  - f. XIII is activated by thrombin and Ca^{2+}
- **XIIIa is a transglutaminase - it forms covalent cross-links between α and γ chains of fibrin**
  - forms a bond between glutamine and lysine, releasing ammonia
  - the amide of glutamine reacts with the NH_2 of lysine → **γ-glutamyl-ε-lysyl bridge** + NH_3; without it there is a risk of depolymerization
- the reinforced mesh can withstand blood pressure

Coagulation regulation

- there is a need to maintain a balance of procoagulation and anticoagulation processes
- major anticoagulant factors
  - **blood flow**- makes it difficult to concentrate coagulation factors in one place
  - **endothelial cells** - produce prostacyclin, NO and thrombomodulin
  - **antithrombinIII**
  - **protein C a protein S**

Antithrombin III

- α_2-globulin, the most important inhibitor of coagulation
- **belongs to serpins - acts as an inhibitor of serine proteases**
- binds and inactivates thrombin and factors IXa, Xa, XIa and XIIa
  - **heparin** (sulphatedGAG) **increases the ability of AT III to bind factors**
- congenital AT III deficiency - thrombophilic condition

Protein C a protein S

- slows the blood clotting process by eliminating non-enzymatic coagulation factors Va and VIIIa
- vitamin K dependent
- protein S is a cofactor of protein C
- protein C is activated by thrombin-thrombomodulin complex to activated protein C (APC)
    - APC cleaves non-enzymatic factors Va and VIIIa upon binding to phospholipids
    - without Va and VIIIa is a dysfunctional inner tenas and prothrombinase complex

Factor V Leiden mutation V

- AD hereditary mutation, manifested by increased blood clotting
- by this mutation one of the cleavage sites disappears from the Va - this leads to the APC cleaving Va much more slowlyi
- the most common congenital thrombophilic condition
- **people with this mutation are at risk if combined with other procoagulant factors such as hormonal contraceptives or cigarettes**

Anticoagulants in medicine

- removal of free Ca2 + ions by means of **citrate, EDTA or oxalate** - this principle is used eg for collecting non-coagulated blood for laboratory processing
- **heparin** strengthens AT III - binds thrombin
- vitamin K analogues - coumarin preparations (**warfarin, pelentan**) block posttranslational modifications dependent on vitamin K

Congenital defects of coagulation

- haemophiliaA (XR) – defect f. VIII
- haemophiliaB (XR) – defect f. IX
- haemophiliaC (AR) – deficiency f. XI
- von Willebrand's disease - vWF defect - poor platelet adhesion and impaired function of f. VIII
- slides: haemophilia = a spectrum of diseases, only some X-linked; defective synthesis or reduced activity of a factor
    - **haemophilia A** – the most common (1/5 000 men); **B** – 1/35 000 men; **C** – rare, **CAVE! autosomal recessive**
    - in the Czech Republic about 1 000 patients, 80–85 % haemophilia A
    - severity: factor **< 1 % = severe** form – spontaneous bleeding into **joints (haemarthrosis)**, CNS, muscles and soft tissues, life-threatening bleeding after injury/procedures; **1–5 % = moderate** – similar, but mostly without spontaneous bleeding
    - no causal treatment – regular administration of the missing factor

Fibrinolysis (thrombolysis)

- blood plug cannot stay in the body forever - therefore there are mechanisms by which the body removes the coagulum – fibrinolysis
- the principle is the cleavage of the insoluble fibrin network into soluble fibrin-degradation products by the action of the **enzyme plasmin**
    - plasmin is derived from plasminogen and, when activated, acts as a serine protease specific for fibrin and fibrinogen
    - the soluble fibrin degradation products include **D-dimers**

Measurement of haemocoagulation

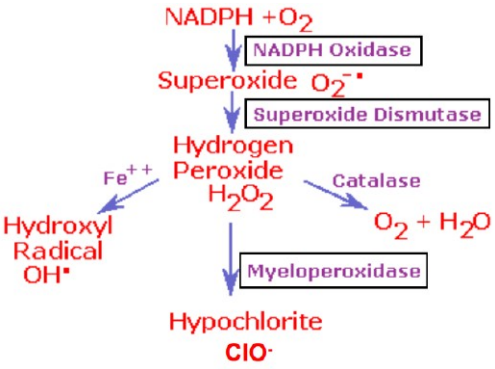
- aPTT (activated partial thromboplastin time) is used to evaluate the intrinsic pathway - standard 26 - 50s
- used to assess the external path
    - quick test - standard 14s (which represents 100% for INR)
    - INR (international normalized ratio) = 80 - 120%

2.21. Biochemistry of leukocytes

- **granulocytes**- neutrophils, eosinophils and basophils
- **agranulocytes** lymphocytes (B, T), monocytes (-> macrophages)
- the blood standard is 4,000 - 9,000 white blood cells per microliter
    - neutrophils- 45-75%
    - lymphocytes- 23-45%
    - monocytes- 2-11%
    - eosinophils- 1-4%
    - basophils- 0-1%

Neutrophils

- their job is to eat a foreign organism, kill it and then die í
  - **that is why, neutrophils are equipped with hydrolytic enzymes within their numerous lysosomes**
- have few mitochondria - are glucose dependent due to NADPH production
  - NADPH is used to produce reactive oxygen species for more reliable killing of bacteria
- **respiratory burst**- such enrage of neutrophils, when with increased consumption of O2 and glucose, neutrophils start to produce huge amounts of free radicals to annihilate anything they have just engulfed
- in addition, nitrous oxide is also produced in neutrophils to kill bacteria
  - arginine-> citrulline+ NO
    - enzyme - nitric oxide synthase
  - this can be dangerous in large infections - neutrophils can produce up to toxic amounts of NO, leading to septic shock
- **proteases and antiproteases**-
  - **Proteases**- serve as homing beacon for neutrophils, produced by phagocytic cells
    - elastase, collagenase, cathepsin G
  - **Protease inhibitors**- they are plasma proteins
    - α_1-antitrypsin, α_1-antichymotrypsin, α_2-makroglobulin
  - The protease / anti-protease ratio affects the rate of inflammation



Basophils

- granules contain heparin and histamine
  - **Histamine** is produced by the decarboxylation of histidine
    - induces vasodilation, swelling at the place of contact with allergen, redness, itching
  - **heparin** is sulphated by GAG - due to sulphate groups (negatively charged) it has very acid character
    - blocks blood clotting (strengthens AT III, see above)

2.22 IRON AND HEME METABOLISM

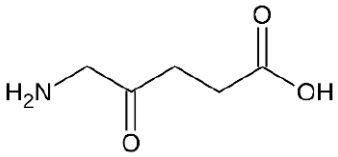
doc. MUDr. Jan Trnka, Ph.D.

2.22.1 Metabolism of heme

- heme is one of the iron-containing coenzymes (mainly reactive Fe^{2+} ion)
  - Fe^{2+} is extremely reactive, Fe^{3+} is poorly soluble at a neutral pH
- **Heme is a cyclic tetrapyrole** – consists of 4 pyrrole nuclei, which are connected by methine bridges
  - various substituents are attached to the pyrrole rings
  - there is a system of conjugated double bonds in the heme, giving the molecule its red colour
  - If we remove iron from the heme, it becomes **porphyrin**
  - If the iron in the heme is substituted with magnesium, the heme becomes **chlorophyll**

Synthesis of heme

- the main components are glycine and succinyl-CoA
- the first reaction takes place in the matrix, then the synthesis moves to the cytosol and returns to the last two reactions in the matrix

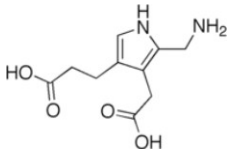


1. Step: glycine + succinyl-CoA -> 5-aminolevulinic acid (5-ALA) + CO_2 + CoA

- enzyme - **ALAS (ALA Synthase)**
- cofactor – pyridoxal phosphate
- It is a regulatory step of synthesis - there are many mechanisms that support / block synthesis of heme
  - the most important regulation is the feedback inhibition of heme - we do not want heme in cells except for erythrocytes - therefore we have two ALAS enzymes
  - **ALAS 1** – basically in all cells, is negatively inhibited by heme
  - **ALAS 2** – only in erythrocytes, it is not inhibited by heme, it is regulated by iron availability (more bellow)
- 5-aminolevulinic acid is then transported from the mitochondria out into the cytosol

2. Step: condensation: 2x 5-aminolevulinic acid -> porfobilinogen + 2 H_2O

- a pyrrole nucleus is formed to which propionate (P), acetate (A) and an amino group are attached (propionate and acetate are not the correct names but have already attached themselves, so too late)
- enzyme - **ALAD (ALA Dehydrogenase)**

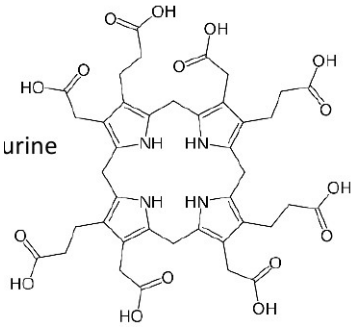


3. Step: deamination: 4 molecules of porfobilinogen join together to form hydroxymethylbilan

- it is not porphyrin yet because hydroxymethylbilane is tetrapyrrole but it is not linear
- **hydration** occurs at the last cycle (-CH_2-NH_2 is formed from -CH_2-OH)
  - the enzyme is **porfobilinogen-deaminase**

4. Step: cyclization: hydroxymethylbilan -> uroporphyrinogen

- **uroporphyrinogen I** - cyclic tetrapyrrole, having propionate (P) and acetate (A) in the following order A-P-A-P-A-P-A-P (simply the alternation between A and P)
  - uroporphyrinogen I doesn't form heme - it is unnecessary and excreted in urine
  - is produced non-enzymatically in the body by spontaneous cycling of hydroxymethylbilane
- **uroporphyrinogen III** - also cyclic tetrapyrrole, but one of the pyrrole nuclei is reversed, so that the substituents are in order A-P-A-P-P-A-A-P (see on picture)
  - enzyme - uroporphyrinogen synthase + uroporphyrinogen III cosynthase



5. Step: Two step decarboxylation of substituents

- the first decarboxylation causes all acetyls to become methyls (coproporphyrinogen III is formed)
  - enzyme uroporphyrinogen III decarboxylase
- followed by the transfer of the molecule to the mitochondria
- in the mitochondria, two propionates turn into vinyls to **protoporphyrinogen**
  - protoporphyrinogen has a total of 4 methyls, 2 vinyl groups and 2 propionates

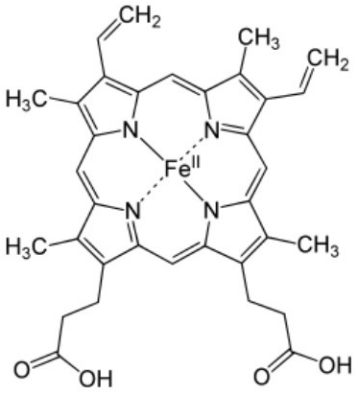
6. Step: dehydrogenation: protoporphyrinogen -> protoporphyrin + H_2

- enzyme - protoporphyrinogen III oxidase (or protoporphyrinogen dehydrogenase)
- protoporphyrin already has a conjugates double bond system (therefore it is coloured)

7. Step: Add iron: protoporphyrin + Fe^{2+} -> hem

- **enzyme - ferrochelatase**

Degradation of heme



- relatively short process
  - 1. Step: cleavage of the ring: heme -> CO + Fe^{3+} + biliverdin (linear tetrapyrrole)
    - enzyme - hemoxygenase
  - 2. Step: reduction: biliverdin -> bilirubin
- **In the spleen,** we form bilirubin by the degradation of heme -> it is an **unconjugated (indirect) bilirubin**, which is transferred via **albumin** into the **liver**, where it is **conjugated** with **glucuronic acid**, resulting in a **conjugated (direct) bilirubin**, which is soluble in water and excreted by bile out of the body
- **Jaundice** – symptom caused by a higher level of bilirubin in the blood
  - Prehepatic jaundice – the cause of jaundice is before/in front of the liver
    - E.g. in conditions that cause an increased degradation of heme
    - In the blood, there will be an increased concentration of indirect bilirubin
  - Hepatic jaundice – the cause of jaundice is in the liver
    - E.g liver damage, typically cirrhosis
    - In the blood, there will be increased concentrations both types of bilirubin
  - Post hepatic jaundice – the cause of jaundice is beyond the liver
    - E.g. if the biliary tract is blocked
    - In the blood, there will be an increased concentration of direct bilirubin

Heme synthesis disorders

- **porphyria** – a very old group of diseases
  - decreased activity of one of the enzymes - symptoms vary depending on which enzyme is defective
  - the urine of patients suffering from certain types of porphyria is purple (in Greek porfyrios is purple)
  - if one of the later enzymes of heme metabolism is defective, some intermediates may spontaneously cyclize and settle in the body
  - a common symptom of porphyria is **photosensitivity** - metabolites may settle in the skin and when sun rays hit them photochemical reactions take place that damage the skin (blisters, redness)
    - by the way – this is probably the origin of vampire myths
- **ALA Dehydrogenase** – second enzyme in the synthesis of heme
  - its cofactor is zinc, but it can also be replaced by other metals which causes ALAD to lose its functionality
  - in lead poisoning (among other things) ALAD stops working causing the accumulation of 5-aminolevulinic acid and its excretion of urine = excellent diagnostic marker

2.22.2 Iron metabolism

- in nature, it mostly occurs in the form of Fe^{3+}, but it is insoluble in water and therefore difficult to obtain
  - for life, we need Fe^{2+} which is very reactive and almost absent in organisms
- in humans, iron intake is very closely regulated – it enters the body in the form of food in two forms
  - **hemic iron** – much easier to use
  - **free iron** (Fe^{3+}) – must undergo treatment in the stomach

Absorption of free iron

- for iron to enter enterocytes, it must be reduced from Fe^{3+} to Fe^{2+}
  - many things help with reduction, such as reducing agents (typically Vitamin C) and low pH
  - directly in the membranes we find DcytB (duodenal cytochrome B), which also reduces Fe^{3+} to Fe^{2+}
- absorption into enterocytes occurs in the vast majority of the duodenum via **using DMT1 transporters**
- in the enterocytes, iron becomes one of three things
  a) enterocyte uses this iron for its own metabolism
  b) iron is stored in a Fe^{3+} crystal form and the whole crystal is encapsulated by a ferritin protein
  c) is exported to the blood using ferroportin transporter
- as soon as iron exports from the enterocyte into the blood, it is captured by its **transporting molecule – transferrin**
  - transferrin however transports iron in the form Fe^{3+}, therefore it is necessary for Fe^{2+} to be oxidized, before it is bound to trasnferrin
    - this oxidation is ensured by haphaestin (name given by the Greek God of fire and blacksmith Hefaistos) directky during the excretionof iron from the enterocyte
  - transferrin has a great affinity for iron – at normal pH, transferrin and iron cannot be separated
  - each molecule of transferrin binds 2 iron molecules

Iron uptake by cells

- if the cell wants iron, it exposes the transferrin receptor on its surface

- the receptor captures the transferrin that carries the iron, and the entire receptor-transferrin-iron complex is endocytosed
- the resulting endocytic vesicle has proton pumps in the membrane that pump protons inside and drastically lower the pH
- inside the endocytic vesicle, the pH drops to such an extent that iron from transferrin simply falls off and is transported from the vesicle to the cytosol, again using the DMT1 transporter
    - during this step, iron is simultaneously reduced back to Fe^{2+}, although it is not entirely clear how
- the receptor-transferrin complex is recycled back to the membrane, the transferrin releases without iron and travels through the blood again

Elimination of iron

- because of the effort required in nature to obtain iron, our body is set up to eliminate as little iron as possible
- The only two ways to get rid of iron are by **bleeding and through getting rid of cells** (intestinal, skin)
- There can also be too much of iron - for example, in multiple blood transfusions
    - In this case, chemical chelators come into play

Regulation of iron intake

Enterocyte level

- **Takes place at the level of ferroportin** - enterocyte can get stuffed with iron, but that does not mean that it is released into the blood
- the frequency of ferroportins is influenced by the hepatic hormone hepcidin (hepcidin promotes the degradation of ferroportins)
    - The liver produces hepcidin in response to iron levels in the body
    - hepcidin production is impaired in hereditary hemochromatosis - degradation of ferroportins does not work and over time toxic amounts of iron accumulate in the body

At the level of cell

- **takes place at the level of post-translation adjustments**
- **IRP (iron response element binding protein) - protein, which binds to iron response element on IRE,** which is a sequence on mRNA and can detect the iron level in a cell
    - IRP1 - as FeS cluster, where iron is bound, if there is a lot of iron in the vicinity (and therefore if there is little iron in the surroundings, iron will not be bound to FeS cluster)
        - when iron falls off the FeS cluster, a conformational change will occur allowing IRP to bind to IRE
- the effect of IRP binding to IRE depends on the location of the IRE on the mRNA
    - **if the IRE is upstream** of the coding sequence (i.e., in front of it - at the 5 ‘end), an IRP that sits on the IRE would block the translation of that mRNA strand and thus the **presence of IRP on the IRE would have an inhibitory effect**
    - **if the IRE is downstream** of the coding sequence (i.e., behind it - at the 3 'end), an IRP that sits on the IRE would block degradation of this mRNA strand (because degradation of the mRNA strands occurs from the end) and hence the **presence of IRP on IRE has a stimulating effect**

2.23 BIOCHEMISTRY OF ERYTHROCYTES

prof. RNDr. Eva Samcová, CSc.

Number of blood elements per 1µl

- **erythrocytes**- 4-6*10^6
  - Haematocrit - the ratio between the volume of red blood cells and the volume of whole blood
    - men - 42-52%
    - women - 37-47%
- **thrombocytes** - 150-400*10^3
- **leukocytes** - 4-9*10^3

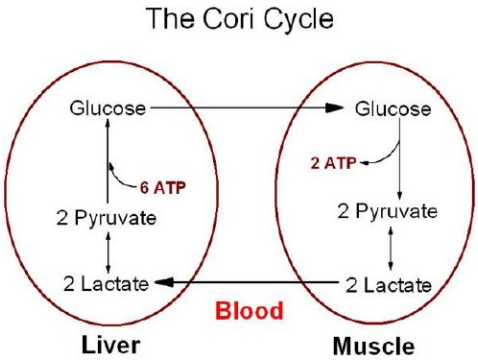
- slide values: erythrocytes **men 4.5–5.5 × 10^{12}/l, women 3.8–4.8 × 10^{12}/l**; haematocrit men 44 ± 5 %, women 39 ± 4 %
- diameter 7.2–7.5 µm, central thickness 0.6 µm, edge thickness 2.6 µm
- **mean corpuscular volume (MCV) 84–96 fl**; MCV > 96 fl = **macrocytes**, < 84 fl = **microcytes**

2.23.1 Erythrocytes

- 2 million new erythrocytes enter the circulation per second
- among the new erythrocytes are so called reticulocytes - young erythrocytes, which still contain ribosomes and ER components
  - increased representation of reticulocytes in the blood is a sign of haemolytic anaemia (haemolysis is increased)
- there are antigens on the surface of the erythrocytes that are responsible for determining the blood type
- erythrocyte production is stimulated by **erythropoietin** (EPO) - hormone synthesized in the kidney
- erythrocytes have large and elastic surface (for better gas diffusion)

- shape and flexibility are provided by **spectrin and ankyrin** (peripheral membrane proteins forming a "scaffolding" on the inner side)
  - defect → e.g. **hereditary spherocytosis** (spectrin defect) → decreased resistance of erythrocytes, haemolytic anaemia
- mature erythrocytes have no organelles for haemoglobin synthesis

- for erythrocytes, blood osmolarity is important
  - isotonic solution (285 mOsmol / l) - all right
  - hypertonic solution (> 285 mOsmol / l) - water flows out of erythrocytes and causes them to shrink
  - hypotonic solution (<285 mOsmol / l) - water pours into erythrocytes, which will fill and burst -> haemolysis
- **membrane carriers**
  - **Na^+/K^+-ATPase**
  - **GLUT 1** - non-insulin dependent
    - consists of 12 transmembrane helical segments
  - **Anionic transporters** – e.g. Cl^-/HCO_3^-



Metabolism of erythrocytes

- erythrocyte recovers all energy from glucose
- 90% anaerobic glycolysis
- 10% from the pentose cycle

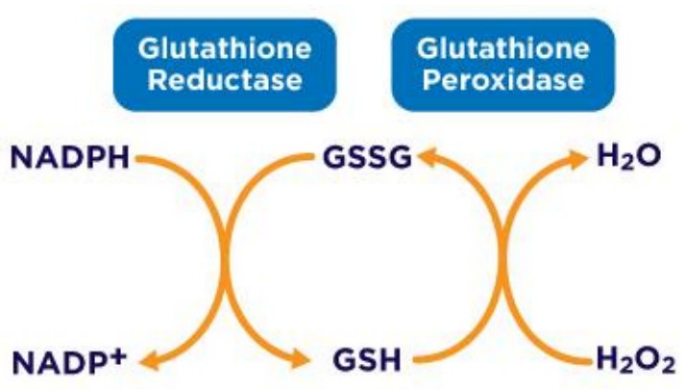
- slides: **7–12 %** of glucose enters the pentose cycle
- glycolysis gives only **2 ATP**; most ATP is used for **ion transport** (Na^+/K^+-ATPase)

- NADPH is also created to protect against oxidative stress
- erythrocytes participate in the Cori cycle
- **2,3-bisphosphoglycerate(2,3-BPG)** - produced by isomerization of 1,3-BPG (intermediate glycolysis)
  - **2,3-BPG is formed in the erythrocyte when blood enters the oxygen-deficient tissue -> at that time 2,3-BPG binds to hemoglobin and decreases its affinity to oxygen, releasing O2 from haemoglobin into the surrounding tissue**
  - **2,3-BPG shunt** bypasses the **phosphoglycerate kinase** step of glycolysis (normally 1 ATP) → it decreases energy production in the erythrocyte
  - 2,3-BPG binds to the **β-chain** of Hb; synthesized more at **high altitude** or after physical activity

Function of erythrocytes

- erythrocyte has only the minimum necessary organelles and cytosol for survival, the rest of the cell is packed with haemoglobin, which ensures the transport of various substances through the blood
- **oxygen transport (O_2)** - reactive oxygen species (ROS) are formed which are dangerous and erythrocytes prevent them from producing NADPH

- **transport of carbon dioxide (CO_2)**
- **transport of hydrogen (H^+)** - has significant buffering properties (35% buffering capacity of blood)



Antioxidant enzymes

- **SOD - superoxide dismutase**
  - $O_2^{\cdot} - O_2^{\cdot} + 2H^+ \rightarrow H_2O_2 + O_2$
- **CAT - catalase**
  - $H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$
- **GPx - glutathione peroxidase**
  - $2\ GSH + H_2O \rightarrow GS\text{-}SG + 2\ H_2O$
  - $2\ GSH + R\text{-}O\text{-}OH \rightarrow GS\text{-}SG + H_2O + ROH$
- **GR - glutathione reductase**
  - Regeneration of GSH
  - $GS\text{-}SG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$

Methaemoglobin reductase

- in erythrocytes, it ensures a backward reduction of methaemoglobin to haemoglobin, or "the return of haemoglobin to life"
- $Hb\text{-}Fe^{3+} + e^- \rightarrow Hb\text{-}Fe^{2+}$ (coenzyme NADH or NADPH)

Glc-6-β dehydrogenase defect

- Glc-6-P is the first pentose cycle enzyme (conversion of Glc-6-P to 6-P-gluconolactone)
- its malfunction or lack means absence of reducing cofactors NADPH, which serves as a glutathione reductase coenzyme in GSH regeneration
- consequently, GSSG is not reduced to GSH, resulting in increased sensitivity to oxidative damage -> resulting in haemolytic anaemia due to excessive oxidative damage to erythrocytes
  - G6PD deficiency = **favism**; the most common enzyme defect (~400 million people)

Pyruvate kinase defect

- a pyruvate kinase defect causes phosphoenolpyruvate not to convert to pyruvate
- this defect may be the cause of haemolytic anaemia

Erythrocyte exposure to oxidative stress

- damage to the erythrocyte membrane leads to impaired molecule exchange and haemolysis

2.23.2 Haemoglobin (Hb)

- haemoglobin is a haemoprotein **composed of the globin protein and the prosthetic group of heme**
  - has a quaternary structure (4 subunits - 2α and 2β) and each subunit contains one prosthetic group (heme)
  - subunits are joined by **H-bridges and ionic bonds**; every globin binds exactly one heme with a central **Fe^{2+}**
  - has a quaternary structure (4 subunits - 2α and 2β) and each subunit contains one prosthetic group (heme)
- myoglobin is something different than haemoglobin - myoglobin has only one globin chain and one hem, is found only in muscles (stores oxygen for the muscle) and binds oxygen more firmly than haemoglobin (has higher affinity for oxygen)

Oxygen saturation of haemoglobin

- due to the quaternary structure, the affinity of oxygen to haemoglobin is allosterically modified
  - **saturation curve shows sigmoidal dependence** - once one oxygen molecule binds, the affinity of haemoglobin to oxygen increases (e.g. the other oxygen is easier to bind)
- Oxygen-free haemoglobin is in the T-conformation and has a low affinity for oxygen
  - once one oxygen molecule connects to heme on one subunit, the affinity of heme for oxygen on all other subunits increases
  - he more oxygen attached to the heme, the greater the affinity of heme between heme and oxygen
  - haemoglobin, which is fully saturated with oxygen, is in the so-called R-conformation

Types of haemoglobin and its subunits

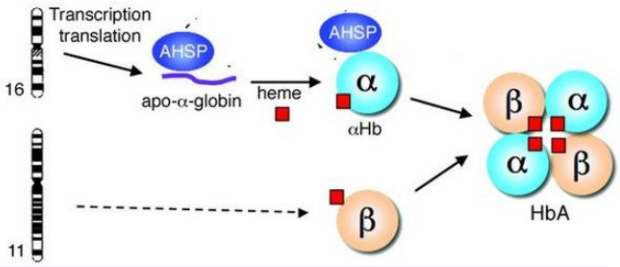
- **adult haemoglobin - HbA**
  - HbA_1 - α2β2
  - HbA_2 - α2σ2 - this is much less frequent (about 2% of total Hb adults)
- **Fetal haemoglobin - HbF**
  - HbF - α2γ2
  - HbF has a higher affinity for O2 than HbA - therefore the fetus is able to receive oxygen from the mother's blood even at lower partial pressure
  - physiologically occurs in the child's body up to 6 months of age

- slides: HbA1 is the major form in adults and children **over 7 months**; HbF forms up to **70 % of Hb in newborns**, its saturation curve is **shifted to the left**

- **embryonal Hb** – **Gower I (ζ2ε2), Gower II (α2ε2), Portland (ζ2γ2)** – first weeks of intrauterine development, then replaced by HbF
- Hb is always made of two pairs of chains (α, β, γ, δ, ε, ζ); chains of one pair are identical

Synthesis of haemoglobin

- occurs in bone marrow in erythrocyte precursors (not in erythrocytes themselves)
- The 4 separate subunits are joined by non-covalent bonds to form the tetramer Hb
- Hb synthesis disorders
  - **thalassemia** - a hereditary disease with impaired formation of one of the type of Hb chains (both α and β-thalassemia exist)
  - **Anaemia** - anaemia indicates a reduced ability of blood to carry O_2
    - **sideropenic anemia** - lack of haemoglobin due to formation of abnormal haemoglobin HbS, point exchange of one amino acid (glutamic acid) for another (valine)



    - **note: HbS causes sickle-cell anaemia (sideropenic anaemia = iron-deficiency anaemia)**
    - HbS: point mutation in the **β-chain gene – Val instead of Glu at position 6**; deoxy-HbS polymerizes into long thick filaments → **sickle shape** → loss of elasticity, capillaries can be clogged
    - erythrocytes with HbS live only **10–20 days**; heterozygotes are more resistant to **malaria** (Plasmodium)
  - **thalassaemia** – defect in the **synthesis** of globin chains → **microcytic hypochromic anaemia**, splenomegaly
    - relatively common monogenic diseases; severity depends on the number of damaged alleles (minor / major forms)
    - the chain with preserved synthesis is in relative excess → **precipitates** → damages erythrocytes; heterozygotes also more resistant to malaria
    - **α-thalassaemia** – α-chain is in both adult and fetal Hb → tetramers of β chains or γ chains form (poor O_2 transport); can damage the fetus, even fatally
    - **β-thalassaemia** – later onset (when β-chain synthesis starts); in severe forms (no β-chains) adults have **HbF and HbA2**
- **physiological concentration of Hb in the blood**
  - women 120-162 g/l, men 135-172 g/l

Haemoglobin function

- carries O_2 and part of CO_2
  - partial pressure O_2 - 100mmHg in arterial blood and 40mmHg in venous blood
- partial CO2 pressure - 40mmHg in arterial blood and 46mmHg in venous blood
- buffering blood - histidine residues in Hb can take up free H + to form deoxyhaemoglobin HHb

Transport of blood gases (slides)

- **oxygen**
  - physically dissolved – **1.5 %** (only ~3.2 ml O_2 per litre of blood; depends linearly on pO_2)
  - chemically bound – **98.5 %**: each Fe^{2+} binds 1 O_2; 1 mol Hb binds 4 mol O_2; **1 g Hb carries ~1.35 ml O_{2}**; 1 l of blood carries ~**216 ml O_{2}**
  - saturation: blood leaving the lungs **97 %**, mixed venous blood **75 %**
- **carbon dioxide** (the slide percentages differ from those below)
  - physically dissolved – **5 %** (arterial blood ~30 ml/l, venous ~35 ml/l); CO_2 is 22× more soluble than O_2
  - chemically bound – 95 %: **~83 % as HCO_{3}^{-}**, ~12 % bound to Hb and plasma proteins (carbamino compounds)
  - carbonic anhydrase reaction also proceeds without catalysis, but very slowly; >70 % of HCO_3^- leaves the erythrocyte via the HCO_3^-/Cl^- antiport
  - **deoxyHb is more basic** than oxyHb → binds protons more readily (buffer)

Transport CO_2 in blood

- **as bicarbonate HCO_3 - 70%**
  - $CO_2 + H_2O <-> H_2CO_3 <-> HCO_3^- + H^+$
- **bound to haemoglobin - 23%**
  - HCO_3 reaction may take place, whereby bicarbonate then leaves the cell and instead closes (to maintain pH) Cl^-
  - if CO_2 is bound to haemoglobin, the whole molecule is called carbaminohemoglobin

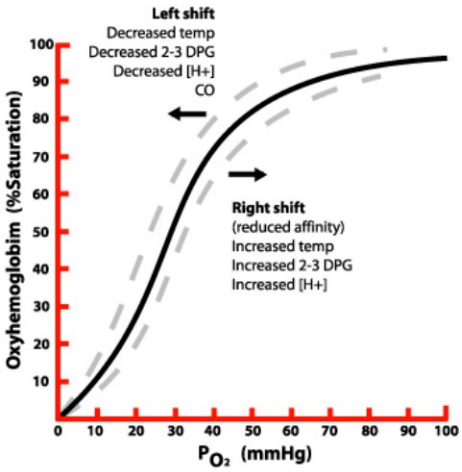
- **loosely dissolved - 7%**

Reactions taking place in erythrocytes

- pulmonary alveoli - the enzyme carbonate dehydratase, or carbonic anhydrase
  - O_2 + HHb -> HbO_2 + H^+
  - H^+ + HCO_3^- -> H_2CO_3 -> H_2O + CO_2
  - Essentially oxygen exports H^+ from haemoglobin, HCO_3 returns from the plasma to erythrocyte, where it combines with H^+ to form H_2CO_3, which decomposes to H_2O and CO_2
- in tissues on the periphery
  - H^+ + HbO_2 -> HHb + O2 -> aerobic metabolism
  - CO_2 + H_2O -> H_2CO_3 -> HCO_3^- + H^+
    - HCO_3^- formed in erythrocyte is transported to plasma by an anionic transporter in exchange for Cl^- to maintain the same charge in erythrocyte and plasma
    - Exchange of HCO_3^- and Cl^- between plasma and erythrocyte called **Hamburger effect or chloride shift**
    - in the lungs due to the Hamburger effect, HCO_3 from plasma enters erythrocyte and Cl^- from erythrocyte enters plasma

Factors affecting haemoglobin oxygen saturation

- CO_2 eescapes in the lungs, so there will be a more **alkaline environment** (pH 7.6) -> alkaline environment together with **increased pO_2** stabilizes **R-conformation** thus increasing the affinity of Hb for oxygen and enabling O_2binding O_2
- on arrival of bicarbonate to the periphery releases H^+ -> more acidic environment (pH 7.2) -> **acidic pH together with higher temperature** and increased pCO_2 stabilize **T-conformation**, thus decreasing affinity of Hb to oxygen and enable CO_2 binding
- **Bohr effect - shift of the saturation curve to the right** (lower affinity of Hb to oxygen) **at lower pH**



- **temperature** – increased temperature decreases affinity (right shift) → helps O_2 release in the periphery

Pathological derivatives of haemoglobin

- **metHb - methaemoglobin**- has Fe3 + instead of Fe2 + and therefore cannot transport oxygen
  - is found in the blood all the time, pathological is above 3%
- **HbA_{1c} - glycated haemoglobin** - glucose-linked haemoglobin
  - everyone has it in small amounts, it rises with long-term elevated blood glucose and is pathological above 6%
  - reacts with NH_2 group to form Schiff base (reversible) and further reaction, which is no longer reversible
- **COHb - carbonylhemoglobin/ carboxyhaemoglobin**- in carbon monoxide poisoning
  - CO competes with O_2 for the heme binding site, hindering oxygen transport
  - pathological limit of COHb is above 2%
- **sulfhaemoglobin, cyanhemoglobin**- occurs in poisoning with sulphate, hydrogen cyanide or cyanides

- slide colours: **oxyHb bright red**, **deoxyHb dark red**, carbaminoHb dark red (CO_2 bound to the –NH_2 of globin), **carboxyHb crimson ("cherry red" skin)**
- metHb normally **0.5–1 %**; children under 1 year have an underdeveloped antioxidant system → **methaemoglobinaemia**, e.g. from tap/well water with **nitrates** (converted to nitrites that oxidise heme Fe)
- HbA1c – glucose bound **non-enzymatically**; slides: healthy **2.8–4.0 %** of total Hb; used to check how well a diabetic patient is controlled (compliance)

Carbon monoxide poisoning

- CO has a 200x higher affinity for Hb than O_2
- maximum allowed concentration in air is 0,003%
- Consequences - Reduction of oxygen transfer capacity Hb, reduced oxygen supply of cells
  - CO can bind directly in cardiac and skeletal muscle (direct toxicity) and nervous system components, causing demyelination and neurological symptoms
  - slides: CO binds Fe^{2+} **200–300×** more tightly than O_2; oxyHb regeneration can be sped up by higher pO_2 – **hyperbaric chamber**
- the degree of CO intoxication depends on the pCO in the air and the duration of exposure
  - pCO 0.04% = severe headache, unconscious after 2-3 hours
  - pCO 1% = death within minutes

2.24 CONNECTIVE TISSUE

MUDr. Eva Maňáková, Ph.D.

- **Connective tissue is composed of cells and a significant amount of intercellular matter, which is divided into interstitial fluid and extracellular matrix - each type of connective tissue has different proportions of these components**
- Different connective tissue – connective tissue proper / cartilage / bone / adipose tissue
- general classification (slides): **connective tissue proper, cartilage, bone, blood, haemopoietic tissue, lymphoid tissue**
- connective tissue proper: **embryonic** (mesenchyme, mucous) and **adult** (loose; dense regular/irregular; reticular; elastic; adipose)
- functions
  - **supporting** (support and connection**) and nourishing** (some tissues do not contain blood vessels, they obtain nutrients by diffusion from connective tissue)
  - **storage of metabolites** - fats, minerals (calcium in bone)
  - **defence**- immune and inflammatory response
  - **Reparation after damage**
- Characteristics
  - large amounts of extracellular matrix and few cells
  - Presence of blood vessels and good regeneration (except for cartilage)
  - origin from embryonic connective tissue = mesenchyme
  - histogenesis from **mesoderm** (except ectomesenchyme); contains blood vessels and nerves; slow turnover
  - specific intermediate filament: **vimentin**
  - protective function also as a **physical barrier**; trophic function = mediator between blood vessels and organs/cells
- is lecture also deals with purely connective tissue proper

Connective tissue proper

- **cells**- contain Vimentin protein in their intermediate filaments
  - **fixed**- fibroblasts, reticular cells, fat cells
    - Fibroblasts are cells that produce reticular fibers
  - **mobile** - macrophages, plasma cells, mast cells, white blood cells
  - slides: **resident** population – fibroblasts, myofibroblasts, reticular cells, macrophages, mast cells, adipocytes; **transient** population – white blood cells (lymphocytes, plasma cells, neutrophils, eosinophils, basophils, monocytes)
  - "-blast" = active, synthesizing cell; "-cyte" = mature, resting cell
  - **reticular fibres are produced by reticular cells** (a variant of fibroblast – stellate, typically in lymphoid organs)
  - **mesenchymal cells** – irregular star-shaped cells interconnected by processes, large euchromatic nucleus with several nucleoli; give rise to other connective tissue cells
- **extracellular matrix = ECM**
  - **fibrillar component**- made up of fibers - collagen / reticular / elastic fibers
    - Collagen and reticular fibers are both collagen but stain differently
  - **amorphous component**- glycoproteins (thin fibers), proteoglycans (huge molecules, bind water) and tissue tissue
  - ground substance: highly hydrophilic, transparent, viscous; **negatively charged → eosinophilic**; it is washed out during routine histological processing
  - **interstitial fluid ≈ 11 l** (25 % of total body water)

2.24.1 Fibrous component of extracellular matrix

Collagen fibres

- a bundle of parallel collagen fibrils
- one collagen fibril is a triple helix of 3 peptide chains
- the individual fibrils are secreted separately into the extracellular mass and joined together there
- the most common types of collagen are I, II and III, but there are about 28 of them
- strong when pulled on (like a steel wire) - used to attach or form a net
- macroscopically **whitish-silvery**, stains eosinophilic; collagen ≈ **30 % of dry body mass**; minimally extensible
- fibrils **10–300 nm** in diameter (15–130 nm on the Grygarová slide), with a characteristic **64 nm banding periodicity**; fibrils associate into woven fibres that stain acidic

Reticular fibres

- **composed of thin bundles of collagen fibrils (mainly type III)**
- form networks around cells of parenchyma organs, capillaries, fat cells, muscle fibers,
- are part of the basal lamina (specifically lamina fibroreticularis)

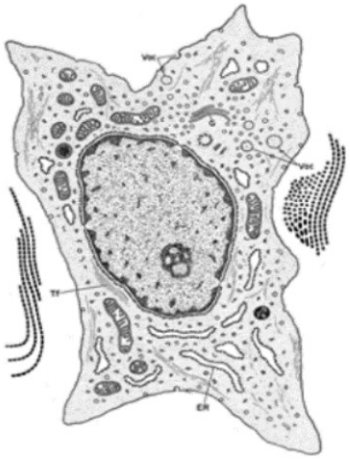
- **staining- AgNO3** - reticular fibres are called argyrophils because of their affinity for silver (Greek silver = árgyros)
- reticular fibres do not bundle into fibres; also **PAS positive**; found in reticular tissue of lymph nodes, bone marrow and spleen, loose connective tissue, around smooth muscle, in the **endoneurium** (Schwann cells)

Elastic fibres

- **formed by elastin surrounded by microfibrils**
- staining- resorcin-fuchsin, orcein -> turns brown-violet
- unlike collagen fibers, they have a rubber-like function -> they can be reversibly stretched and normally return to their original state after deformation
- elastin has specific amino acids - desmosin and isodesmosin, which form cross bridges between elastic fibers
- elastin synthesizes fibroblasts on their ribosomes as a globular tropoelastin consisting of two secondary structures (β-spiral and α-chain) and is subsequently secreted from the cell
    - immature elastic fibers arise after interaction of elastin and microfibrils (which are made up of fibrilin protein), which form mature elastic fibers by aggregation
        - fibrilin can also occur independently to elastic fibres, for example, in the eye apparatus
- macroscopically **yellowish**; low resistance to tension, highly extensible
- locations: elastic membranes of large arteries (**aorta – lamellar layers**), bronchioles and interalveolar septa, **ligamenta flava**, wall of the vagina

Fibroblast

- spindle cell with oval nucleus (only the nucleus is often seen in the specimen)
- **fibroblast is active cell, fibrocyte is inactive cell (in G0 phase, easy to activate and divide)**
- **produces both fibrous and amorphous matter**
    - proteosynthetically active - light nucleus with nucleoli, basophilic cytoplasm, GER and GA - collagen, elastin, proteoglycans and glycoproteins
- **myofibroblast**- modified fibroblast, actively forms extracellular matrix and shows similarities to smooth muscle cells (has a lot of actin fibres) = tissue with myofibroblasts is capable of long-term contraction



- slide picture of a fibroblast: **stellate** (star-shaped), large clearly visible nucleus, abundant rER and Golgi; **fibrocyte** = resting form (structural support)

Collagen synthesis

- **contains hydroxyproline, hydroxylysine, glycine -> characteristic of collagen**

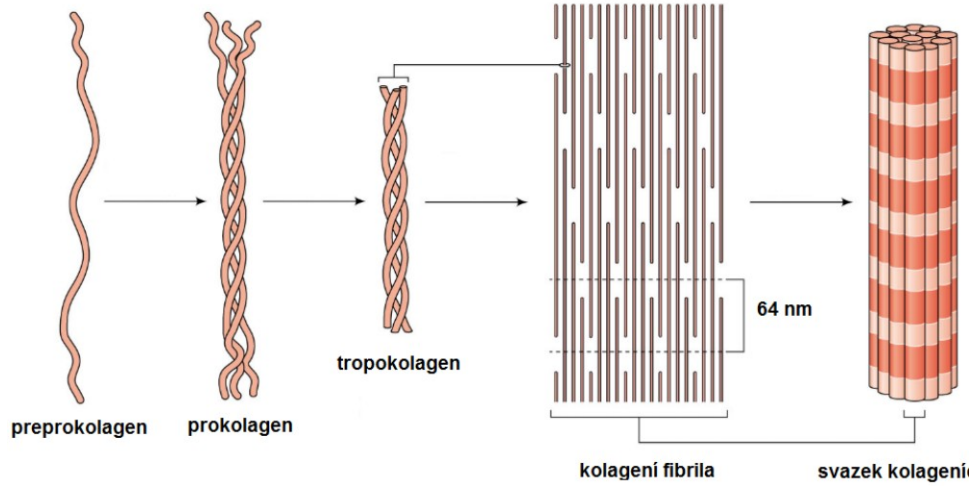
1. The mRNA, after leaving the nucleus, associates with a ribosome and translation occurs 2 A preprocollagen protein is synthesized, which carries a signal sequence which sends the entire protein to RER immediately after generation 3 In RER, the pre-collagen will become a procollagen by post-translation modifications
    - cleavage of the signal sequence
    - hydroxylation of proline and lysine amino acids to hydroxyproline and hydroxylysine (cofactor Vitamin C)
    - glycosylation of hydroxylysines formed in the previous step (glucose or galactose attached)
    - after glycosylation, the 3 chains are joined together to form a left-handed triple helix of procollagen

4. procollagen continues to Golgi apparatus, where it adds an oligosaccharide to it, envelops it with a vesicle and sends it out of the cell 5 Once the procollagen is **outside the cell,** the membrane enzymes of the procollagen peptidases capture the procollagen and cleave its free globular ends (shortening it) - what remains is called tropocollagen

- slide summary: **intracellular** – uptake of proline and lysine, mRNA in the nucleus, translation of preprocollagen on RER, hydroxylation and glycosylation in RER, triple helix of procollagen in RER, packing in trans-Golgi and exocytosis; **extracellular** – cleavage of the non-helical terminal ends by **procollagen peptidase** → tropocollagen → assembly into fibrils

6. **enzyme lysyl oxidase** ((extracellular copper-dependent enzyme) mediates bonds between several tropocollagen

- o polymer of tropocollagen is collagen



Types collagen

- done, **collagen I.** – tendon, teeth, skin
- hyaline **collagen II.** - and elastic cartilage
- lamina fibers **collagen III.** - reticular and reticular
- basal lamina **collagen IV.** -
- **collagen V.** - chorion and amnion
- descending by size - collagen I. is the thickest, each next is thinner than the previous one

- slide version (Grygarová): **type I ≈ 90 %** – dermis, bone, tendons, capsules; **II** – hyaline and elastic cartilage, **notochord**; **III** – vessel walls, reticular fibres; **IV** – basement membrane (barrier); **V** – placenta, together with type I in smaller amounts; **type VII** – basement membrane of stratified epithelia (**anchoring fibrils**)

Clinical significance

- vitamin C - necessary for hydroxylation of collagen, a problem in scurvy
- **Ehlers-Danlos syndrome** skin hyperelasticity and joint hypermobility due to lack of procollagen peptidase or lysyl hydroxylase (no cross-bridges and therefore no collagen polymerization)
- **Marfan syndrome** - autosomal dominant defect of fibrilin, myopia (nearsightedness), ectopia (occurrence beyond its usual site) lenses, skeletal defects, arachnodactyly, mitral valve prolapse, dilatation of the aortia ascendens
  - slides: Ehlers-Danlos syndrome – **defective genes for collagen** (I); Marfan syndrome – also **dilatation of the ascending aorta (risk of rupture)**

2.24.2 Amorphous component of extracellular matrix

- also, known as amorphous matrix
- fills the spaces between the fibers
- **glycoproteins**- fibronectin, chondronectin, laminin…
- **glycosaminoglycans (GAG)** - chondroitin sulfate, heparan sulfate, dermatan sulfate, keratan sulfate
  - **Hyaluronic acid** - forms a skeleton to which other components bind
- **proteoglycans**- aggrecan, perlecan, versican, syndecan - core protein and GAG

Glycosaminoglycans

- long chains from disaccharide units
- **consist of alternating hexosamine and uronic acid**
- sulfonated, attached by covalent bonds to the protein
- chondroitin sulphate (cartilage), dermatan sulphate (dermis), keratan sulphate (cornea), heparan sulphate (liver)

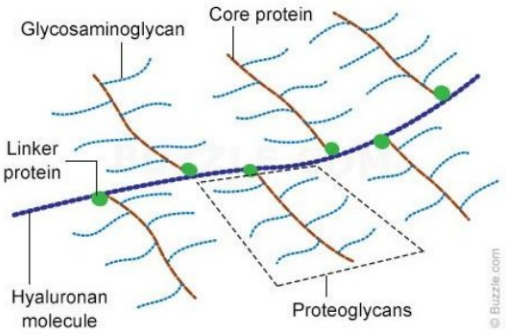
- slide locations: **chondroitin sulphate** – cartilage, bone, heart valves, serum, skin; **heparan sulphate** – basal lamina, blood vessels, lungs; **dermatan sulphate** – dermis, tendons, cornea
- **functions of ground substance components**
  - GAGs – bind water (negatively charged)
  - proteoglycans – **resist compression**, permit movement of cells, **slow down movement of bacteria**, form a **molecular filter**, binding sites for signalling molecules
    - **syndecan** – attached to the cell (transmembrane protein), binds **FGF**
  - glycoproteins – bind ECM components (collagen, proteoglycans) to each other and to cells
    - **fibronectin** binds collagen, GAGs and **integrins**; **laminin** – main adhesive component of the basal lamina; **chondronectin** – adhesion of chondrocytes to collagen; **tenascin** – binds syndecan, marks migratory pathways during development
- tissue-specific ground substance
  - **cartilage** – chondroitin sulphate, heparan sulphate, HA, aggrecan, chondronectin; binds water maximally (**80 % of wet weight**); the negative charge attracts Na^+ and water
  - **bone** – keratan sulphate, HA; glycoproteins **osteonectin, osteocalcin, sialoproteins** bind calcium in mineralization

Hyaluronic acid

- abbreviation HA (hyaluronic acid)
- consists of 250 - 50 000 disaccharide subunits
- it is neither sulfonated nor bound to a protein
- abundant in the **vitreous body, Wharton's jelly and synovial fluid**
- has a huge volume in aqueous solution, forming a viscous gel
- **function**
  - viscous gel - water supply, turgor retention, joint lubricant
  - connects proteoglycan molecules into proteoglycan aggregates
  - interacts with cell surface receptors, thereby stimulating migration and proliferation
- biosynthesis - formed by the enzyme HA synthase, which is fixed on the inner surface of the cell membrane

Proteoglycans

- Linear polysaccharide composed of core protein to which glycosaminoglycans (GAGs) bind
- **attaching a lot of proteoglycans to hyaluronic acid creates a proteoglycan aggregate**
- proteoglycans have the appearance of bottle-washing brushes
- binds water and growth factors
- they are components of the basal lamina



Interactions between cells and extracellular matrix

- the extracellular matrix is attached to the cells by integrins
- **integrins**- receptors for extracellular matrix molecules, components of hemidesmosomes and focal adhesions
- inside the cell integrins are attached to cytoskeletal fibres (paxillin, vinculin, talin, β-catenin))

Extracellular matrix

- is not completely stable and must be adjusted
- **degradation and remodelling of ECM is done by Metalloproteinases** (MMP) collagenase, cathepsin, stomelysin, gelatinase
  - **Metalloproteinases are lysosomal enzymes produced by various types of cells, including macrophages**
- Importance- invasion of microorganisms (streptococci) - enzymes enable crossing through extracellular matrix
  - in the invasion of tumors, the basement membrane will be degraded (cells lose integrins) and thus the attachment of cells to the basal laminate will be lost

Regeneration

- occurs after tissue damage or trauma
- healing and scar formation
  - a blood clot is formed immediately after injury, platelets release growth factors and cytokines
  - the first neutrophils and macrophages enter the wound to kill bacteria and tissue debris
  - The process of granulation - proliferation of fibroblasts and blood capillaries through damaged wound sites
  - some fibroblasts turn into contractile myofibroblasts, bringing the wound edges closer
  - At the same time, re-epithelialization occurs - the epithelial cells travel from the wound edges towards the center until the entire wound area is covered and a new basement membrane is formed (which takes about 5 days)
  - finally, myofibroblasts disappear by apoptosis
- scar retraction - scar reduction / retraction - pathological but commonly occurring phenomenon (myofibroblasts remained functional for too long)

2.24.3 Cells of connective tissue

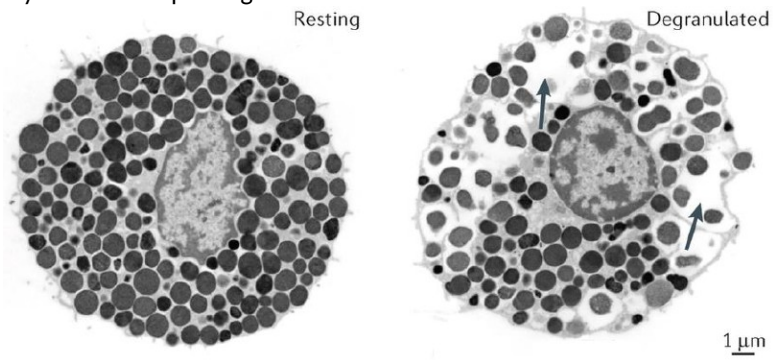
Macrophages

- phagocytic cells
- contain lysosomes and phagosomes (vacuoles)
- accumulate vital dyes - trypan blue, ink (dark blue)
- **function** - break down fibres and other components of intercellular matter
  - they are part of the immune system - they are among the antigen presenting cells (MHC II, more in the 3rd course)
  - **produce signalling molecules (cytokines) - interleukin 1, TNF-α**
- **macrophages are formed by conversion from monocytes**

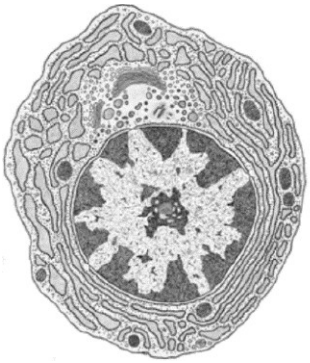
- monocytes are formed in the bone marrow and are washed out into the bloodstream, after about 8 hours they enter the tissues and there they turn into macrophages
- tissue macrophages then show numerous heterogeneity depending on the tissue and are therefore called differently in each tissue
  - histiocyte in connective tissue, osteoclast in bone, microglia in nerve tissue, Kupffer cells in liver, Langerhans cells in skin
- other members of the monocyte–macrophage system: **dendritic cells**; macrophages can form **epithelioid cells and multinucleated giant cells** (segregation of foreign bodies)
- size 10–30 µm, irregular shape, kidney-shaped nucleus, abundant rER, Golgi, lysosomes; remove old and damaged cells

Mast cells - mast cells

- arise from bone marrow
- have an oval shape and an oval core, are basophilic
- **contain a large number of granules, by which they gain their basophilicity**
- their granules contain vasoactive mediators - histamine, heparin and leukotrienes (chemotactic mediators of inflammation)
- **metachromatic granule -** characteristic of some basic dyes, dye changes its colour after binding to some biological tissue (eg blue dye binds to metachromatic granule and turns black)
  - **granules are metachromatic due to the high content of acid residues in heparin glycosaminoglycan**
- responsible for the immune response and early allergic response
- **mast cell types**
  - mucosal mast cells (lungs and intestine) - smaller, containing chondroitin sulphate
  - connective mast cells
- **functions**- they are mostly negative, but there are exceptions (for example, they can inactivate snake poisons)
  - **histamine secretion** (causes itching in the swelling) **& heparin** ((prevents the formation of thrombus in the swelling)
  - **produce cytokines, growth factors and arachidonic acid derivatives**
- activation- antigen binding to IgE receptor - allergic reaction -> effector cells of early type allergic reaction
- slides: mast cell **20–30 µm**, lifespan **8–18 days**; basophilic granules obscure the nucleus; **mast cells are not basophilic granulocytes**; activation needs antigen binding to **two IgE receptors** (cross-linking) → release of histamine, proteases, proteoglycans
- synthesis of inflammatory mediators - prostaglandins and leukotrienes



Plasma cells



- **nucleus is placed eccentrically** (wheel/kernel shaped nucleus, imagine a 300-year-old wheel on a car)
- striking nucleus, lumps of heterochromatin sit on a nuclear envelope
- arises from activated B-lymphocyte – immunoblasts
- they have almost no cytoplasm, but after activation they turn into large cells by immunoblasts
- **photosynthetically active - GER a GA - production of antibodies**
- **ach plasma cell has its own antigen against which it produces antibodies**
- in mucosal tissue, in immune organs

2.24.4 Types of connective tissue proper

- **embryonic** - mesenchyme, jelly-like tissue (Warton's jelly)
- **adult** - collagenous connective tissue (dense / thin), reticular connective tissue, elastic connective tissue
- **specific** - brown and white adipose tissue

Embryonic connective tissue

- **mesenchyme**- embryonic tissue with star cells and a small amount of extracellular matrix
  - **consists of glycoproteins and hyaluronic acid, which allows easy cell migration**
  - **is the basis for the development of all types of binders, blood elements and blood vessels (blood and lymphatic)**
- **Wharton jelly**- fetal connective tissue, collagen V, hyaluronic acid
  - mostly amorphous matter, less fibres, almost no cells
  - occurs in all fetal tissues - placenta, chorion, umbilical cord, ...
  - Wharton's jelly (slides) contains **fibroblasts** and a lot of hyaluronic acid (umbilical cord)

Adult connective tissue

Collagen

- **loose** - cells (even mobile) and less fibres - occurrence eg in lamina propria, perimysium in muscles, etc.
  - slide locations of loose tissue: lamina propria, **papillary layer of the dermis**, interstitial spaces of glands, tunica submucosa, **endomysium, endoneurium**; cells and ground substance predominate over fibres
- **dense** - more fibers and fewer cells
  - **regular** - **tendons** (connection of muscle and bone), **fascia** ((connective tissue muscle), **aponeurosis** (flat tendon)
  - **irregular** - **dermis**
  - regular: parallel bundles of **collagen type I** in the direction of mechanical stress (tendons, ligaments, fascias)
  - irregular: collagen I bundles in multidirectional pattern – **reticular layer of the dermis, sclera, organ capsules** (kidney, liver, spleen, lymph nodes, adrenals, testes, ovaries)
- **tendons** - transfer of tension between muscles and bones
  - matrix - collagen fibres, less elastic fibres and proteoglycans
  - between the fibres there are rows of spread fibroblasts and tendinocytes (tendon cells) that are interconnected by gap junction
  - tendons are wrapped with a layer of thin ligament (epitenonium), from which extend into the septum from the thin ligament (peritenonium), which divides the tendon into bundles of fibres
    - Nerves and blood vessels enter the tendon through the septum

Elastic

- bundles of branched elastic fibers accompanied by small amounts of collagen fibres and fibroblasts
- **in elastic arteries** ((the artery is divided into muscular and elastic; this maintains blood pressure stability; more in the sophomore)
  - **ligamenta flava** (yellow ligament with admixture of elastic fibre) **in places with large range of movement** (aorta)
  - **ligamentum suspenzorium penis - suspension ligament of the penis**
- there is less collagen in the elastic ligament and there are thinner types of collagen

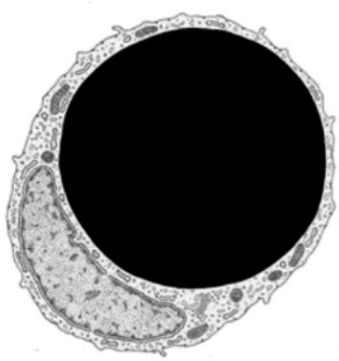
Reticular

- three-dimensional mesh made up of fibroblasts (called reticular cells) and fibers (collagen III)
  - suitable environment for haemopoesis and lymphatic organs (red bone marrow, spleen, lymph nodes)
- reticular connective tissue is sparse at the blood site, dense at the accumulation of cells
- the reticular fibers themselves may also be present outside the connective tissue - for example in the basalis laminate

Special connective tissue

- **low intercellular matter, fat cells predominate, separated by fibrous septa**

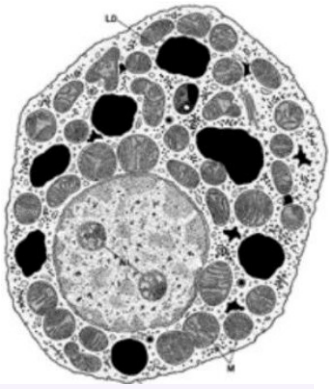
White addipose tissue



- **consists of adipocytes -** the nucleus is flattened and placed eccentrically (off centre); almost a whole drop of fat is filled by a single drop of fat, which is not separated by a membrane, but by a monomolecular layer of phospholipids and perilipine (a protein that prevents lipolysis)
- the connective tissue is richly vascular
- hormonal control - insulin, catecholamines, prostaglandins
- adrenaline stimulates lipolysis - can deactivate perilipine
- insulin stimulates fat storage
- **function-** energy supply, insulation against heat loss (not important in humans), filling free spaces (organ fixation), mechanical pressure absorption (structural fat) and synthesis of signalling molecules and hormones
- slides: unilocular adipocyte **50–150 µm**, surrounded by a **basal lamina**; white fat = **10–20 % of body weight**, yellow

macroscopically
- hormones acting on it: insulin, **GH, thyroxine**, catecholamines, **glucocorticoids**, prostaglandins
- it is **endocrinely active** – synthesizes **leptin, adiponectin, oestrogens**; mechanical support e.g. of the kidney and eye, shock absorber in the soles

Brown fatty tissue



- adipocytes are multivacular - they contain many fat droplets
- **inside the cell, the core is stored in the middle of the MIT**
- present in foetuses and newborns - between shoulder blades, back of neck
  - in small quantities, even in adults
- rich vascular supply and sympathetic noradrenergic innervation
- function - thermogenesis - thermoregulation in newborns
  - locations (slides): **mediastinum, interscapular region, around the aorta and adrenal glands**; rapid lipid mobilization
  - **uncoupling protein 1 - uncovering protein thermogenin - heat is generated in MIT instead of ATP**
  - animals have a lot of brown fat just because of thermogenesis - mainly bears and others who resort to winter sleep

2.25 METABOLISM OF CONNECTIVE TISSUE

prof. RNDr. Eva Samcová, CSc.

Connective tissue

- Forms and maintains shape of body
- forms an extracellular matrix that connects and binds cells and organs
  - The extracellular matrix is the major component of connective tissue
  - Has **fibrillar** (fibrous) **component, amorphous** (glycosaminoglycans) **component and tissue fluid** (water)
  - the connective cells are embedded in it
- **connective tissue thus contains cells** (connective cells, fibroblasts ...), **fibers and amorphous matter in different proportions, which creates differences between individual types of connective tissue** (e.g. bone, adipose tissue, cartilage, vitreous gel...)
- a large part of the tissue is always ECM and the cells are scattered in it

Function of connective tissue

- **structural** - creates capsules enveloping organs, tendons, ligaments and thin ligament
  - Bone, adipose tissue and cartilage are specialized types of connective tissue
- **immune** - phagocytic and immunocompetent cells, antibody producing plasma cells
- **nutritional** - environment for metabolite exchange between cells and blood circulation,
- animal connective tissues are highly variable
  - tendon and skin range are firm and elastic, bone is hard and dense, cartilage absorbs impacts and is elastic, eye jelly is soft and transparent
- in all these cases a large part of the tissue is made up of the extracellular matrix (ECM) and the cells are scattered in it

Extracellular matrix

- **in other tissues the amount of ECM is insignificant, but in connective tissue the ECM is the most important part of the whole tissue**
  - e.g. ECM is responsible for kidney filtration properties - it creates a glomerular membrane that is negatively charged and repels positive charges
  - ECM also minimizes joint friction and allows cell movement
- **variability is determined by the type of collagen, its amount and the presence of other molecules**

Function of ECM

- determines the shape of the tissues by binding the cells and filling the space between them
- some embryonic cells must migrate through the ECM
- Biochemical changes in ECM occur during inflammation and aging
- ECM also plays a role in metastasis - allows cancer cells to migrate into blood and lymphatic circulation
- ECM molecules are involved in rheumatoid arthritis and osteoarthritis

ECM includes

- **Structural proteins - collagen** (tensile strenght)
- **specialized proteins** (adhesive proteins) - **fibronectin, laminin and elastin** (fibrous protein)
- **proteoglycans- consist of GAGs attached to protein core**
- proteoglycans can bind in bulk to hyaluronic acid and thus form a proteoglycan aggregate

Formation of ECM

- **the synthesis proceeds intracellularly and only outside the cells the ECM molecules assemble into huge formations**
- from the cell the non-synthesized molecules are exocytosed
- fibroblasts - skin and tendons
- osteoblasts - bone
- chondroblasts - cartilage

2.25.1 Fibrillar component of ECM

Collagen fibers

- accounts for about 25% of all mammalian proteins - the most abundant protein in our body
- at least **28 types** of collagen; **over 90 %** of body collagen is **type I** (skin, tendon, vasculature, organs, bone)
- is present in discontinuous connective tissue, bones, tendons, skin, veins and opaque sclera
  - contains **33 % glycine a 21 % proline and hydroxyproline as well as lysine and hydroxylysine**
- Mammals have many different types of collagen

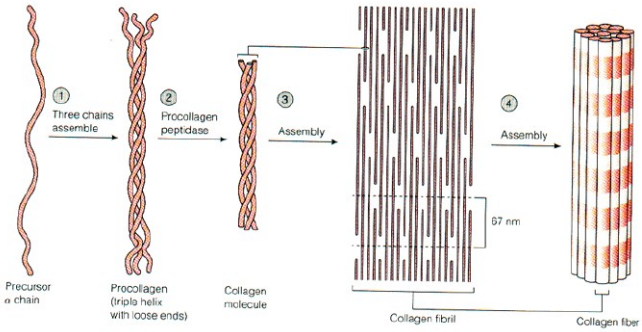
- **are produced by different cell types, but most by fibroblasts**
- the structure of one molecule of collagen (tropocollagen) is in the form of a right-handed triple helix and consists of smaller molecules - three left-handed peptide helixes
- 1 tropocollagen consists of about 1,000 amino acids - every third amino acid is **glycine**
    - the other two important molecules of collagen are **4-hydroxyproline** (or 3-hydroxyproline - both containing an amino group = an amino group in the ring) and **5-hydroxylysine**
- repeating sequence **Gly-X-Y** is an absolute requirement for the triple helix
    - X is often **proline**, Y is often **hydroxyproline or hydroxylysine**
    - prolyl residues can be hydroxylated at **C-3 or C-4**
    - hydroxylysines can be glycosylated with **galactose or galactosyl-glucose** via an O-glycosidic bond

Collagen synthesis

- **collagen is synthesized on ribosomes as a precursor preprocollagen**
- the signal sequence introduces it to GER, where **preprocollagen** is changed to procollagen by posttranslational modifications
    - cleavage of the signal sequence
    - hydroxylation of proline and lysine amino acids to hydroxyproline and hydroxylysine
        - **enzymes lysyl hydroxylase and prolyl hydroxylase with cofactor Vitamin C**
    - glycosylation of hydroxylysines formed in the previous step (glucose or galactose attached)
    - after glycosylation, the 3 chains join and together form a left-handed triple helix of **procollagen** by creating disulphide bridges between individual molecules - thanks to SH groups of cysteines
        - the helix is formed from **two α1 chains and one α2 chain**; S-S bonds form between the **C-terminal extension peptides**
    - At this point, procollagen is long and has untangled ends rich in cysteine
- procollagen continues to Golgi apparatus, which adds oligosaccharides to it, envelops it with a vesicle and sends it out of the cell
- **once the procollagen is out of the cell, the membrane enzymes capture it and cleave its free globular ends** (thus shortening it) **- what remains is called tropocollagen or collagen fibres and what is cleaved is called the propeptide**
    - Tropocollagen is stabilized by inter and intra-chain cross-links
- **the enzyme lysyloxidase** extracellular enzyme dependent on copper) mediates the bonds between several tropocollagen, thus forming the finished collagen (collagen is therefore a polymer of tropocollagen))
    - the cross-links (aldol reaction) link **hydroxylysine and lysine** residues; many collagen fibrils → collagen fibres
    - collagen is attached to cell membranes by fibronectin, laminin, **fibulin** and integrins
- **collagen degradation** – **collagenases** (in the ECM, pancreatic juice, bacteria)
    - the formation of bonds between tropocollagen occurs via aldehydes, their condensation, schiff base formation etc...

Types of collagen

- **collagen I.** - skin, tendons, bones, dentin
- **collagen II.** - cartilage, vitreous humour
- **collagen III.** - fetal skin, uterus, blood vessels
- **collagen IV.** - glomeruli, basement membranes kidneys, capsule lenses,
    - Type IV does not form striated fibers - unlike for others, it has a globular structure



Disorders of collagen synthesis

- defects at the level of triple helix folding and stabilization
- **Ehlers-Danlos syndromes**- it is a group of about 10 clinically and genetically distinguishable diseases that are associated with manifestations of structural weakening of connective tissue (joint hypermobility, translucent fragile skin ...)
    - **type VI** - lysylhydroxylase deficiency (EDS type VI) - transverse bonds are less stable, the clinical picture is hyperextensibility of the skin and joints, poorly treatable injuries and deformities of muscles and bones
- **scurvy**- ack of vitamin C in the diet for proline hydroxylation, manifestations are subcutaneous and other bleeding, muscle weakness
    - slides: major signs – **bleeding gums (loss of teeth)**, subcutaneous haemorrhages, **poor wound healing**
    - slides: Ehlers-Danlos = inherited disorders of proteins involved in synthesis/assembly of **collagen I, III and V** – hyperextensible skin, abnormal tissue fragility, increased joint mobility

Fibronectin

- during tissue development, the cells (fibroblasts) move along the already formed collagen fibers, stretch and stretch it into the ropes, affecting the arrangement of the collagen fibers and at the same time the collagen fibers affect the distribution of fibroblasts
- fibronectin is responsible for the ability of cells to attach to the collagen fibers
- **it is an adhesive protein** (glycoprotein) - a fibrous dimer found in both ECM and blood
    - fibronectin molecules are divided into several domains and form bonds with cell membrane (via integrins), collagen, fibrin and various proteoglycans
- A sudden drop in fibronectin concentration may indicate cancerous growth
- **fibronectin is secreted by the same cells as collagen**
- **binding to the cell surface is realized by receptors for fibronectin- integrins**
- integrins are heterodimers, have different types of α and β polypeptide chains
- fibronectin interacts indirectly with actin microfilaments present in the cytosol, contacting the intra-cellular space
- also participates in cell migration by providing a binding site

Elastin

- **a protein conferring extensibility and shrinkage in tissues**
- found in the lungs, large arteries, elastic ligaments, skin ..
- **contains a large amount of proline and Gly, little OH-proline, no OH-lysine, is not glycosylated**
- prolyl hydroxylase hydroxylates some prolines (Vitamin C is the cofactor)
- **produced by fibroblasts, after secretion from the cell oxidative deamination of lysyl residues** (enzyme **lysyloxidase**)
- he main cross-links are **desmosins** (condensation of 3 lysine-derived aldehydes and one normal lysine)
- formed mainly during embryonic development - later minimal metabolism (elastase inhibited)
- cross-linked form of elastin is insoluble, very stable and resistant (with low metabolic turnover)
- elastin contains repeating sequences (pentapeptide Val-Pro-Gly-Val-Gly and hexapeptide Val-Ala-Pro-Gly-Val-Gly)
- slides: also in elastic cartilage and **urinary bladder**; precursor **tropoelastin** (linear, small nonpolar AAs – Gly, Ala, Val, Pro, Lys) → exported to the ECM → interacts with **fibrillin** microfibrils → lysyl oxidase → desmosine cross-links → mature elastic fibres
- synthesis decreases in adults; one of the **most stable proteins** known
- degraded by **elastase** (released by neutrophils, also in pancreatic juice)

Fibrilin

- **is an adhesive protein, a large glycoprotein that is a structural component of microfibrils**
- fibroblasts secrete it into the ECM where it is incorporated into insoluble microfibrils
    - they create a kind of scaffold that stores tropoelastin, which matures here into elastin
- fibrilin defect results in a decrease in elasticity and strength of the elastic ligament
- **Marfan syndrome** is caused by mutation of the **fibrillin** gene (15th chromosome))
    - Eyes are effected (ectopia lentis – dislocation of lens), skeletal and cardiovascular system are effected as well (weakened aorta)

Laminin

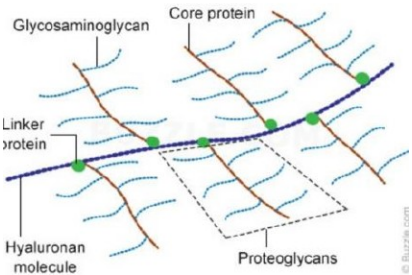
- after collagen IV, laminin is the most common protein in the basement membrane, a specialized type of ECM
- laminin has the ability to bind to other ECM components
- **entactin** (nidogen), a glycoprotein that is the major factor connecting cells to the lamin, binds to laminin
- is composed of 3 different peptide chains linked to a cross structure

Glomerular membrane

- **has 3 layers**
    - **epithelium**
    - **basement membrane** - its primary components are 3 proteins - laminin, entactin and collagen IV
    - **endothelium**
- reduces the penetration of large molecules by the glomerulus into the renal tubule (small molecules pass)
- **larger molecules such as albumin** (greater than 8nm) **do not pass due to negative charge in the laminate or pass only in very small quantities**
    - Negative charge repels mostly plasma proteins and is caused by heparan **sulphate**
- when the membrane is damaged (e.g. by glomerulonephritis), the pore size changes and the charge is distributed differently -> passes albumin and other plasma proteins (albuminuria), which we can easily test

Basal lamina (membrane)

- 40-120nm thick layer of specialized ECM
    - supports epithelial cells, separating them from the connective tissue
    - surrounds muscle, endothelial and fat cells



- two layers of epithelia are adjacent to each other in the kidney glomeruli and in the alveoli - the basement membrane is made of 2 basal laminations
- determines cell polarity, affects metabolism and organizes proteins in the plasma membrane

2.25.2 Amorphous component of ECM

Proteoglycans

- prevent compression and serve as a filler
- **contain up to 95% carbohydrates and 5% protein (lots of carbohydrates, the rest protein)**
- **proteins containing covalently linked glycosaminoglycans (GAG)**
  - **GAG - hyaluronic acid, chondroitin sulphate, keratan sulphate, heparin, heparan sulphate and dermatan sulphate**
  - **an unbranched polysaccharide consisting of repeating dimers**
  - **have negative charges**
  - **they are long polymers of dimers of amino sugar (D-glucosamine or D-galactosamine) and uronic acid**
  - amino sugar: **D-glucosamine or D-galactosamine** (amino group usually **acetylated**); uronic acid: **D-glucuronic acid or L-iduronic acid**
  - sulfation on C-4 or C-6 or on non-acetylated nitrogen; COO^- + sulfate groups → strongly negative → repel each other, surrounded by water shell → **slippery consistency** of mucus and synovial fluid
  - GAGs are synthesized in the **Golgi apparatus**
  - **with the exception of hyaluronic acid, they contain sulphate groups**
- found in synovial fluid, vitreous humour, artery walls, bones, cartilage and cornea
- often have the group $-SO_3-$ and thus carry a negative charge (also COO- group)
- **are hydrated and act as lubricants because they bind large amounts of water and sodium**
- functions (slides): **"sponge"** – pressure expels the fluid, it is sucked back when the pressure stops; bind collagen fibrils; form networks for cell adhesion and migration; take part in development; **hydroxyapatite and CaCO_{3}** bind to them in bone

Classification of GAGs (slides)

- **heparan sulphate** – extracellular, basement membrane, ubiquitous on cell surfaces
- **heparin** – **intracellular** component of mast cells, anticoagulant; sulfate on C-3 or C-6 of glucosamine
- **keratan sulphate** – contains **galactose (no uronic acid)** + GlcNAc; sulfate on C-6
- **chondroitin sulphate** – **most abundant GAG**; cartilage, tendons, ligaments, aorta; sulfate on C-4 or C-6; in cartilage it binds collagen into a tight, strong network
- **dermatan sulphate** – skin, blood vessels, heart valves; sulfate on C-4 of GalNAc
- **hyaluronic acid** – synovial fluid, cartilage; unsulfated

Structure of proteoglycans

- all GAGs except HA are covalently attached to a core protein = **proteoglycan monomer** ("bottle-brush")
- link: trisaccharide **Gal-Gal-xylose** and a **serine** – O-glycosidic bond between xylose and Ser-OH
- **aggrecan** (chondroitin + keratan sulphate on a core protein, cartilage) monomers associate with HA **non-covalently (ionic interactions)** → proteoglycan aggregates

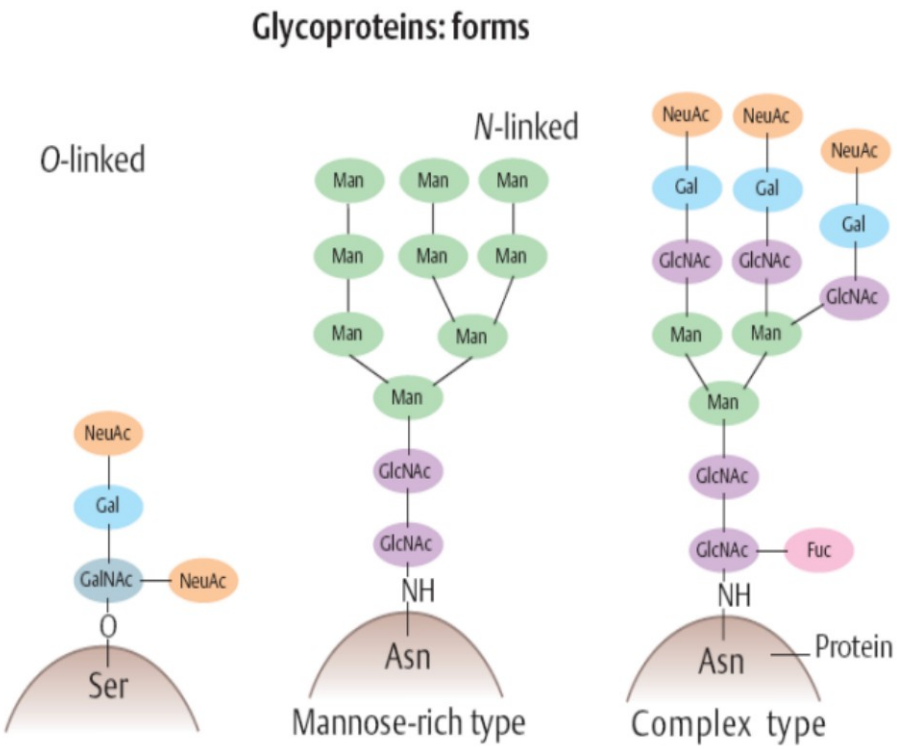
Synthesis and degradation of GAGs

- sulfation happens after incorporation of the sugar into the chain – **sulfotransferases**, sulfate donor **PAPS** (3'-phosphoadenosyl-5'-phosphosulfate)
- **L-iduronic acid** is formed from D-glucuronic acid already in the chain (epimerase)
- degradation: phagocytosis → fusion with a lysosome → **acid hydrolases** (pH ~5): endoglycosidases (**hyaluronidase**) → oligosaccharides → sequential cleavage from the non-reducing end (**β-glucuronidase**); the **sulfate is removed first** by a specific sulfatase
- **mucopolysaccharidoses** – deficiency of any lysosomal hydrolase degrading heparan and/or dermatan sulphate
- they are secreted from the cells to the ECM after synthesis
- Negatively charged GAG chains repel each other, thus taking up a lot of space and acting as molecular sieves that determine which substance enters or leaves the cell
- degradation (both glycoproteins and glycolipids) occurs in the cell after endocytosis by lysosomal fusion of the endocytes
- Chondroitin sulphates in proteoglycans are an important component of cartilage

Glycoproteins

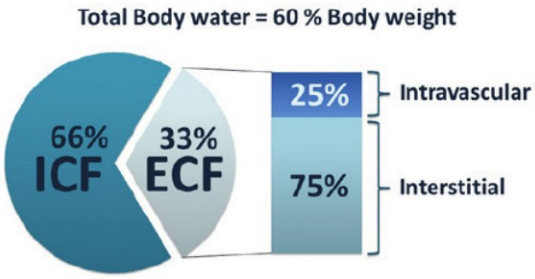
- **proteins that have covalently linked oligosaccharide (glycan) chains to the central protein chain)**
- **almost all human plasma proteins are glycoproteins (except albumin))**
- glycoproteins may contain more than 50% carbohydrates, but generally the protein component should predominate
- 7 monosaccharides predominate in human glycoproteins - Glc, Man, Gal, NeuAc, Fuc, GalNAc, GlcNAc
- oligosaccharide chains are short (**2–10 sugar residues**), branched; attached co- or post-translationally (glycosylation) in

the ER and Golgi
- sugars include D-hexoses, **L-fucose**, **xylose**, **sialic acids** (N-acetylneuraminic acid, NANA)
- **functions**
    - membrane glycoproteins – cell surface recognition (cell–cell, virus–cell, hormone–cell), antigenicity (**AB0 blood groups**)
    - ECM components; **mucins** – protective lubricants in GIT and urogenital tract
    - soluble glycoproteins – plasma proteins (**ceruloplasmin, transferrin**), hormones (**FSH, LH, hCG, TSH, EPO**)
- **N-glycoproteins** – bond via the amide group of **asparagine**; synthesized in the rER using **dolichol** (lipid made from an intermediate of cholesterol synthesis) carrying a pyrophosphate-linked oligosaccharide (GlcNAc, mannose, glucose)
- **O-glycoproteins** – bond via –OH of **serine or threonine**; sugars added **gradually in the Golgi**, without dolichol
    - activated sugars: UDP-Glc, UDP-Gal, UDP-GlcNAc, UDP-GalNAc, GDP-mannose, GDP-L-fucose, CMP-NANA; glycosylation starts with **GalNAc** on Ser/Thr; specific glycosyltransferases build the chain
    - types: **mucins** (most common, 50–90 % sugar, mucous secretions of GIT, urogenital, respiratory, ocular and auditory systems) and the **proteoglycan type**
- degradation in **lysosomes**: endopeptidases (cathepsins B, D, E), exopeptidases (cathepsins A, H, C…), endo- and exoglycosidases (β-galactosidase, α-neuraminidase, mannosidase, hexosaminidase)
- glycoproteins of connective tissue: fibronectin (also plasma fibronectin and platelet granules), **chondronectin** (cartilage only), laminin, **thrombospondin, tenascin, fibrillin**, and **osteocalcin, osteonectin, osteopontin** (osteoblasts – bone mineralization)



2.26 COMPONENTS OF BLOOD PLASMA AND THEIR FUNCTIONS

MUDr. Josef Fontana



2.26.1 Definitions of basic terms

Whole Body Water (WBW)

- **forms 55 - 60% of body mass**
- women have less water than men (because they have more fat)
- small children and pregnant women have a higher WBW
- in older persons, total body water decreases
- **we distinguish between two components of WBW**
  - **intracellular fluid (ICF)** - forms 2/3 WBW
  - **extracellular fluid (ECF)** - forms 1/3 WBW
    - **intravasal fluid (IVF)** – found in vessels (plasma and lymph) and forms 1/4 ECF
    - **interstitial fluid (ISF)** – tissue fluid, forms 3/4 ECT

Plasma vs. Serum

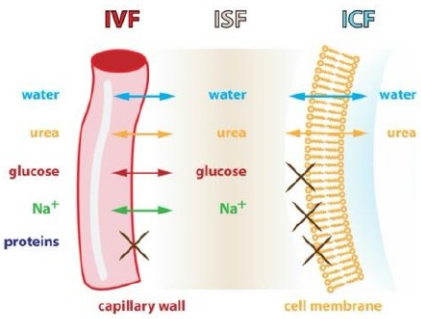
- **plasma is blood after the removal of cells**
  - anticoagulants (calcium binding agents e.g. citrate) and centrifugation is used to separate blood elements from the plasma
  - we can conduct blood clotting tests on plasma (as it contains coagulation factors)
  - examination of blood plasma is used in emergency/urgent medicine
  - **„-aemia" = concentration of something in the plasma/blood**
- **serum is a liquid without clotting factors**
  - serum is suitable for most biochemical tests; therefore, it is most often used compared to plasma
  - **„-rrhachia" = concentration of something in cerebrospinal fluid**

composition of plasma

- **water (up to 92%)** – free or hydrating (bound to molecules)
- **low molecular weight components** - ions (minerals), energy substrates, metabolites
- **high molecular weight components** - proteins, lipoproteins

2.26.2 Osmolarity in plasma

- indicates the number of osmotically active particles per litre
  - two possible units - mOsmol/l and mmol/l, they are the same, where mol/l = M (therefore 10 µM is 10 µmol/l)
- osmolarity has strict limits – in a healthy individual it is 280-295 mmol/l
  - osmolarity is regulated mainly by the pituitary gland via antidiuretic hormone (ADH)
- **osmolarity is usually not measured, but it is calculated from an imperfect formula, which assumes that osmolarity is mainly influenced by low molecular weight (LMW) components**
  - from each group of low molecular weight components (ions, nutrients, metabolites) we take the main one and form a formula
  - **2 * Na^+** (twice because ions are positive and negative) **+ urea** (most common metabolite) **+ Glc** (most common nutrient)
- **osmotic gap (OG) – difference b between measured and calculated osmolarity**
  - calculated osmolarity is not accurate, most often it comes with discrepancies
  - physiological value of osmolar window is 4 - 12 mmol/l
  - high OG means there is an unfamiliar, osmotically active substance in the blood, which is interfering with our balance
    - 1% ethanol increases osmolarity by 23 mmol/l
- helps us determine if a patient underwent poisoning
- **headaches when drunk** - firstly hyperosmolarity, then hypoosmolarity

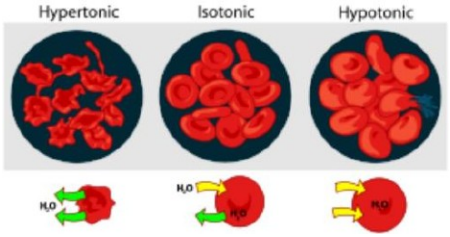


Transition between blood and cells

- Water and urea pass freely between cells and blood. The problem lays in other substances.
- e.g. glucose and Na^+ are trapped inside cells, therefore their concentration in blood does not reflect their situation/concentration inside cells.

Clinical significance of plasma osmolarity

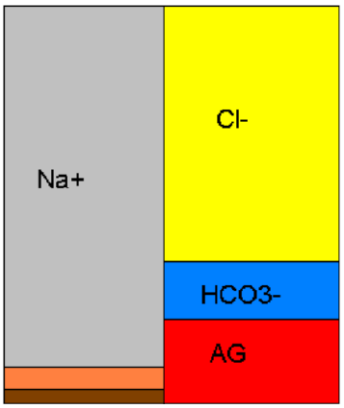
- isotonic solutions have approximately the same osmolarity as plasma
  - **physiological solution** - 0,9% NaCl has the same osmolarity, but decreases the pH, which is not the best.
  - slides: hypotonic solutions can cause **haemolysis**, hypertonic solutions can **irritate the vessel wall**
  - **5% solution of glucose** – osmolarity is also okay, but after the processing of glucose only water remains, therefore a hypotonic environment.
  - **balanced solutions – Ringer or Hartman solutions** – their compositions resemble that of plasma in all aspects
- it is important to respect the tonicity of solutions administered to patients, because the water content in blood interferes with the water content in cells
  - **hypotonic environment (high water in blood) – water enters cells, which then bulk up**
  - **hypertonic environment (low water in blood) – water exits cells, which then shrinks**



- **quick changes in osmolarity can be dangerous on our brain**
  - rapidly receiving a large number of cells leads to:
    - swelling of brain
    - bad blood flow into the brain
  - rapidly decreasing the osmolarity leads to
    - decrease in cell volume – typical is the damage to myelin sheath in the pontine region of the brain (so-called pontine demyelination)
    - motor and sensory problems

2.26.3 Low molecular weight components of blood plasma

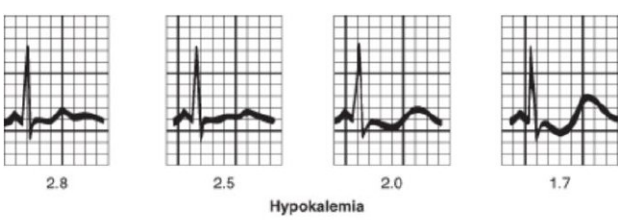
- **cations** - Na^+, K^+, Ca^{2+}
- **anions** – mainly Cl^-, HCO_3^- and in lower concentrations also HPO_4^{2-}, HSO_4^-
- **metabolites** - urea, creatinine, uric acid, bilirubin
- **nutrients** – glucose, fatty acids, ketone bodies
- **others** - vitamins, hormones, xenobiotics…

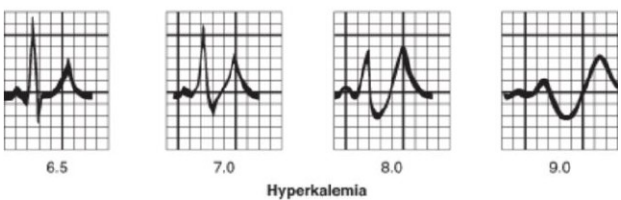


  - slides also list **Mg^{2+}** among cations and **uric acid** among waste products
- **law of electroneutrality: there must be a balance between anions and cations**
- **anion gap (AG)** = other anions which are not Cl^- or HCO_3^-
  - AG = $(Na^+ + K^+) - (Cl^- + HCO_3^-)$
  - Increases during the overproduction of ketone bodies in the body

Sodium cation - Na^+

- **Main cation of ECF (135 mM - 145 mM)**
- Along with Cl^-, it is responsible for 80% of the osmolarity of the plasma → binds water → determines the volume of plasma
- sodium + water → blood pressure
- **hormones regulating natremia** (concentration of sodium in blood)
  - **aldosterone** – increases the reabsorption of sodium in kidneys
  - **atrial natriuretic polypeptide (ANP)** – decreases the reabsorption of sodium in kidneys
  - slides: regulation by the **renin-angiotensin-aldosterone system** and **natriuretic peptides (ANP, BNP)**
    - produced by the heart (heart produces hormones)
    - increases the secretion of Na+ and water into urine, thus reduces the total blood volume
    - decreases blood pressure





Chloride Anion - Cl^-

- **main anion of ECF (97 - 108 mM)**
- accompanies sodium – maintains osmolarity and decreases pH
- **important for maintaining acido-basic balance (ABR)**
- chloride anion co-forms gastric juice (hydrochloric Acid - HCL)

- neutrophil granulocytes produce hypochlodic acid during respiratory bursts from free blood Cl^- and H^+
  - (slide: hypochlorous acid is formed from Cl^- and **hydrogen peroxide**)

Potassium cation - K^+

- **typical ICF cation - 98% in ICF (c = 155mM), only 2% v ECF (c = 3.8 - 5.2 mM)**
  - this gradient is extremely important due to the role it plays in the membrane potential
  - **together with magnesium it is one of the main ICF cations**
- **potassium is important especially in excitatory tissues,** especially the heart, where hyperkalaemia is more dangerous than hypokalaemia
  - K^+ is also regulated by the renin-angiotensin-aldosterone system; the Na^+ (EC) / K^+ (IC) distribution is maintained by **Na^{+}/K^{+}-ATPase**

Calcium cation - Ca^{2+}

- **largest gradient – its level in ECG is 10 000x higher than in ICF**
  - **ECF (2.25 - 2.75 mM)**
  - **99 %** of body Ca^{2+} is in bones; Ca^{2+} is also a **second messenger**
- In plasma occurs in free (ionized) and bound **fractions**
  - **~46% Ca^{2+} binds to plasma proteins** (albumin)
  - **~6% Ca^{2+} form complexes with small anions** ($HCO3^-$, citrate, lactate...)
  - **~48% Ca^{2+} forms free (ionized fractions)** which are the only physiologically active ones
- **fractions ratio (during constant total calceamia) affects**
  - **pH** – increase in pH (e.g during hyperventilation) leads to the decrease of free (ionised) Ca^{2+} → convulsions
    - mechanism: Ca^{2+} **competes with H^{+} for binding sites on albumin** – at higher pH more Ca^{2+} binds to albumin
  - **proteinemia** - loss of proteins → decrease in bound fraction
- regulation of Ca^{2+}
  - to increase calcaemia: parathormone and calcitriol
  - to decrease calcaemia: calcitonin
- significance of Ca^{2+}
  - stabilization of membranes of excitatory tissues
  - muscle contraction (no muscles contact in the absence of calcium)
  - haemocoagulation – activates coagulation factors
  - necessary for lactation
  - part of inorganic bone matric (hydroxyapatite)

Bicarbonate - HCO_3^-

- **concentration in blood (22 - 26 mM)**
- bicarbonate is an important blood buffer
- it forms half of the bloods buffer capacity
- its concentration decreases during the increase of ketone bodies

Energy substrates (values are during fasting period)

- **glucose = 3.3 - 5.6 mM**
- **fatty acids = 0.6 - 1.7 mM**
  - the longer the chain, the more albumin is needed for transport in blood
  - the high molecular weight molecules "lipoproteins" transports other lipids
- **ketone bodies**
  - similar to glucose, so they are water soluble and transport freely in blood
  - **concentration of 3-hydroxybutyrate < 0.5 mM**
  - **when concentration of 3-hydroxybutyrate > 3 mM → KETOACIDOSIS**
- **amino acids = 2.3 - 4.0 mM**
  - Mainly: Glutamine (Gln = 0.6 mM) because it carries ammonia in blood
  - and Alanine (Ala = 0.3 mM)

Nitrogenous catabolites

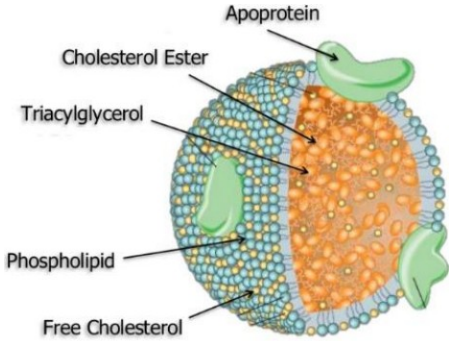
- **ammonia (12-50 µM)**
  - transported in blood as **glutamine and alanine**
  - most of ammonia comes from deamination of AA
- **urea (2,5-8,3 mM)**
  - occurs in the body in high concentrations, is formed in the liver
- **creatinine (50-120 µM)**

  - it is a waste product from muscle metabolism
  - itself is not harmful
  - high creatinine is a sign that something is wrong in the body
  - creatinine is used for the **calculation of glomerular filtration**
  - **note: gout and crystal arthropathy are caused by excess uric acid, not creatinine – uric acid 150–360 µM**, product of **purine degradation**
  - if it is in excess "gout" is formed, at higher concentrations it starts to crystalize → arthropathy
- **bilirubin (17 µM)**
  - waste product of haemoglobin metabolism
  - during bilirubinaemia above 40µM there is jaundice (latin icterus)
  - bilirubin depletes albumin binding capacity and begins to diffuse into tissue

2.26.4 High molecular components of blood plasma

Lipoproteins

- **fatty acids with short chains (up to 12C) can freely move in plasma, longer fatty acids need to be bound to a transporter protein albumin**
- other lipids need to be distributed by lipoproteins
- **lipoproteins – composed of lipids and proteins**
  - In the core of lipoproteins are non-polar substances
    - **triacylglycerol's** (TAG)
    - **cholesterol esters** (CE)
  - Surface of lipoproteins consists of
    - **Phospholipids**
    - **Apoproteins**
    - **Cholesterol**



- classes of lipoproteins (by density and apoproteins)
  - **chylomicrons** – TAG and CE from the intestine to tissues
  - **VLDL** – newly synthesized TAG from the liver to peripheral tissues
  - **IDL** – formed from VLDL
  - **LDL** – CE from the liver to tissues
  - **HDL** – collect CE from tissues and bring them to the liver → bile acids → excretion

Metabolism of cholesterol and lipoproteins (separate lecture – not in the script)

Importance of cholesterol

- component of cell membranes – about **1/4 of all membrane lipids**; amphipathic; **stabilizes the membrane and reduces its fluidity**
- important derivatives: **bile acids, steroid hormones, vitamin D**
  - steroid hormones: gestagens (**progesterone**), androgens (**testosterone**), oestrogens (**oestradiol**), glucocorticoids (**cortisol**), mineralocorticoids (**aldosterone**)

Cholesterol synthesis

- de novo synthesis covers about **70 %** of the daily need (mainly **liver and intestine**, ~800 mg/day), **30 % from the diet** (eggs, meat, dairy; ~300 mg/day)
- localization: cytosol (start), peroxisomes and smooth ER
- 3 phases
  - 1. **AcCoA → isopentenyl diphosphate** ("active isoprene"): 2 AcCoA → acetoacetyl-CoA (β-ketothiolase) → + AcCoA → **HMG-CoA** (HMG-CoA synthase, **cytosolic** – unlike the mitochondrial one in ketogenesis) → **mevalonate** (**HMG-CoA reductase**, 2 NADPH) → isopentenyl diphosphate
  - 2. condensation of **6 isoprene units** (dimethylallyl-PP → geranyl-PP → farnesyl-PP) → **squalene**
  - 3. cyclization of squalene → lanosterol (first sterol) → (≈19 reactions) → **cholesterol**
- **HMG-CoA reductase = the main regulatory enzyme**

Regulation of cholesterol synthesis

- feedback regulation of the activity and amount of HMG-CoA reductase by cellular cholesterol
  - 1. **transcription** – transcription factor **SREBP-2** (low cholesterol activates it, high inhibits)
  - 2. **translation** – inhibited by non-steroid metabolites of mevalonate
  - 3. **degradation** – high cholesterol starts proteolysis of the reductase
  - 4. **phosphorylation reduces activity** – **AMPK** stops synthesis when ATP is low; **insulin** activates (dephosphorylation), glucagon inhibits
- **statins** (simvastatin, lovastatin) – **competitive inhibitors** of HMG-CoA reductase (K_i ≈ 1 nM)

Apoproteins

- stabilize the particles
- 1. **structural**: Apo B100, Apo B48
- 2. **enzyme cofactors**: Apo C-II (for **LPL**), Apo A-I (for **LCAT**)
- 3. **receptor ligands**: Apo B100 and Apo E (LDL receptor), Apo E (scavenger receptor), Apo A-I (HDL receptor)

Fate of the individual lipoproteins

- **chylomicrons** – go first into **lymph** → thoracic duct → blood; **lipoprotein lipase (LPL)** hydrolyses their TAG to MAG + FA (FA enter cells) → **chylomicron remnants** (smaller, more CE) → taken up by hepatocytes (ligand **Apo E**)
- **LPL** – on endothelial cells (mainly adipose tissue and muscle); cofactor **Apo C-II**; chylomicrons → remnants, VLDL → IDL
- **VLDL** – formed in the liver, carry liver TAG; LPL converts VLDL → IDL; **CETP** (cholesterol ester transfer protein) moves CE from HDL to VLDL and TAG in the opposite direction
- **IDL** – more CE, less TAG; either taken up by the liver, or **hepatic lipase (HL)** hydrolyses their TAG → **LDL**
- **LDL** – from IDL (or small part de novo); deliver CE to tissues – cells that need CE express **LDL receptors** (for Apo B100 and Apo E)
  - **"bad", atherogenic** lipoprotein – plasma level should be **< 3.0 mM** (in diabetics < 2.5 mM)
- **HDL** – **reverse cholesterol transport** from tissues to the liver → excretion
  - formed in the liver and enterocytes as flat empty discs = **nascent HDL** (Apo A-I, A-II..., ± Apo C-II, Apo E)
  - **LCAT** (lecithin-cholesterol acyltransferase; acyl donor lecithin, acceptor cholesterol; cofactor **Apo A-I**) esterifies cholesterol and fills HDL with CE → HDL3 → HDL2α → (CETP) HDL2β → liver; HL turns HDL2β back to HDL3
  - **"good"** lipoprotein – should be **> 1.0 mM in men, > 1.2 mM in women**; sex hormones protect women before menopause

Familial hypercholesterolaemia

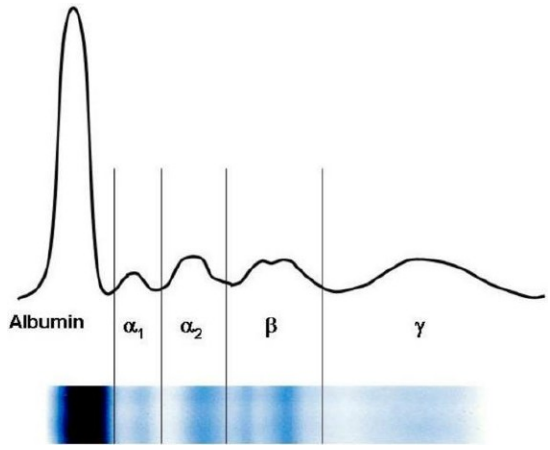
- see **2.31** for the full description; the cholesterol lecture adds:
  - international project **MedPed** ("Make Early Diagnosis to Prevent Early Deaths"); diagnosis by clinical symptoms, cholesterol level and mutation analysis of the LDL-receptor / Apo B genes
  - the most severe (homozygous) forms are treated by **LDL apheresis**



[slides] Tendon xanthomas, xanthelasma of the eyelids and arcus lipoides corneae

Proteins

- **The total plasma concentration ("total proteins") ranges from 60 - 80 g/l**
- "total protein" = more than **300 proteins**, almost all **glycoproteins**; slides (Kvasnicová) give **64–83 g/l**
- proteins are **ampholytes** – at physiological pH they are **negatively charged (anions)**
- about **25 g** of plasma proteins are synthesized daily; other sources: plasmocytes (Ig), other cells (e.g. β_2-microglobulin)
- Plasma proteins are synthesized in the liver (except for immunoglobulins, which are formed by plasma cells)
- Function of plasma proteins:
  - maintaining oncotic pressure at around 25 mmHg or 2.2 kPa)
  - taking part in hemocoagulation and fibrinolysis
  - immunity: antibodies, complement cascade, and acute phase reactants (APP, see below)
  - transporting poorly water soluble substances
  - buffer of blood
  - are antioxidants
  - diagnosis of diseases (enzymes- creatinine kinase, markers)
- **plasma proteins are divided based on serum electrophoresis (separation according to mobility in an electric field)**
  - using agarose gel as a medium yields 5 to 6 fractions
    - **1) albumin**
    - **2) alpha-1 fraction**
    - **3) alpha-2 fraction**
    - **4) beta fraction** (sometimes divided into beta-1 and beta-2 fractions)
    - **5) gamma fractions**



- densitometry of a normal pattern: albumin **60 %**, α1 **3 %**, α2 **9 %**, β **12 %**, γ **16 %**
- **prealbumin** moves before albumin but is not visible (low concentration); **fibrinogen** appears only when plasma (not serum) is analysed

Albumin

- relatively small molecules (Mr = 68 000)
  - Sits on the border of permeability of glomerular filtration membranes.
  - During the destruction of the membrane, albumin is the first plasma protein to filtrate into the urine
- forms 60% of all plasma proteins
- **35 - 53 g/l**
- function of albumin
  - maintaining oncotic pressure of plasma (oncotic pressure is the osmotic pressure caused by proteins)
    - albumin forms up to 75% of its value
      - hypalbuminaemia= leakage of fluid into the intestine, causing swelling
    - "storage" of proteins
      - half-life is 21 days (takes a while before changes in its level take effect)
    - buffer
    - **transport of substances**
      - **albumin is the most important transporter of substances in the plasma**
      - **takes part in the transport of unconjugated bilirubin, fatty acids, thyroid hormones, Ca, Mg, Zn, drugs...**
  - also important drugs (**penicillin, digoxin, salicylates**)
  - slides (Kvasnicová): reference 32–45 g/l; plasma buffer (7 %), antioxidant function; **negative acute phase protein**; ↓ in acute phase response, severe liver damage, malnutrition, increased loss (kidney, GIT, skin); ↑ in dehydration

Prealbumin (transthyretin)

- 0.2–0.4 g/l, from the liver; carries **thyroid hormones (mainly T4)** and **retinol-binding protein** with retinol
- **negative acute phase protein**; ↓ also in severe liver damage and protein malnutrition

Alpha 1 globulins

- **α1-antitrypsin**
  - protease inhibitor
  - belong to APPs/RAF (acute phased reactants)
- **α1-fetoprotein (AFP)**
  - used in prenatal diagnostics
  - in adults with carcinoma of liver there are high levels of it

- α_1-antitrypsin = alpha-1-protease inhibitor (API, AAT) – inhibits **elastase, collagenase**; acute phase protein
- **α_{1}-acid glycoprotein (orosomucoid)** – 0.5–1.4 g/l, acute phase protein
- **α_{1}-lipoprotein = HDL**
- α_1-antitrypsin deficiency – see **2.31**; slides add ZZ homozygote frequency: 1/2500 in CZ, 1/1600 in Sweden, 1/6000 in USA

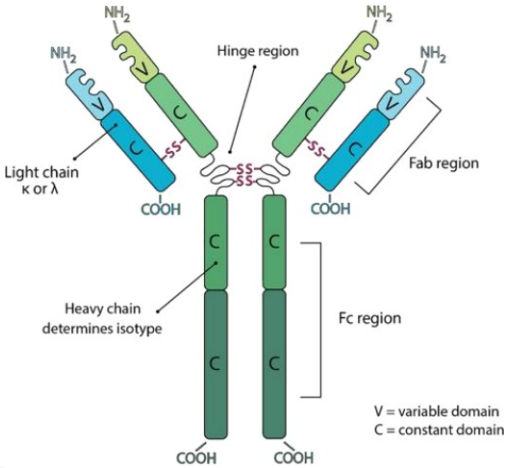
Alpha 2 globulins

- **haptoglobin** – binds free haemoglobin, APPs
- **ceruloplasmin** – main transporter protein of Cu, Fe^{2+} -> Fe^{3+}
- **α2-makroglobulin** – protease inhibitor
  - α_2-macroglobulin: inhibits **trypsin, chymotrypsin, plasmin**; its large molecule hardly passes the glomerulus → ↑ in **nephrotic syndrome**; ↓ in acute pancreatitis
  - haptoglobin (0.35–2.0 g/l) – ↓ in **intravascular haemolysis**
  - ceruloplasmin (0.22–0.6 g/l) – contains **6–8 Cu atoms**, ferroxidase activity; ↓ in **Wilson's disease**, ↑ in pregnancy
  - **ferritin** (30–284 µg/l) – iron-storing protein in cells; serum level reflects **iron stores**; also a tumour marker

Beta globulins

- **protein transferrin**
  - transmits Fe^{3+}
  - without a bound iron
  - acts as an antioxidant
  - transferrin 1.8–4.0 g/l; ↑ in iron deficiency, ↓ in iron excess; **negative acute phase protein**; in alcoholics **carbohydrate-deficient transferrin**
- **C-reactive protein (CRP)**
  - APP – normal concentration is below 10 mg/l, during acute bacterial inflammation its level increases rapidly
  - Binds substance C of Streptococcus pneumoniae

- **fibrinogen**
  - belongs to coagulation factors (factor I
  - APP
  - normal concentration is 1.5-4.5 g/l
- **hemopexin**
  - secure binding
  - transport of free heme



- β_2 fraction also contains **complement C3, C4**, **β-lipoprotein (LDL)**, **β_{2}-microglobulin** (part of the HLA system, Mr 11 800)
- CRP (Kvasnicová: < 5 mg/l) – sensitive and fast; much higher in **bacterial** than viral infections; not stained in electrophoresis
- fibrinogen – also an **independent risk factor of atherogenesis**

Gamma globulins

- **produced by plasma cells (the only plasma proteins that are not synthesized in the liver)**
- **their structure consists of two pairs of heavy and two pairs of light chain interconnected by S-S bridges**
- we differentiate between 5 classes of gamaglobulins, based on their heavy chains – **IgG, IgA, IgM, IgD and IgE**
- IgG 7–18 g/l; polyclonal or **monoclonal** (paraprotein – lymphatic tissue tumours / myeloma; specified by **immunofixation**)

Other clinically used proteins

- **cystatin C** – glomerular function (0.5–1.2 mg/l); **myoglobin** – fast cardiomarker; **troponins** (cTnT, cTnI) – cardiomarkers
- **Apo A1** (surface protein of HDL), **Apo B** (B-48, B-100), **lipoprotein (a)** – risk factor for coronary disease
- **thyroglobulin**; **CEA** (carcinoembryonic antigen) and **CA** markers – tumour markers

Factors influencing plasma protein concentration

- rate of synthesis and degradation, distribution in body fluids, loss into the "third space", elimination, hydration
- pre-analytical: physical activity (by 12 %), **standing position (+10–15 %)**, supine position ↓, tightening of the arm during sampling, storage of the specimen; plasma > serum (fibrinogen)
- ↓ in children, pregnant women, after starvation (albumin, transferrin, C3)
- synthesis ↑: inflammation, hyperthyroidism, hypercortisolism, ↑ GH, iron deficiency, protein loss, clonal Ig production; ↓: liver damage, nutritional deficit, hypothyroidism, diabetes mellitus, alcoholism
- consequences of abnormal levels: changed **erythrocyte sedimentation**, swelling (oedema), polyuria, sensitivity to infections
- distribution: continual movement into the interstitium (pinocytosis, interendothelial junctions; the basal membrane = molecular sieve); e.g. ~42 % of albumin is intravascular, ~92 % of α_2-macroglobulin
- interstitial proteins: lymph has less than plasma; **CSF 200× less**; pathological fluids: **transudate < 30 g/l, exudate > 30 g/l**
- catabolism: liver (**desialylation of glycoproteins**), kidneys, endothelium – pinocytosis and lysosomal hydrolysis; rate = **biological half-life** (longest in structural, shortest in regulatory proteins)
- elimination: kidney (physiological loss **< 150 mg/day** in urine), GIT, skin

2.26.5 Enzymes in plasma

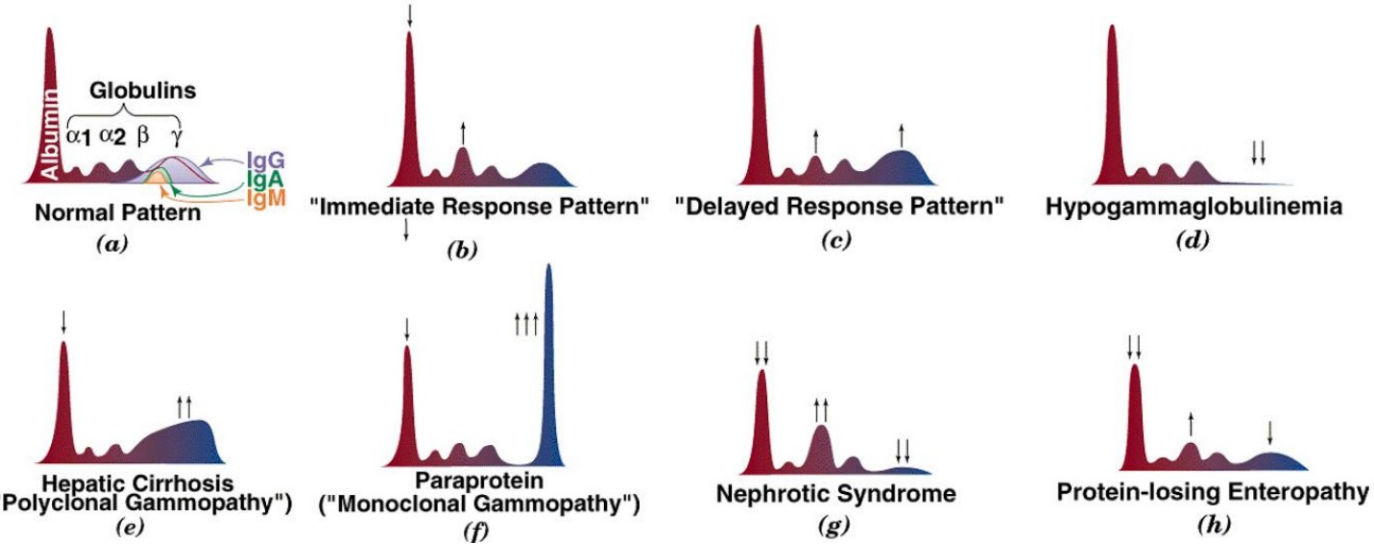
- **during the damage and breakdown of cells, intercellular enzymes are released into the ECF and these enzymes can be found in the plasma**
- we determine the activity [μkat/l], which is the measure of tissue damage – the more IC proteins in ECF, the higher the damage
- the problems lay in the specificity – the same enzyme is released from multiple tissues, but some are tissue-specific
  - **liver tests**
    - **ALT** – alanine-aminotransferase, from cytoplasm
    - **ASP** – aspartate-aminotransferase, from mitochondria
    - **GMT a ALP** - gama-glutamyltransferase and alkaline phosphatase, mainly from cells around the gallbladder
  - **Heart examination** - **CK** (creatine kinase) and **isoenzyme CK-MB** (myocardial specific creatine kinase)
    - **AST, LDH** (lactase dehydrogenase)- only historical significance
  - **Pancreatic examination** – **pancreatic amylase and lipase**
  - **muscles** - **CK** (creatine kinase)

Basic set

- determined in most hospitalized patients
- gives the physician an overview of the patient's metabolic status and organ function
- includes
  - Na, K, Cl, glycaemia
  - liver tests - ALT, AST, bilirubin, ALP, GMT
  - kidney function - urea, creatinine
  - blood count and sedimentation (FW)
  - urine chemically + sediment

2.26.6 Acute phase reactants

- **acute phase reaction is a nonspecific immune reaction of the organism against a pathogen**
- **the liver is stimulated in order to produce acute phase proteins (APPs)**
- Stimulants: pro-inflammatory cytokines (IL-1, IL-6 a TNF-α)
- APPs:
  - CRP, α1-antitrypsin
  - haptoglobin
  - coagulation factors
  - complement components (C3, C4)



A – Healthy state B – Acute inflammation – mainly an increased production of APP, which results in an increase of both alpha factions

- due to an increase in catabolism we may detect a decrease in albumin levels

C – chronic inflammation – we may observe an increase of gamma-fraction and a slight increase in alpha fraction (formation of APP) **D - hypogammaglobinaemia** – occurs due to deficiency of antibody production or excessive immunoglobulin loss **E - chronic hepatopathy** – typically occurs in severe lover fibrosis and cirrhosis

- synthetic function of the liver is reduced, which is reflected in the decrease of protein production in the liver
- gamma-fraction increases during the course of chronic inflammation
- there is also an increase in IgA – on the electrophoretic curve in the area between beta and gamma fractions (the so called β-γ bridge)

F - monoclonal hyperimunoglobulinaemie (monoclonal gammapathy)

- develop in tumour diseases, in which one type of Ig is cloned
- a narrow high band of monoclonal antibody can be found anywhere in the beta and gamma fractions

G - nephrotic type – during the destruction of filter glomerular membrane (nephrotic syndrome), renal protein loss occurs

- small proteins have the highest level of loss (albumin, gamma-globulins)
- proteins of a higher Mr, on the other hand, increase their levels (also due to the started proteosynthetic processes trying to replenish the losses)

H – malnutrition type – albumin and some other fractions decrease significantly in long-term starvation states, deprivation of food deprivations

2.27 REGULATION OF METABOLIC PATHWAYS AT CELL LEVEL

MUDr. Josef Fontana

2.27.1 General principles of regulation

- metabolic pathways are constantly monitored and adjusted
- the synthesis and degradation of metabolites meet the requirements of both cells and the organism
  - **it is never possible to satisfy everyone** - most cells or the most important organs are prioritized
- **cell-level regulatory mechanisms**
  - compartmentation of metabolic processes
  - change in the concentration of the active regulatory enzyme
    - change in absolute enzyme concentration
    - modulating the activity of an existing regulatory enzyme
      - Covalent modification of the enzyme molecule
      - interventions that alter enzyme kinetics

Compartmentation of metabolic processes

- eukaryotic cell is divided into compartments
- only certain metabolic pathways take place in each compartment
  - there is a different representation of enzymes in each compartment
  - compartment borders are often **impermeable** to substances
  - transport of excess **citrate** from mitochondria to the cytoplasm transfers AcCoA and also **inhibits glycolysis (PFK-1) and activates FA synthesis**
- **Specific transmitters in membranes ensure proper distribution of substrates and products**
  - allow the cell to affect several metabolic pathways at once - e.g. transport of Acetyl-CoA from mitochondria to the cytoplasm promotes the Krebs cycle (takes product) and FA synthesis (adds substrate)
- **compartmentation facilitates the regulation of contradictory events**
  - For example, FA synthesis occurs in the cytoplasm, whereas MT degradation occurs in mitochondria
- **subsequent processes take place close to each other** – e.g. Krebs cycle and respiratory chain - both pathways take place in the matrix of mitochondria

Change in concentration of active regulatory enzyme

Change in absolute enzyme concentration

- **it proceeds through the induction (activation) or repression (inhibition) of the expression of the gene which encodes the enzyme**
- mostly transcription factor (TF) is involved
  - the effect of TF is usually reversible and often dependent on ligand binding (or ligand-dependent TF)
  - **Ligands are signalling molecules or various metabolites**
- the effect lasts longer (hours, days) than the regulation of the activity of an existing enzyme (seconds, minutes)
- examples of this regulation
  - glucocorticoids induce the expression of genes encoding gluconeogenesis enzymes
  - enzymes of the monooxygenase system are induced by xenobiotics (foreign substances that have nothing to do in the body)
  - heme inhibits the synthesis of δ-aminolevulate synthase

Modulation of the activity of an existing enzyme

Covalent modification of enzyme molecule

- **formation of active enzymes from inactive precursors - irreversible activation**
  - **converting an inactive proenzyme into an active enzyme by partial proteolysis**
    - part of the inactive precursor is cut off -> what remains is the active enzyme
  - examples include digestive enzymes, caspases or coagulation factors
  - rapid increase in the concentration of active enzyme
  - via proteolytic breakdown we can inactivate an enzyme
- **enzyme interconversion** (between active and inactive form) - **reversible activation**
  - **Fast switching of the enzyme to active and inactive form (activation / inhibition) due to another enzyme**
  - mechanism of enzyme interconversion is reversible ATP-dependent phosphorylation and dephosphorylation of enzymes
    - Enzymes that provide interconversion are protein kinases (phosphorylate) and protein phosphatases (dephosphorylate)
    - phosphorylated amino acids: **Ser, Thr and Tyr**

- phosphorylation activates or inhibits, depending on the particular enzyme
- often response to hormonal signal

Interventions that affect enzyme kinetics

- **effect of substrate and product concentration and KM, pH, temperature, etc.**
  - the more substrate or less product, the faster the reaction will proceed (see lecture 2.3)
  - methods of removing the product from the reaction
    - reaction number 2 takes product of reaction number 1 (classic case of metabolic pathways))
    - use of the product of one metabolic pathway in another
      - e.g. NADH from β-oxidation to the respiratory chain
    - transport of product to another cell compartment – e.g. from cytoplasm to mitochondria (pyruvate)
- **effect of the presence of activity modulators (activators or inhibitors)**
  - The accumulation of an intermediate or end product of pathway metabolism leads to inhibition
    - **so-called feedback control**
    - e.g. acetylCoA inhibits its own synthesis
  - The intermediate (or product) of one metabolic pathway affects the rate of the other (often contradictory) metabolic pathway
    - **so-called cross regulation**
    - e.g. malonylCoA (an intermediate of FA synthesis) inhibits the degradation of FA
  - The intermediate affects one of the following enzymes in its metabolic pathway
    - **so-called step-forward regulation**
    - e.g. nucleotide synthesis

2.27.2 Regulation of individual metabolic pathways at the cell level

Respiratory chain regulation and aerobic phosphorylation

- availability of O_2
- ratio of cofactors NADH / NAD^+
- availability of ADP for ATP synthesis
- UCP (uncoupling proteins, viz. 2.7.2)

Regulation of Krebs cycle

- availability of substrates (acetyl-CoA and oxaloacetate) = **substrate control**
- collection of products (NADH, FADH) by subsequent reactions (respiratory chain) = **respiratory control**
- ratio ATP / ADP = **energetic control**
  - A high ratio (much ATP, little ADP) indicates enough energy in the cell and will slow the Krebs cycle
  - low ratio (low ATP, much ADP) indicates a lack of cell energy and will accelerate the Krebs cycle
- **regulatory enzymes - citrate synthase, isocitrate dehydrogenase** (the main regulatory enzyme), **2-ketoglutarate dehydrogenase**

Regulation of oxidative decarboxylation of pyruvate

- Interconversion **pyruvate dehydrogenase (PDH) complex** - phosphorylation (inactive) / dephosphorylation (active)
  - insulin activates dephosphorylation - the main regulatory mechanism
  - glucagon activates phosphorylation - wants to increase blood sugar concentration = must prevent processing of sugar in cells
- competitive inhibition by product (acetyl-CoA)
- inhibition by increased NADH / NAD^+ - at a high ratio, the cell has enough energy and may not produce additional

Regulation of glycolysis

- **hexokinase / glucokinase**
- **6-phosphofructo-1-kinase (PFK)** - the main regulatory enzyme
  - activation
    - increased AMP / ATP ratio
    - Presence of Fru-2,6-bisP (occurs when insulin / glucagon ratio increases)
    - insulin
  - inhibition
    - increased ATP / AMP ratio
    - presence of citrate in the cytosol (citrate in the cytosol means that it is so much in mitochondria that it is already climbing out))
    - acidic pH
- **pyruvate kinase**

Regulation of gluconeogenesis

- **pyruvate carboxylase**
  - activation- cortisol, glucagon, excess Acetyl-Co
  - inhibition- insulin
- **phosphoenolpyruvate carboxykinase**
- **Fru-1,6-bisphosphatase**- he main regulatory enzyme
  - the regulation of Fru-1,6-bisphosphatase is exactly the opposite of that of PFK
- **glucose-6-phosphatase**- activation by cortisol and glucagon, inhibition by insulin

Regulation of glycogen metabolism

- **degradation of glycogen**- **glycogen phosphorylase -** main regulatory enzyme
  - activation - glucagon, adrenaline (phosphorylation), increased AMP / ATP ratio, Ca^{2+} (in muscle)
  - inhibition- insulin
- **glycogen synthesis** - **glycogen synthase**
  - activation- insulin
  - inhibition- glucagon, adrenaline (phosphorylation)

Regulation of pentose cycle

- he regulatory enzyme is glucose-6-phosphate dehydrogenase (G6P DH), which requires $NADP^+$ for its activity
  - inhibition- increased NADPH / $NADP^+$ ratio
  - activation- insulin

Regulation of lipolysis

- **hormone sensitive lipase** an important regulatory enzyme in fat cells, it is activated in stressful situations
  - Activation - catecholamines (adrenaline, noradrenaline), glucagon (phosphorylation)
  - inhibition - insulin

Regulation of β-oxidation

- Initiation of β-oxidation requires the supply of FA (see regulation of lipolysis)
- the rate of β-oxidation is regulated more or less by the rate of transport of FA into mitochondria
  - **Carnitine-acyl transferase I (CAT I) -** inhibited by malonyl-CoA and increased insulin / glucagon ratio
- Acetyl-CoA, FADH2 and NADH must also be collected by subsequent reactions, otherwise β-oxidation will slow down

Regulation of ketogenesis

- regulation at the level of substrate availability - Acetyl-CoA
  - is closely related to lipolysis and beta-oxidation

Regulation of MK synthesis

- **Acetyl-CoA carboxylase** - the main regulatory enzyme, in ER
  - the first enzyme of the entire metabolic pathway - ensures the transport of long fatty acids into the mitochondria
    - **note: acetyl-CoA carboxylase is cytosolic and produces malonyl-CoA; it is malonyl-CoA that blocks the transport of FA into mitochondria (CAT I)**
  - inhibition - palmitoyl-CoA (generally acyl-CoA = when we have enough FA, we do not have to make new ones), glucagon (phosphorylation, repression) and AMP
  - activation - citrate, insulin, low-fat, high-energy, high-carbohydrate diet

Ornithine cycle regulation

- the ornithine cycle enzymes are activated by a protein-rich diet or by metabolic changes during fasting
- urea synthesis is a proton productive reaction = it is inhibited by acidosis
- regulatory enzyme - **carbamoyl phosphate synthetase I**
  - activation - N-acetylglutamate
    - N-acetylglutamate is formed by the enzyme **N-acetylglutamate synthetase**, which is activated by arginine

Actions of insulin and glucagon – slide summary ("Remember forever!")

- **insulin – anabolic**: increases the number of **GLUT-4**; glucose storage (mainly liver); activates **glycolysis, glycogen formation and the pentose cycle**; blocks gluconeogenesis and glycogen degradation → glycaemia ↓
- **insulin – lipoanabolic**: inhibits **lipolysis, β-oxidation and ketogenesis**; converts excess saccharides to FA and TAG; transports TAG to adipose tissue; **proteoanabolic**
- **glucagon** – antagonizes insulin (via **cAMP**); keeps glycaemia between meals: stimulates **liver glycogenolysis** and **gluconeogenesis** from lactate, AAs (protein catabolism) and glycerol (lipolysis)

2.28 ENERGY METABOLISM - THE OVERVIEW

MUDr. Josef Fontana

Important terms of energy metabolism

- metabolism is a set of catabolic and anabolic reactions
- **intermediates** are part of metabolic pathways
  - e.g. pyruvate can be converted to lactate (lacking in oxygen), or can provide the amino acid alanine, participate in glucose production or be converted to acetyl-CoA
- types of metabolic reactions
  - **catabolic** (Greek kata = Down) - fission, degradation, exergonic reactions - e.g. sugar-> CO_2 a H_20
  - **anabolic** (Greek ana = Up) - synthetic, endergonic reactions - e.g. glycogen synthesis, urea formation
  - **amphibolic** (Greek amphi = both sides) - sequence of reactions, some of which are catabolic and some anabolic

2.28.1 Basic reactions and metabolic intermediates

Main metabolic reactions

Catabolic reactions

- **glycolysis**- degradation of carbohydrates to pyruvate / lactate
- **glycogenolysis**- degradation of glycogen to glucose
- **lipolysis**- degradation of triglycerides to glycerol + fatty acids
- **beta-oxidation**- degradation of fatty acids to acetyl-CoA
- **ketone bodies breakdown** - in case of liver starvation they produce so-called ketone bodies which send other tissues for degradation
- **degradation of proteins and amino acids**

Anabolic reactions

- **gluconeogenesis**- glucose synthesis
- **glycogenesis**- glucose synthesis
- **fatty acid synthesis**
- **lipogenesis** synthesis of TAG from fatty acids and glycerol
- **ketogenesis**- production of ketone bodies in the liver
- **proteosynthesis**- protein synthesis
- **ornithine cycle**- urea formation

Amphibolic reactions

- **pyruvate dehydrogenase reaction**- converts pyruvate to Acetyl-CoA
- **Krebs cycle** - uses Acetyl-CoA to produce CO_2, NADH (NADH should be correctly written as NADH + H^+) and $FADH_2$
  - important- Krebs cycle has a second name - Citrate cycle
- **respiratory chain**- consumes NADH and $FADH_2$ to produce ATP and H_2O

Main metabolic intermediates

- metabolism is basically a flowchart - you know where a molecule will go and what it will be, but there are a few that stand at the crossroads - they can participate in more than one reaction, the most important of which are Pyruvate, Acetyl-CoA and NADH
- **pyruvate**
  - arises from glycolysis, degradation of some amino acids, transamination of alanine...
  - Acetyl-CoA (energy production), alanine (photosynthesis), lactate (anaerobic glycolysis), oxaloacetate (gluconeogenesis) or ethanol (alcoholic fermentation, thus producing beer and wine)
- **acetyl-CoA**
  - arises from sugars (glycolysis through pyruvate), fats (β-oxidation), ketone bodies and some amino acids
  - may enter the Krebs cycle (energy production) of fatty acid / ketone / cholesterol synthesis
- **NAD a NADH** (NAD should be written as NAD^+ and NADH as NADH + H^+)
  - t is not an intermediate in itself, just an electron carrier
  - NAD is an uncharged (oxidized) state, NADH is a charged (reduced) state
  - In some reactions, it is necessary to supply / remove electrons, in which case the appropriate electron transmitter is needed
    - e.g. in glycolysis we need to remove electrons - therefore we need free NAD and in case of lack of glycolysis it stops
  - there is also a phosphorylated version - NADP and NADPH- NADP a NADPH ($NADP^+$ a NADPH + H^+)

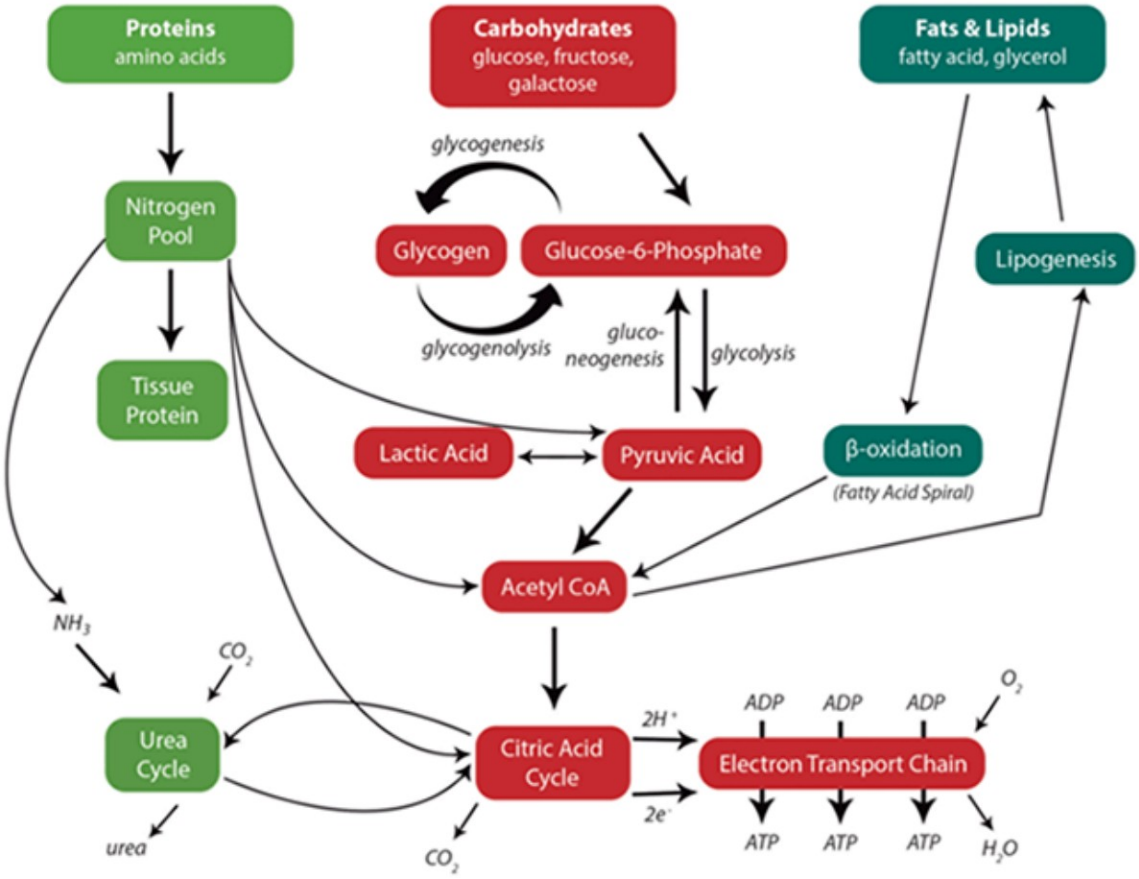
- the slides name the three **major intermediates**: **acetyl-CoA, pyruvate and NADH**

2.28.2 Overview of metabolism

- main nutrients - carbohydrates, fats and proteins - have a common metabolism (less enzymes are then needed)

Saccharides vs. lipids

- **respiratory quotient (RQ)** = produced CO_2 / consumed O_2
  - saccharides **RQ = 1.0** (glucose + 6 O_2 → 6 CO_2 + 6 H_2O)
  - TAG / fatty acids **RQ = 0.71** (palmitic acid + 23 O_2 → 16 CO_2 + 16 H_2O); short-chain FA have RQ higher than 0.71
- **energy content** (calorimetry): saccharides **4.1 kcal/g = 16.7 kJ/g**, lipids **9 kcal/g = 37.7 kJ/g**, proteins 4.1 kcal/g = 16.7 kJ/g (incompletely oxidized in the body)
  - lipids are more reduced – a high proportion of **saturated bonds** in TAG molecules



General course of nutrient processing

1. extracellularly, complex nutrients break down into smaller ones - proteins into amino acids, polysaccharides to monosaccharides…
2. The individual TAGs, amino acids and monosaccharides reach the cell
3. they are processed into components of the Krebs cycle (mostly Acetyl-CoA) in the cell
4. CO_2, small amount of energy and reduced cofactors NADH and $FADH_2$remain from Acetyl-CoA citrate cycle
5. Reduced cofactors continue into the respiratory chain, where oxidative phosphorylation produces ATP

Saccharide metabolism

- monosaccharides are absorbed by the intestinal wall into the bloodstream and transported to the liver
  - they are degraded or transformed into glucose in the liver
- glucose is leached back to the blood in a controlled way, excess glucose forms glycogen or storage fat in the liver
  - cells can take glucose from blood and use it for energy, fatty acid synthesis, ribose…
  - Once glucose enters the cell, it will never leave it (liver cells are the exception)
  - **Glucose is stored in the liver and muscle cells in the form of glycogen**

Catabolism of saccharides

- **glycolysis**- a process in which glucose in a cell is degraded to pyruvate to release energy in the form of ATP
  - in the cytoplasm, NAD is needed, 2 pyruvates, 2 ATP and 2 NADH are produced from one glucose
- **pentose phosphate cycle** - it does not serve as an energy source but provides pentoses (ribose) for nucleotide formation and NADPH + H^+

Saccharide Anabolism

- autotrophic organisms - have the ability to synthesize carbohydrates from inorganic substances during photosynthesis
- heterotrophic organisms - if necessary, they form glucose from simple organic substances (from pyruvate, glycerol...)
    - this process is called **gluconeogenesis**, occurs in the liver after glycogen exhaustion
    - gluconeogenesis is identical to glycolysis except for a few reactions, just running in the opposite direction

Lipid Metabolism

Lipid catabolism

- TAG is first broken down into glycerol and fatty acids
    - glycerol is phosphorylated to the glycolysis metabolite (glycerol -> glycerol-3-P -> dihydroxyacetone-P)
    - fatty acids undergo β-**oxidation**, resulting in a lot of Acetyl-CoA, NADH and $FADH_2$
        - β-oxidation occurs in mitochondria

Lipid anabolism

- the synthesis of TAG is based on Acetyl-CoA - it gradually builds up fatty acids of the required length, which are then connected one after the other to glycerol until the whole TAG is formed
    - this is done in the cytoplasm, and NADPH is needed

Protein Metabolism

Protein catabolism

- proteins in the intestine hydrolytically cleave enzymes up to amino acids
- when AA is used as an energy source, the amino group is first removed by deamination, the resulting carbon skeleton is metabolized to various products, which sooner or later travel to the Krebs cycle
    - Each AMK has its own path of degradation
- ammonia enters the ornithine cycle where it is converted into urea, which is then expelled from the body
    - the ornithine cycle is linked to the Krebs cycle via a couple of intermediates

Protein anabolism

- non-essential AAs can be synthesized in the liver
- each cell can produce proteins from the AMK it already has (which it receives from the blood)

Krebs cycle (citrate cycle)

- it is a sequence of reactions in which **Acetyl-CoA degrades to CO_2, ATP and reduced coenzymes NADH a $FADH_2$**
- occurs in the matrix of mitochondria
- always released during the Krebs cycle: í 2x CO_2, 1x ATP, 3x NADH and 1x $FADH_2$

Respiratory chain

- **hydrogen bound in NADH and $FADH_2$ is oxidized by oxygen to form water and ATP**
- occurs on the inner membrane of mitochondria
- he main suppliers of reduced cofactors are the Krebs cycle and β-oxidation of fatty acids
- hydrogen is not directly combined with oxygen, oxidation is carried out gradually through several stages (redox systems)
- the hydrogen atoms are split into protons and electrons, which are then transferred through several proteins until they finally join with oxygen at the end to form water

2.28.3 Specialization of individual organs in energy metabolism

Liver

- **they have an incredible number of functions** (in terms of metabolism) **and so it is so difficult to create an artificial substitute for them**
- play a vital role in maintaining homeostasis, the synthesis and conversion of nutrients, and the regulation of storage and release of energy

Liver and carbohydrate metabolism

- **glucostatic function**- the liver regulates blood glucose (blood glucose)
    - **high post-meal glucose = glucose uptake and glycogen production**
    - **Low fasting blood glucose = breakdown of glycogen and release of glucose into the blood**
- the supply of glycogen in the liver is about 80-100 g - enough for about 24 hours

- Gluconeogenesis begins when the glycogen stores are depleted - glucose synthesis from non-sugar substrates

Liver and protein and AA metabolism

- the deamination, transamination and subsequent conversion of the carbon skeleton of all AAs takes place in the liver
- ensures removal of ammonia from the body by urea synthesis in the ornithine cycle (ammonia is toxic)
- non-essential amino acids are synthesized and most plasma proteins are produced

Kidneys

- high energy consumption - concentration of urine and transport of substances is not easy
- during prolonged fasting, gluconeogenesis may produce glucose
- have enzymes of amino acid metabolism

Muscles

- muscle contraction requires a lot of ATPP
- ATP sources
    - creatine phosphate - phosphorylates ADP to ATP, waste product is creatinine
    - anaerobic glycolysis
    - aerobic glycolysis + oxidative phosphorylation
    - muscle glycogen - 300-1000g of glycogen is standard in the muscles)
    - nutrients from circulation - glucose and fatty acids

Muscles and metabolism of amino acids

- BCAA (Branched-Chain Amino Acids; Leucine, Isoleucine and Valine) - can be transaminated and used directly in muscles, so bodybuilders are so mad about them
    - carbon skeletons are used in energy metabolism (Krebs cycle)
    - Ammonia is incorporated into Ala, Glu and Gln and released into the blood - the liver catches, deaminates and forms urea
- Alanine cycle (see below)

Adipose tissue

- Storage of TAG (postprandial - after meal)
- lipoprotein lipase (endothelial) - catalyses the degradation of TAG
- de novo lipogenesis (= from the beginning) - from glucose
- lipolysis - release of fatty acids (FA = Fatty Acids) and glycerol (during fasting)
- hormone sensitive lipase - enzyme catalysing the breakdown of TAG, activated by glucagon

Brain

- egoist- 2% body weight, 20% energy, 25% total oxygen
- glucose is the main source of energy for the brain - it has a daily consumption of 120g
    - neuroglycopenia - a state of sugar deficiency in the brain, leading to unconsciousness, brain damage and death
- in the adapted starvation (3 weeks) also the oxidation of ketone bodies, which cover up to 50% of energy needs

Inter organ metabolic pathways

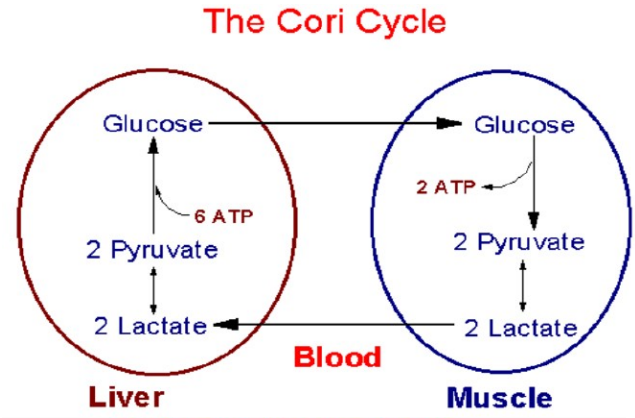
- there are many inter-organ connections within energy metabolism
- two examples - the Cori cycle and the Glucose-alanine cycle

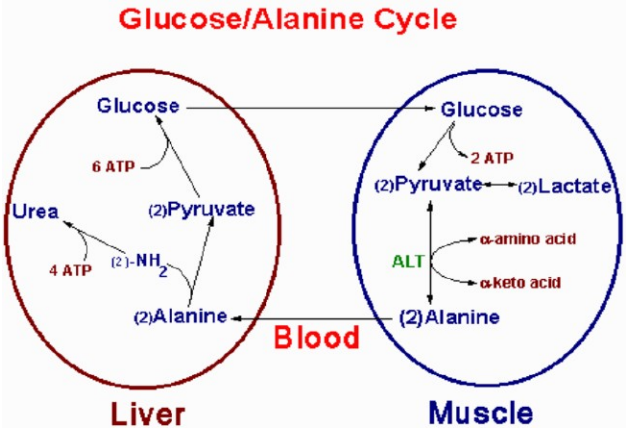
Cori cycle

- **the way in which erythrocytes and muscles are dehydrated**
- Lactate is sent to the liver, processed into glucose and sent back

Alanine cycle

- **the way in which muscles are supplied with energy while also extracting toxic ammonia**
- ammonia is attached to pyruvate, alanine is produced, which travels to the liver, where ammonia is disconnected and processed into urea
- pyruvate is converted to glucose by gluconeogenesis and sent back to the blood





2.28.4 Overview of hormonal regulation of energy metabolism

- the effect of insulin and counter-regulatory hormones on energy metabolism
- on the one hand - insulin
- on the other hand - counter-hormones glucagon, cortisol, adrenaline, growth hormone

Insulin action

- **All this leads to a reduction in blood glucose**
- increases glucose storage (mainly in the liver)
- activates glycolysis and blocks gluconeogenesis
- activates glycogen production
- activates the conversion of carbohydrates into fatty acids
- promotes the transport of TAG into adipose tissue
- blocks lipolysis and ketogenesis
- promotes protein production and tissue growth - insulin is therefore technically an anabolic hormone

Action of glucagon

- antagonize the effect of insulin (through cAMP, more in signals)
- **all leads to increased blood glucose** - the point is to maintain blood glucose between meals and during increased glucose consumption
    - Decomposition of hepatic glycogen and glucose excretion
    - stimulation of gluconeogenesis
    - ketogenesis - an alternative fuel for cells

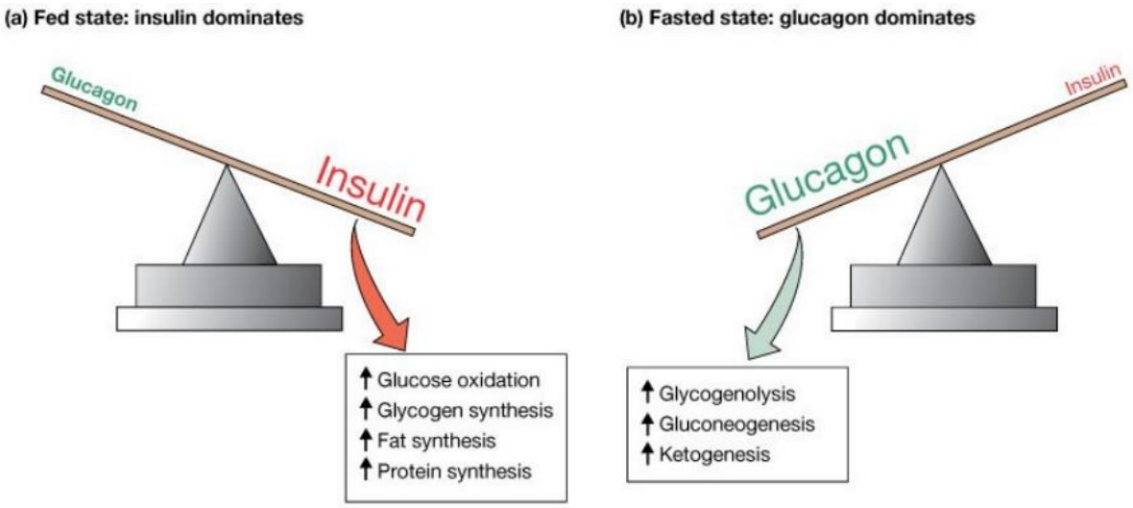
Basic knowledge

Insulin rises after a meal

food gets into the intestines -> glucose is absorbed -> blood glucose rises -> we need to get it down -> pancreas expels insulin into the blood

Glucagon rises while starving

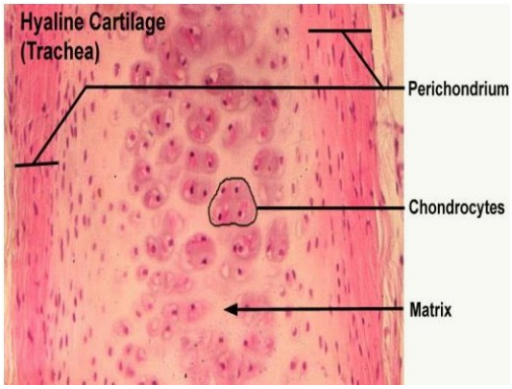
man has not eaten for a long time -> glycemia is low -> need to get it up -> pancreas eliminates glucagon into blood


(a) Fed state: insulin dominates
Glucagon
Insulin
↑ Glucose oxidation
↑ Glycogen synthesis
↑ Fat synthesis
↑ Protein synthesis
(b) Fasted state: glucagon dominates
Insulin
Glucagon
↑ Glycogenolysis
↑ Gluconeogenesis
↑ Ketogenesis

2.29 CARTILAGE, BONE AND OSSIFICATION

MUDr. Eva Maňáková, Ph.D.

2.29.1 Cartilage



- **cells**
  - chondroblast – young cell, differentiates from mesenchymal cell, produce the extracellular matrix
  - chondroclast – degrade the cartilage (macrophages)
- **fibres**
  - collagen type II - hyaline and elastic cartilage
  - collagen type I - fibrocartilage
- **inorganic part of ECM** – from glycoproteins (chondronectin, hyaluronic acid, chondroitin sulphate) and proteoglycans
- **it is an avascular tissue nourished by the diffusion from the perichondria**
- **bad regeneration** – extensive damage heals through collagen scars
- basic degenerative process of cartilage is calcification, but sometimes it may be physiological (e.g. during ossification)
- **high compression elasticity, as 80% is water**
- fresh hyaline cartilage is **bluish-white and translucent**; mature cartilage is **avascular** – nutrition from vessels of the perichondrium
- chondrocytes: round cells with processes, **anaerobic metabolism**
- the arrangement of collagen fibres can be visualized under a polarized microscope

Chondrocytes

- round cells with a light nucleus and a prominent nucleolus
- **chondrocytes** are responsible for the synthesis of everything in the extracellular matrix
  - has a proteosynthetic apparatus - GER, GA, vesicles
- **anaerobic metabolism** – storage of energy through glycogen stores and lipid droplets
- **lacunae** – in histology a lacuna is a small cavity in which chondrocytes (in cartilage) or osteocytes (in bone) sit.
  - chondrocytes fill up the whole lacunae – on a slide, there if often free space, but that is only an artefact

Pericellular matrix

- chondrocytes in cartilage are surrounded by a thin layer called **pericellular matrix**, which chemically and mechanically differs from the extracellular matrix (for example contains a soft network of collagen type VI)
- divided into
  - **territorial matrix** – thin layer, basophilic, a lot of proteoglycans, neighbours with chondrocytes
  - **interterritorial matrix** – further from chondrocytes, lighter, more extracellular matrix

Extracellular matrix

- formed by collagen, proteoglycans, glycoproteins and water (binds to proteoglycans, forms up to 80% of total volume)
  - **proteoglycans** - chondroitin-4-sulphate, chondroitin-6-supltate a keratan sulphate, which along with hyaluronic acid forms proteoglycan aggregates (e.g. aggrecan)
  - **glycoproteins** - chondronectin -> responsible for the adhesion of chondrocytes to collagen type II

Isogenous groups

- **group of cells which do not separate post division and stay in groups**
- formed via interstitial growth (proliferation of chondrocytes) – chondrocytes are located in lacunae, if they divide, then both chondrocytes will remain in the same lacunae – this is what we call Isogenous groups

Perichondrium

- **dense connective tissue around cartilage – ensure growth and nourishment**
- formed from 2 layers
  - **stratum fibrosum** – a lot of fibroblasts -> dense collagen tissue = captures tensile strength
  - **stratum chondrogenicum** – inner layer right in undifferentiated mesenchymal cells
  - (slides) outer layer – dense connective tissue with blood supply and nerves; inner layer – proliferating chondroblasts → **growth to the sides (appositional)**
- does not cover articular cartilage – surface of cartilage is covered by an extracellular matrix
- contains collagen type I

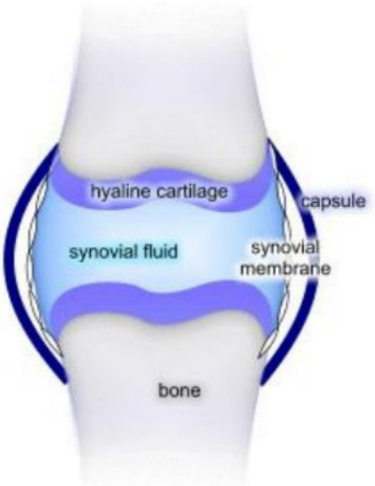
Cartilage growth

- **interstitial growth – division of chondrocytes**
  - used during development (epiphyseal discs) or in articular cartilage

- **appositional growth** – **mesenchymal cells from perichondrium differentiate into chondroblasts**
  - this type of growth is more significant

Articular cartilage

- **its surface is not covered by perichondrium**
- functions as a shock absorber on the bone
- **collagen fibres are arranged in an arcade style** (= are layered)
  - **tangential zone** – heavily intertwined collagen fibres are organized parallel to the surface of the cartilage which gives it smoothness and strength
  - **transitional zone**
  - **radial zone** – collagen fibres are orientated radially and cross the calcified zone to the bone, to which they are firmly connected
  - **zone of mineralized cartilage** – firmly sits on the bone and protects it



- most articular surfaces are hyaline cartilage, **except the mandibular (temporomandibular) joint and the clavicular joints** (acromio- and sternoclavicular) – fibrocartilage
- in the space between the cartilages of bone is bone joint cavity which is filled with viscous fluid so called synovial fluid

Types of cartilage

- by the composition of extracellular matrix, we can differentiate between three types of cartilages
- **hyaline cartilage**
  - attachment of ribs, nasal cartilage, trachea, bronchi and majority of cartilages in larynx, skeleton before officiation, joints
  - oval chondrocytes in groups, strongly basophilic matrix
  - light microscope cannot visualize collagen fibres
  - **the most frequent type** in humans; in the embryo it is the **temporary skeleton**; forms the **epiphyseal plate** (bone growth)
- **elastic cartilage**
  - elastic fibres + collagen II, epiglottis, external auditory canal, auditory (Eustachian) tube
  - matrix has the same composition as hyaline cartilage but has a network of elastic fibres which extend to the perichondrium and increase elasticity and flexibility during applied pressure and movement
  - elastic matrix is **yellow**; also in the **auricle** and small cartilages of the larynx; isogenous groups are smaller / less common than in hyaline cartilage
- **fibrocartilage**
  - collagen I + extracellular matrix, chondrocytes sit individually
  - intervertebral disks, joints, symphysis
  - without perichondrium
  - tissue intermediate between dense collagen connective tissue and hyaline cartilage; chondrocytes in **long columns** (according to mechanical force); isogenous groups rare
  - also in the **menisci** and the anulus fibrosus
  - clinical relevance: **torn menisci** after knee trauma heal poorly

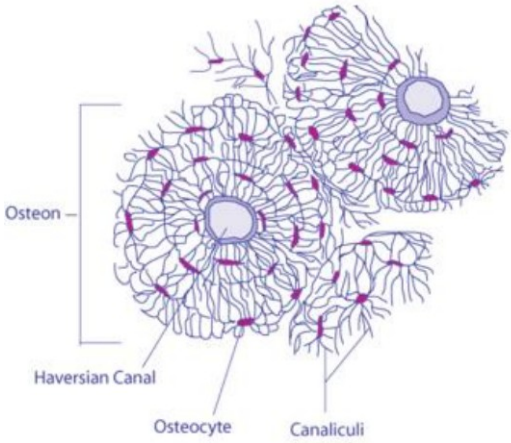
2.29.2 Bone

- strong support of our body, contains high amounts of inorganic components
- cells - osteoblasts (synthesize ECM), osteocytes (inactive osteoblasts), osteoclasts (break down ECM)
- functions (slides): support, body's **reserve of calcium, phosphates and ions**, **haematopoiesis**
- inorganic part: crystals of calcium hydroxyapatite, **amorphous calcium phosphate** and other elements

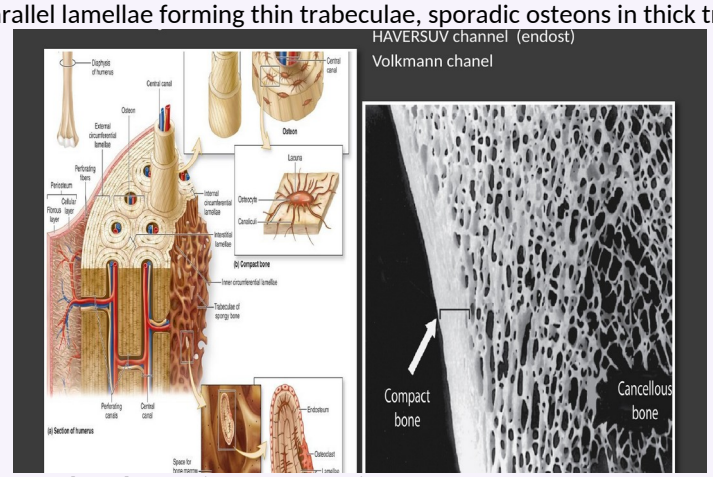
Extracellular matrix

- **main component - collagen type I, crystals of hydroxyapatite, glycoproteins** (sialoprotein, osteocalcin) **and proteoglycans** (chondroitin sulphate a keratan sulphate)
- **important concept - bone lamellae – plate of mineralized collagen fibres organized in the same direction**
- distinguishes fibrous and lamellar bone as they are made from the same material, their difference lays in the orientation of the fibres
  - **fibrous/woven bone** – intertwined collagens fibres in bundles
    - fibres randomly arranged

        - where – primary bone (at the beginning of development), during the healing of fractures, sites of bone and tendon connections
        - gradually turns into lamellar bone
    - slides: woven bone has **less inorganic component**, more osteoblasts/osteocytes; persists in **skull sutures, tooth sockets, tendon insertions (tuberositates)**
    - it is the first bone formed in embryonic development and in **bone repair**
- **lamellar bone** – collagen fibres and arranged into lamellae
    - where – secondary bone
    - in-between lamellae are osteocytes
    - macroscopically two different forms
        - **spongiosa** – middle of bone, spatial network from thin plates and trabecular, in between trabeculae is bone marrow
        - **compacta** – structure from osteons (also known as Haversian systems, concentric lamellae around a central canal) on the periphery are osteoblasts



- compact bone – **diaphyses** of long bones; spongy bone – **epiphyses** and flat bones (**diploe**)
- compact bone: osteons + **interstitial lamellae** (incomplete, remnants of remodelling) + **outer and inner circumferential lamellae**
- spongy bone: parallel lamellae forming thin trabeculae, sporadic osteons in thick trabeculae

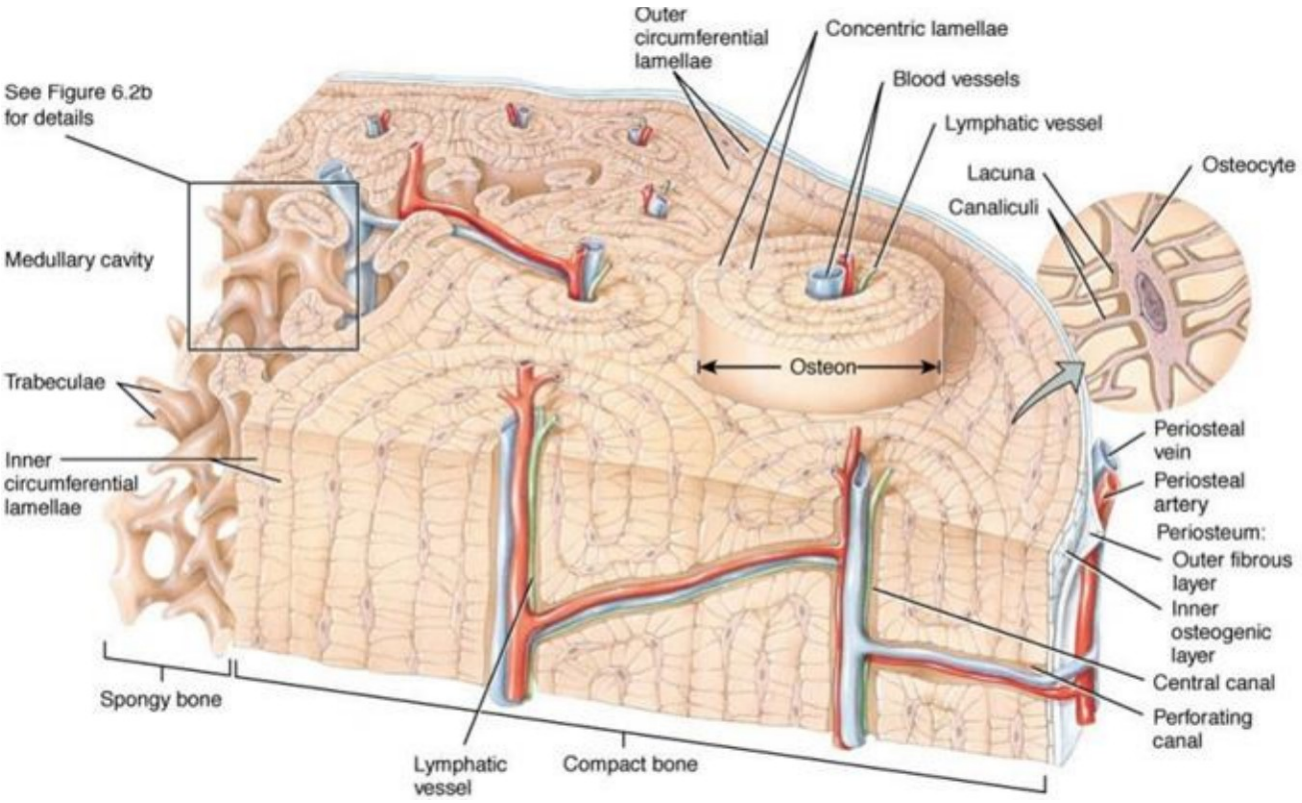


[slides] Osteon (Haversian system), Haversian and Volkmann canals

- **hydroxyapatite** - forms 45% of the mass of bone tissue, longitudinal hexagonal crystal, orientation is determined by collagen fibres
- tensile strength (collagen) and flexibility (hydroxyapatite)

Composition of lamellar bone

- **spongiosa** or trabecular bone tissue
    - **found in the cavity of long bones and kits structure resembles a dense network through which blood vessels and nerves pass**
    - anatomical and functional unit is trabeculae which are aligned towards the mechanical load, this allows the trabeculae to with stand pressure – this specific organisation of bone/architecture of bone, is different in everyone and has the ability to change throughout our lives depending on our current mechanical stresses/loads.
    - spongiosa is significantly responsible for the process of remodelling and healing process of bone tissue
- **compact bone**
    - this hard tissue is found under the periost and is formed mainly by concentric lamellae and central canal of cylindrical appearance called osteons (or Haversian systems the same thing)
    - individual centres of osteons contain **Haversian channels** with blood and lymphatic vessels and nerves. Their function is to ensure proper nutrition, growth and regeneration of bone tissue
    - **Volkmanns channels** – form vertical connections between neighbouring Haversian channels, and provide communication between osteons
    - Volkmann's canals carry the **a. nutricia**



Cells of bone

- **osteoblasts** – form bone matrix, produce collagen I and different proteoglycans and glycoproteins
- **osteocytes** – take up the population majority, do not form the ECM but take part in the metabolism (release minerals from bone)
- **osteoclast** – breakdown bone matrix

Osteoblasts

- produce **osteoid**, which is unmineralized bone tissue which is progressively mineralised (collagen type I)
- **originates from mesenchyme, they are progenitor cells of osteocytes**
  - influence of growth factors - BMP2 (bone morphogenetic protein 2)
- **active osteoblast is a cubical cell rich in RER**
- throughout its life, it accumulates bone matrix around itself and may remain in it (in this case it will turn into an osteocyte)
- on the membrane of osteoblasts is alkali phosphatase, which is important for the mineralization of osteoid
- osteoblasts are **polarized, epithelium-like** cells on the bone surface (also in Haversian canals)
- form from osteoprogenitor cells under the influence of signalling molecules of BMP

Osteocytes

- **closed in mineralised bone matrix, located in lacunae**
- **in contact with other osteocytes through long thin projections, which run through channels of bone matrix - canaliculi**
- osteocytes communicate through gap junctions
  - the processes connected by gap junctions serve **communication and nutrition**

Osteoclasts

- **large, multinucleated free cells, which attach to mineralized bone matrix**
- formed by the fusion of mononuclear precursor cells (monocytes)
- belong to a group of macrophages, their main function is to breakdown bone tissue
- **osteoclast release proteolytic enzymes from cells into matrix and digest the organic components of bone**
  - e.g. collagenase, acidic phosphatase, cathepsin K
  - some matrix fragments are endocytosed
- deepen pits (so called Howship's lacunae) and after reaching a certain depth they widen them, osteoblasts follow them which refill the lacunae with ECM
- have proton pumps, which lower the extracellular pH, which is needed during the destruction of ECM
- on their surface facing bone is a groovy plasmalema which looks like a brush border
- activity of osteoclasts is regulated by various cytokines and hormones - **parathormone** (parathyroid gland, increases the level of calcium in blood) and calcitonin (thyroid gland decreases the level of calcium in blood)
- resorption via the **ruffled border** and lysosomes (**acid phosphatase**); osteoclasts help regulate the **blood Ca^{2+} level**

Periost and endosteum

Endosteum

- layer of mesenchymal cells (precursors of osteoblasts and osteoclasts) and mineralized collagen fibres
- covers all inner surfaces of bones

Periost

- **cover outer surfaces of bone**
- two layers
  - **stratum fibrosum** – outer dense collagen connective tissue
    - periost is attached to the bone through collagen fibres from stratum fibrosum -Sharpey's fibres
  - **stratum osteogenicum** – tightly attached to bone, richly vascularized and innervated, pain sensitive, same cells as in endosteum
  - periosteum and endosteum nourish the bone and are a **"pool" of osteoprogenitor cells**; endosteum = one layer of osteoprogenitor cells (lines the Haversian canals too)

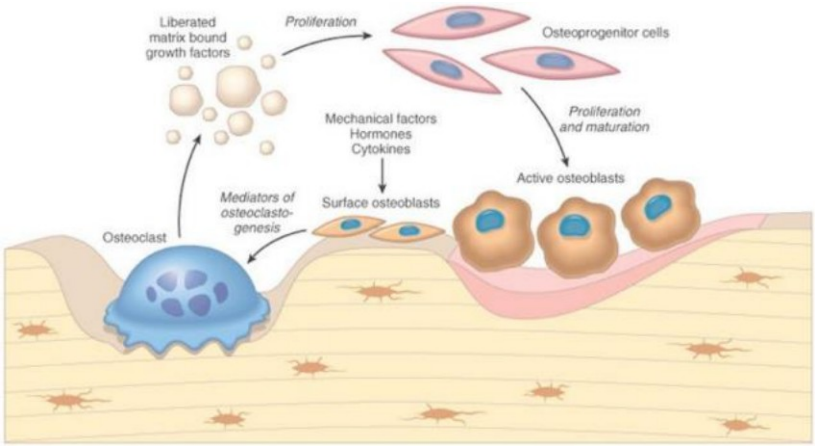
Remodelling bone

- **osteoclasts and osteoblasts are grouped together as they work together to breakdown and renew bone**
  - **osteoclast break down a piece of bone** (osteocytes present in this piece die through apoptosis)
    - formation of pits called **Howship's lacunae,** osteoclasts sit in these lacunae
  - **osteoblasts in this pit start to synthesize osteoid – unmineralized bone tissue where calcium and phosphor attach and crystallise, which finished the process of bone remodelling**
  - some osteoblasts stay in the newly synthesized osteoid which mineralizes around them and closes them in
    - these osteoblasts become osteocytes

- **regulation of remodelling**
  - **cytokine M-CSF** – encourages the proliferation of precursors of osteoclasts, produced by osteoblasts
  - precursors of osteoclasts (macrophages) have a receptor RANK
    - **RANKL** (RANK Ligand) – membrane protein of osteoblasts, binds to RANK and induces the fusion and activation of precursors of osteoclasts (macrophages)
    - **OPG** (osteoprotegerin) - competitive inhibitor of RANKL, its production is stimulated by oestrogen
  - Activated osteoclasts act on osteoblasts through negative feedback
  - **hormones (parathormone, calcitonin) functions through RANKL/OPG**

Clinical correlations

- **osteopetrosis** – genetic diffuse bone disease; **sclerotic thickening** of the skeleton because osteoclasts **cannot create an acidic environment** in the bone matrix
- **osteoporosis** – loss of **bone mineral density** → decreased strength and higher fracture risk (resorption exceeds formation)



2.29.3 Ossification

- there are two type of ossification
- in both, primary (woven) bone is formed first and then rebuilt into definitive **lamellar bone** and the bone marrow cavity
- intramembranous ossification is also responsible for the **appositional (width) growth of long bones**

Intramembranous ossification

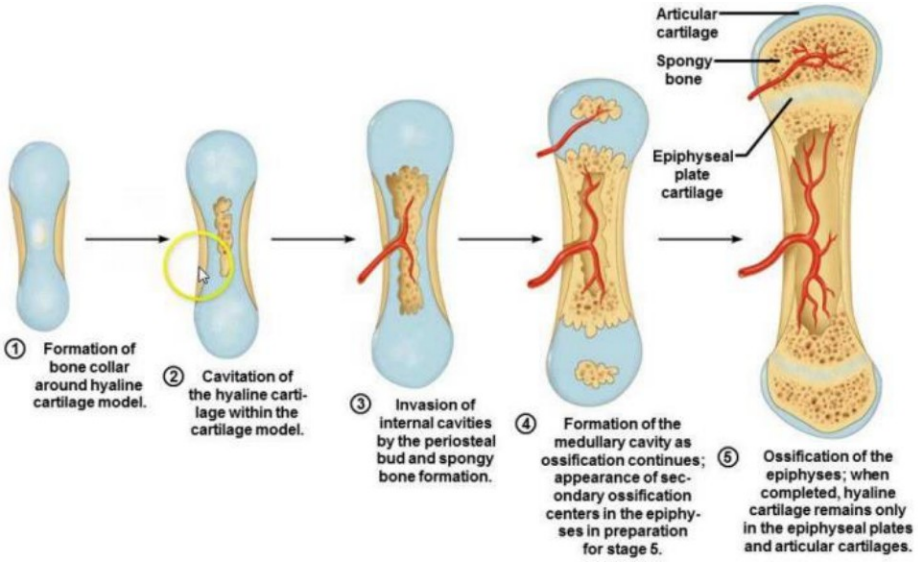
- ossification of mainly flat bones – skull, clavicle, lower jaw
- **it is the ossification of mesenchymal cells straight into bone**
- starts during embryonic period (around week 8 of pregnancy) on the base of the connective tissue
- primary ossification centre
  - selected centrally located mesenchymal cells cluster and differentiate into osteoblasts
  - mesenchyme condenses on the external face of the woven bone and from the periosteum
- synthesized collagen (type I) surrounds osteoblasts and gradually causes the differentiation of osteoblasts to osteocytes and the calcification of osteoid
- first primary spongiosa forms and then through further growth turns into compacta
- this is how we describe oppositional growth -> growth to the sides/width

Endochondral ossification

- mainly used in long bones - femur, humerus…
- **it is the ossification of mesenchymal cells into hyaline cartilage and then the replacement of cartilage to bone**
  - **cartilage is broken down and replaced by bone, it is not the conversion of cartilage but its destruction and the replacement, part of it remain only in the region between epiphysis and diaphysis in so called cartilage growth plate/epiphyseal plate**
- at the end of the embryonic period, primary ossification centres appear at the diaphysis of long bones
- after birth, secondary ossification centres in epiphysis appear
- ossification occurs from the centre towards the all sides
- starts by the formation of a **bone collar** – at the mid-section of long cartilage osteoblasts in perichondrium differentiate, which forms a short collar bone around the diaphysis, this collar growth proximally and distally until the whole perichondrium is replaced and forms a periost – in picture this is highlighted by an arrow and yellow circle
- **principle: replacement (not remodelling!) of the hyaline cartilage model by bone**
  - the bone collar (formed by **intramembranous** ossification under the periosteum) disturbs nutrition of chondrocytes → cartilage degenerates and cells release signals inducing vascular growth
  - **osteogenic buds** (capillaries + osteoprogenitor cells) penetrate the bone collar → **primary ossification centre**; osteoclasts resorb the centre → **bone marrow cavity**
  - later **secondary ossification centres** in the epiphyses; cartilage remains only as **articular cartilage** and as the **epiphyseal (growth) plate**
- **endochondral ossification has 6 zones, through which the bone gradually expands**
  - **reserve zone** – undifferentiated precursors of chondrocytes
  - **zone of proliferation** – active chondrocytes divide and form the ECM around themselves, this causes the formation of isogenous groups
  - **zone of hypertrophy** – chondrocytes grow
  - **zone of calcified cartilage** – extracellular matrix forms pits, which become filled with vessels entering into the cartilage
  - **zone of destruction** – chondroclasts and macrophages breakdown cartilage (chondrocytes carry out apoptosis)
  - **zone of ossification** – migration osteoprogenitor cells and formation of osteoid

Joints – diarthrosis

- allows free movement; articular surfaces covered by cartilage; cavity filled with **synovial fluid**
- capsule: **outer fibrous layer** (dense collagen tissue, continuous with tendons and ligaments) and **inner synovial membrane** with two cell types
  - **A-cells (type A synoviocytes)** – macrophage-like
  - **B-cells (type B synoviocytes)** – fibroblast-like, **produce synovial fluid**


Articular cartilage
Spongy bone
Epiphyseal plate cartilage
① Formation of bone collar around hyaline cartilage model.
② Cavitation of the hyaline cartilage within the cartilage model.
③ Invasion of internal cavities by the periosteal bud and spongy bone formation.
④ Formation of the medullary cavity as ossification continues; appearance of secondary ossification centers in the epiphyses in preparation for stage 5.
⑤ Ossification of the epiphyses; when completed, hyaline cartilage remains only in the epiphyseal plates and articular cartilages.

2.30 ENZYMOPATHY – HERITABLE DISORDERS OF METABOLISM

RNDr. Hana Zoubková, Ph.D.

- Enzymopathies are hereditary diseases caused by the malfunction or absence of one or more enzymes
- **The nature of metabolic diseases**- due to mutation of nuclear or mitochondrial DNA, a dysfunctional protein is produced that cannot convert the substrate into a product, leading to multi-organ damage
- **Nuclear vs Mitochondrial Enzymopathies**
  - **Nuclear**- may be mutated gene from mother or father, Mendel's laws apply here and the whole body is affected
  - **Mitochondrial**- mutation always comes from the mother; Mendel's laws do not apply and the expression of the disorder is variable

General properties of enzymopathies

- Most enzymopathies are autosomal **recessive (**heterozygotes have no symptoms)
  - heterozygotes have about **50 % residual activity** and are healthy; some are **X-linked recessive**
  - **most patients are compound heterozygotes**
- diffusible vs. **macromolecular substrates** – macromolecular substrates stay in the tissue where they accumulate
- the consequences cause acute or slow "poisoning", even sepsis-like states
- The pathological consequences of enzymopathies are due to:
  - **Substrate accumulation -** accumulation of molecules that are not properly degraded and eliminated
  - **Deficiency of the product** - absence of the product in the organism causes pathological behaviour§ of the organism
  - **Alternative degradation of the substrate** - often substances that are toxic to the body (cause poisoning)
  - **Combination of the three**
- Enzymopathies include diseases where the patient is deficient in cofactor or activating / modifying / stabilizing protein - although the enzymes themselves may be perfectly fine, they do not work
- Common for **locus heterogeneity to be present**- mutation of different genes can lead to the same phenotypic manifestation
  - For example, if one metabolic pathway uses 3 enzymes in series, it does not matter which gene mutates the enzyme, because the entire pathway will be non-functional anyway
- **Variable expression** - different diseases that result from a partial or complete enzyme defect
- a patient can lose **more than one enzyme** when they share a cofactor (e.g. **BH4**), a common activator/modifying/stabilizing protein (**GM2 gangliosidoses**), or when a whole group of enzymes or an entire organelle is missing
- **allelic heterogeneity** – identical or similar phenotypes caused by different mutated alleles
- variable expressivity: **classical, intermediate and intermittent** forms
- Does not concern several catalytic ribonucleic acids

Classification

- Approximately 1000 IDMs (inherited metabolic disorders) are currently recognized, of which approximately 100 are treatable or amenable to diet
  - e.g low-protein, lactose-free or gluten-free diet
  - 100 children with IMD are born in the Czech Republic each year
- **IMD amino acids** - hyperphenylalaninemia, tyrosinemia, alkaptonuria, homocystinuria, leucinosis, organic aciduria
- **IMD saccharides** - galactosemia, fructose intolerance, glycogenosis
- **IMD organic acids** - methylmalonic aciduria
- **IMD purines and porphyrins** - adenosine deaminase deficiency (SCID), porphyria
- **IMD steroids** congenital adrenal hyperplasia
- **IMD lipids** - LCHAD, VLCAD, MCAD deficiency
- **IMD vitamins and cofactors** - MTHR deficiency, biotinidase deficiency
- **IMD lysosomes**
  - sphingolipidosis- Tay-Sachs disease, Gaucher disease, Niemann-Pick disease
  - mucopolysaccharidosis - Hurle Syndrome
- **IMD mitochondria**
- slide numbers of known IMDs by group: amino acids **(60)**, saccharides **(100)**, lipids **(21)**, purine/pyrimidine + heme **(35 + 8)**, mitochondria **(260)**, peroxisomes **(18)**, lysosomes **(60)**
- other examples from the slides: **cystinuria** (AA), **ornithine transcarbamylase deficiency** (urea cycle), **lipoprotein lipase deficiency, Smith-Lemli-Opitz syndrome**, LCHAD (lipids), **Lesch-Nyhan syndrome** (purines), **MERRF, NARP** (mitochondria), **Zellweger syndrome, X-adrenoleukodystrophy** (peroxisomes), **Krabbe disease, metachromatic leukodystrophy, Fabry disease**, acid lipase deficiency, α-mannosidosis, MPS II–VII (lysosomes)

2.30.1 IMD Amino acids

Hyperphenylalaninemia

- A group of enzymopathies caused by mutations in the **phenylalanine hydroxylase** gene or a genetic defect in some metabolic step of its **tetrahydrobiopterin cofactor**
- **Three severity levels** - **phenylketonuria (PKU)** (worst form), **variant PKU** (intermediate form) and **non PKU Hyperphenylalaninemia** (least severe form of hyperphenylalaninemia)
- There is no treatment, only diet can prevent symptoms (therefore every new-born in the Czech Republic is tested for these diseases)

- **locus heterogeneity**: 5 mutated genes can cause hyperphenylalaninaemia; diet is effective only if started **immediately after birth**

Phenylketonuria (PKU)

- **Is due to the inability to metabolize phenylalanine to tyrosine**
  - phenylalanine is instead metabolised by a minor metabolic pathway to the toxic phenylpyruvic acid, which irreversibly causes mental retardation and light pigmentation
- There are about 400 alleles which by mutation can cause phenylketonuria
- Phenylketonuria is part of neonatal screening. - Breast milk also contains phenylalanine
- people with phenylketonuria should not eat essentially any protein-rich food

- slides: symptoms – mental retardation, impaired speech and walking, epilepsy, microcephaly, parkinsonism, neurotransmitter deficiency, light pigmentation, **"mouse" odour**
- **allelic heterogeneity** ~500 alleles; gene on **chromosome 16q**(slide); incidence 1:5 000–1:15 000, **1:6 500 in CZ**
- high-Phe foods to avoid: meat, milk, cereals, normal bread, confectionery, nuts, most fruit and vegetables, and the sweetener **aspartame**

Albinism

- **Lack of skin and hair pigment due to missing / non-functional tyrosinase**
- A typical albino has red eyes, white hair and white skin
- types of albinism
  - Type I(A) - tyrosinase is missing in the body
  - Type I(B) - tyrosinase is present but is inactive
  - Type II (most common) - tyrosinase is present but not as effective
- Symptoms are most often **sensitivity to sunshine** (frequent skin cancers) and **sensitivity of eyes** (visual disturbances))

- forms: ocular, oculocutaneous, partial; **photophobia, nystagmus, astigmatism**; incidence 1:120 000 in CZ, 1:4 000 in South Africa
- albinism = **lack of product** (pigment)
- Albinism exists throughout the animal kingdom (and partly also among plants)

Alkaptonuria (AKU)

- due to the lack of the enzyme homogentisate dioxygenase (HGD), homogentisic acid accumulates in the organism, which is then converted to the brown-black pigment alkapton by an alternative route (oxidation) and is stored in the tissues
  - **Ochronosis**- brown coloration of tissues (mainly eyes and cartilage) in alkaptonuria
- **treatment**- limited supply of Phe and Tyr

- enzyme = homogentisate **1,2-dioxygenase**; alkapton is excreted in urine; incidence **1 : 250 000**

Tyrosinemia type I

- Tyrosine metabolism disorder - **fumarylacetoacetate hydrolase (FAH) activity disorder**
  - tyrosine accumulates, converts sideways to toxic **succinyl-acetone and damages the liver and kidneys**
- type I is most widespread (type II and III are rare)
- treatment with diet and Nitison (a medicine that slows down the alternative pathway of tyrosine metabolism)

- toxic derivatives in urine: **succinylacetone, δ-aminolevulinic acid**; symptoms: liver failure, enlarged kidneys, failure to thrive, peripheral neuropathy; odour of **cabbage / rancid butter**; incidence 1:100 000–120 000
- treatment of tyrosinaemia and alkaptonuria: diet, **nitisinone**, liver transplantation

Homocystinuria (classic type)

- Methionine metabolism disorder (commonly metabolised to homocysteine and then to cysteine)
- is due to cystationin β-synthase (CBS) deficiency, which **causes increased urinary homocysteine and methionine levels**
  - The cause of damage is the accumulation of substrate (methionine) in the body
- 4 organ systems are affected - eye, skeleton (bones), vascular endothelium (vascular lining) and CNS

- **marfanoid habitus**, **lens ectopy**, glaucoma, **thromboembolic events**, mental retardation, excessive height and limb length
- **~50 % of patients are vitamin B6-responsive** (pyridoxal-5-phosphate is the cofactor); 6 types (6 genes, 132 mutations), 1:6 400–1:80 000; treatment also **betaine**

Organic Aciduria (Acidemia)

- it is a group of disorders of specific enzymes in catabolism of BCAA (valine, leucine and isoleucine)
- the result is the accumulation of toxic by-products (organic acids) and hyperacidification of the organism occurs, which particularly disturbs the brain

Leucinosis

- maple syrup disease (MSUD; maple syrup urine disease)
- impaired metabolism of branched α-keto acids - disorder of the dehydrogenase in multienzyme complex
- keto acids accumulate in the **body causing odour of urine and ear wax after maple syrup**
- enzyme complex = mitochondrial **BCKD**; ketonuria, food intolerance, vomiting, lethargy, ketoacidosis, coma
- 1:190 000, but **1:760 in Mennonites**; 4 genes for subunits; classical, intermediate and intermittent type
- other organic acidurias: α-methylacetoacetic aciduria, hydroxymethylglutaric aciduria, glutaric aciduria

Ornithine transcarbamylase (OTC) deficiency

- **X-linked recessive** urea cycle disorder; enzyme in the mitochondrial matrix of liver and intestinal cells
- **hyperammonaemia** after protein food → brain damage, sepsis-like state, convulsions, impaired development, vomiting, lethargy → coma

2.30.2 IMD Saccharides

Galactosemia

- is due to malfunction of **galactose-1-phosphate uridyl transferase (GALT)**, leading to the inability to metabolize galactose monosaccharide (a component of lactose)
  - toxic metabolic **by-products are formed that damage the liver, brain, kidneys and eye lenses**
- failure to follow a strict diet could result in malnutrition, cirrhosis of the liver, cataract and mental retardation
- also **hyperbilirubinaemia**; gene on chromosome 9p, 3 types, 160 mutations, 1:18 000–1:70 000
- galactose is present also in fruit, some cereals and vegetables (cabbage, cauliflower, beet, Brussels sprouts, tomatoes, legumes), cocoa, chocolate, ice-cream, almonds, nuts, cinnamon

IMD lipids (slides)

MCAD deficiency

- FA β-oxidation provides **~80 % of energy in starvation**; MCAD deficiency → in fasting hypoglycaemia, acidosis, convulsions, lethargy, vomiting → coma (see also 2.13)
- treatment: **prevent starvation**, corn-starch feeding, parenteral nutrition
- **3 % of sudden infant death** was caused by MCAD; 1:20 700 in CZ

Smith-Lemli-Opitz syndrome

- disorder of endogenous **cholesterol synthesis** – **7-dehydrocholesterol reductase (DHCR7)**; toxic intermediates excreted in urine
- complex disability: intrauterine growth retardation, craniofacial dysmorphia, microcephaly, heart defects, **polydactyly**, CNS malformations, hypospadias, cryptorchidism, micropenis, cleft palate, bifid tongue; **20 % die after birth**
- steroid metabolites in the **maternal urine**; 1:20 000; treatment – cholesterol supplementation (feedback inhibition), statins

2.30.3 IMD Lysosomes

Tay-Sachs disease

- damage of **HexosaminidaseA (HEX A)**, leading to inability to degrade sphingolipids GM2-ganglioside
- o GM2-ganglioside
  - GM2-ganglioside then **accumulates in cells, mainly in lysosomes**
- the nervous system is most affected
- there are several forms of the disease (depending on the age at which the first symptoms begin to appear), where the most severe (infantile type) leads to death within 5 years of life
- **cherry-red spot on the retina**, blindness; incidence 1:360 000, but **1:3 600 in Ashkenazi Jews**

Gaucher disease

- due to the deficiency or absence of the lysosomal enzyme glucocerebrosidase

- substrate (glucosylceramide) accumulates in macrophages
- especially affected - spleen, liver, bone marrow, brain, nervous system, lymphatic tissues
- enzyme = **β-glucocerebrosidase** (β-glucosidase, GCase); **Gaucher cells**; bones shaped like **Erlenmeyer flasks**; anaemia, leukopenia, thrombocytopenia
- chromosome 1, 120 mutations, 1:40 000–50 000 (1:850 in Jewish population); **enzyme replacement therapy (ERT)**

Niemann-Pick disease

- lysosomal storage of **sphingomyelin** – **acid sphingomyelinase (ASM)** deficiency
- accumulation in RES and CNS, **hepatosplenomegaly**, impaired development, **cherry-red retinal spot**
- types A/B (1:300 000, olipudase alfa) and C (1:93 000, miglustat)

Hurle Syndrome

- Glycosaminoglycans (dermatan sulphate and heparan sulphate) accumulate in tissues as a result of α-L-iduronidase I enzyme deficiency, leading to corneal opacity, skeletal changes, hydrocephalus and hepatosplenomegaly
- = **mucopolysaccharidosis I** (Hurler, Hurler-Scheie, Scheie); GAGs excreted in urine
- **gargoyle-like facies**, abnormal skeletal development, dwarfism, kyphosis, hepatosplenomegaly, mental retardation
- manifests around **1 year**, children die around **10 years**; treatment – bone marrow transplantation, ERT; 1:140 000

2.30.4 IMD Purines and Pyrimidines

Porphyria

- a group of diseases caused by mutations in the heme synthesis genes
  - defects in the early phase of the synthesis cause the accumulation of starting materials - 5-aminolevulinic acid and porphobilinogen
    - accumulation of substrates with toxic effect
  - defects in the higher stages of synthesis lead to the accumulation of porphyrinogens
    - accumulation of substrates and by-products with toxic effect
- porphyria is divided according to
  - **most affected organs producing porphyrins**- hepatic, erythropoietic, erythrohepatic
  - **manifestations**- skin, liver
  - **course** - acute, chronic
- Porphyrinogens react with **visible light** to form radicals that damage cellular organelles, including lysosomes
  - this releases lysosomal enzymes that cause **redness, blistering, scarring, etc.**
- caused by deficiencies of **8 enzymes** of heme synthesis; e.g. **congenital erythropoietic porphyria (Günther disease)**

2.30.5 Mitochondrial diseases

- if caused by mutations in nuclear DNA - normal AR inheritance
- if caused by mutations in mtDNA - purely maternal inheritance
  - if a mother is affected, all her children are affected
  - If a father is affected, all his children are healthy
- **mitochondrial disease is a heterogeneous group of diseases with respiratory chain dysfunction**
  - are metabolic disorders and neurodegenerative or muscle deficiencies due to product deficiency (ATP)
- tissues with high ATP consumption (brain, muscles, liver, heart, kidneys, endocrine glands) are most affected
- **the disorder is multisystemic,** exceptionally one organ is affected (LHON syndrome)
- **very serious illnesses with poor prognosis**
- **variable expressivity (different** phenotypic expression) - in division the distribution into daughter cells is random, therefore the distribution of mutated and normal mtDNA is different
  - is typical for mitochondrial diseases
  - in each disease, the disease may have different expressivity – affects different organs, have different severity
  - **Homoplasmia**- cells have only mutant mtDNA or only normal mtDNA
  - **heteroplasmia**- the cell contains a mixture of normal and mutant mtDNA
- mtDNA mutation 10 times more frequent than in nuclear DNA, because it is not so much protected and it lacks some repair mechanisms
- examples of mitochondrial diseases
  - **MELAS syndrome** (mitochondrial encephalopathy, lactic acidosis and cerebrovascular accident syndrome)
    - dementia, hearing loss, small stature, stroke at a young age
  - **LHON syndrome** (leber hereditary optic neuropathy)
    - retinal ganglion degeneration, optic nerve atrophy, vision loss
- a defect in the mitochondrial respiratory chain should be considered in patients who have any unexplained combination of neuromuscular and / or other symptoms, progressing and affecting apparently unrelated organs
- slides: caused by lack of ATP, free oxygen radicals, triggering of apoptosis; **ragged red fibres** in myopathy
- diagnosis from epithelia in urinary sediment, hair follicles and **muscle biopsy**

- **heteroplasmy threshold > 70 %** for the disease to manifest
- **MELAS** – also fatigue, muscle weakness, vomiting, hypotension, ptosis, migraine; mild acidosis, ragged red fibres; mutation **A3243G** in the mtDNA gene for **tRNA^{Leu(UUR)}**
- **LHON** – acute/subacute loss of **central** vision; 1:10 000; **incomplete penetrance (50 % men, 10 % women)**; treatment idebenone (quinone analogue), gene therapy
- other mitochondrial disorders: mitochondrial myopathy, **DAD** (diabetes and deafness), **Leigh syndrome**, **NARP** (neuropathy, ataxia, retinitis pigmentosa, ptosis), **MNGIE** (myoneurogenic gastrointestinal encephalopathy), **MERRF** (myoclonic epilepsy with ragged red fibres)

2.31 BIOCHEMICAL CORRELATIONS OF MONOGENIC DISORDERS

MUDr. Josef Fontana

2.31.1 Introduction to monogenic diseases

Specifics of monogenic diseases

- the inheritance of the disease is linked to one particular gene
- molecular genetic diagnostics is available for most of them
- A causal therapy is usually not available (=therapy which is focused on the cause of the disease)
- Onset of symptoms in childhood: < 10 % start after puberty
- Low overall incidence: approximately 0.36 %(**incidence** = number of new cases of disease over time)

Inheritance of monogenic diseases

1. **Autosomal dominant (AD)**
2. **Autosomal recessive (AR)**
3. **X – linked dominant (XD)**
4. **X – linked recessive (XR)**
5. **mitochondrial**

- most monogenetic diseases are **hereditary metabolic disorders** (enzymopathies)
  - together about 900 diseases
- Annually in the Czech Republic born about 1000 children with some enzymatic defect)
- often treated by diet = limited intake of proteins, sugars (galactose) or cereals (gluten)

Characteristics of inherited metabolic disorders

- almost always **recessive inheritance** - heterozygotes with 50% residual activity of the enzyme are usually without problems
- Symptoms are caused by: **(a) accumulation of the substrate, (b) lack of product or (c) the formation of toxic intermediates / alternative products**

2.31.2 Autosomal dominant diseases

Lactose intolerance

- The most common form is called Adult Lactase Deficiency (Adult Lactose Intolerance) - an enzyme of brush border of enterocyte
- It is a form of a common polymorphism – Decreased expression of lactase during adulthood is essentially a normal state - reflects the decline in role of milk in a diet of mammals after a lactation period
- In some areas (eg. Northern Europe) unfermented milk was an important part of a diet after chioldhood - ability to digest lactose maintained (they have "beneficial" mutations)
- Elsewhere usual decline in lactase activity with age
- Lactase **activity depends on a summation of expression of both alleles** - AD inheritance
- Approximately **75 % of adults** worldwide suffer from a **reduction in lactase activity** (10 % of the adult Caucasian population)
  - **20-40 % adults** suffer wolrdwide with a **severe form**
- Frequency of the defect: 5 % in northern Europe, 71 % in southern Europe, 90 % in some African and Asian countries
- **Consequences**: watery (osmotic) diarrhea and excessive gas production, Accumulation of non-metabolisable substrate in the lumen of the gastrointestinal tract (bacterial lactase breaks down the disaccharide eg. yoghurt)

Familial hypercholesterolemia (FH)

- Defect in metabolism of LDL particles - mutation of a gene for LDL receptor
- Similar is familial defect of apolipoprotein B-100 (FDB) - defective Apo B-100 will not allow the proper binding of LDL to the LDL receptor
  - difference can be assessed on the basis of DNA analysis
  - both are inherited dominanty: expressed even when only one allel is damaged (rare to have 2 damaged allels → very severe disorder
- Consequences: impaired uptake of LDL particles - increased levels of Cholesterol
- Excess Chol is deposited in the walls of blood vessels - the development of atherosclerosis and its complications (MI or stroke) at a young age

- • Skin and tendon xanthomas, xanthelasmas of eyelids and arcus lipoides corneae
- Frequency in the Czech Republic is **0.2 %** (frequency of heterozygotes 1/500)! - affects **about 20 000 people**
- **treatment**: diet, pharmacological treatments (statins – competitive inhibitor of HMG-CoA reductase, which catalyses the main reaction in cholesterol synthesis -> leads to an increased number of LDL receptors and increases absorption of LDL by the liver, in the most severe forms (homozygote) LDL apheresis (methods by which excess LDL particles are removed form blood)

2.31.3 Autosomal recessive disorders

deficiency of α1-antitrypsin

- **Plasma proteinb inhibitor of proteases** (elastase also)
- So called . **Z-mutation** n (substitution of Glu for Lys at position 342) - high frequency of the allele in Caucasian population:
  - in Sweden 5 % of the population MZ-heterozygou
- Normal serum levels of α1-antitrypsin: **20-50 µmol/l,** with the ZZ-genotype drop to **3-6 µmol/l** (10-15 %)
- **Destruction of vulnerable tissues** - eg.. **lung tissue**
- 80-90 % of patients with ZZ-genotype develop **pulmonary emphysema** in young / middle age
- Proteolytic enzymes are released from the macrophages and neutrophils during inflammatory processes
- Emphysema is a result of an imbalance of protease /antiprotease activities in the lungs
- Potentiates with smoking – smoking causes an even worse course of the disease

Congenital adrenal hyperplasia (CAH) – adrenogenital syndrome

- Frequency **1/8-9 000** (carriers approx. 1/40)
- 8 types of defect in enzymes of steroid hormones synthesis in the adrenal cortex - the most common 21-hydroxylase deficiency - 90 % of CAH (rarely 11β-hydroxylase, other only less than 1 %)
- **adrenal glands don't produce enough cortisol** = low level of cortisol = hypophysis produces ACTH to increase the synthesisi of cortisol, but due to the defect of the enzyume the only effect of ACTH is the **hyperplasia of adrenal glands nadledvin**
- **consequences**
  - Insufficient supply of energy (glucose) during stress (diseases, starvation)
  - Mineral dysbalance: Na+ and water is lost in the urine - dehydration;
  - **hyperkalemia** - lethargy, muscle weakness, heart rhythm disturbances
    - coma and death
- excess ACTH does not increase the synthesi of cortisol – the precurosrs accumulate and released into the circularion and are used to create cortical sex hormones (cortical androgens)
- in **females** it leads to **viriliztion** (the appearance of external genitilia begins to be similar to the male sex) so called o. **pseudohermaphroditism femininus** - female genotype, but male phenotype (masculinization)
- Precocious puberty in males (pubertas praecox)
- **Therapy**: supplementation of missing glucocorticoids - compensates the deficit of cortisol and stops the excessive production of ACTH - decreases the formation of excess androgens

Sickle Cell Anaemia

- **Special type of haemoglobin - HbS**
- Point mutation at the gene for β-globin chain – substitution of Glu for Val at the 6th position
  - Val is a non polar AA- non polar compounds like to associate. Hb contain a **sticky spot** – moelcules attach together
- • In the deoxy-form Hb S forms long chains associated in thick strands - change of the ery shape: biconcave to sickle cell **– impaired elasticity** - can not pass through capillaries - obstruction
- Shortened lifespan of ery (10-20 days x 120 days) - hemolytic anemia
- More resistant to infection with the **protozoan Plasmodium - causes malaria** (heterozygote advantage in regions with a high incidence of malaria)

Porphyria

- Mutations in the enzymes that control the production of heme (part of cytochromes and Hb) - AD and AR heredity
- **Symptoms are derived from:**
  - 1)Affected enzyme and severity of the defect
  - 2) Whether the formation of heme is primarily affected in the liver or developing erythrocytes

- **mechanism of damage** - Intermediates of heme formation contain conjugated double bonds - after exposure to photons of visible light (esp. wavelength = 400 nm) results in excitation of electrons
  - Generated reactive oxygen species - damage to cells - release of proteolytic enzymes from lysosomes

- **symptoms** photosensitivity (skin lesions, redness, blistering, hypertrichosis), abdominal pain, liver and neurological impairment (even psychotic conditions)
- **diagnosis** - determination of porphyrins and precursors in urine, faeces and / or red blood cells, red colored urine
- **treatment** - avoiding of provoking factors, adminitsration of hemin (similar to heme complex, but containing Fe3+, supplemets the lack of heme + represses transcription of the gene for ALA-synthase)

Congenital erythropoetic porphyria

- AR diseases caused by the mutation of gene coding for **uroporphyrinogen synthase**
- **Severe photosensitivity** - skin lesions, redness, blistering, hypertrichosis), abdominal pain, liver and neurological impairment (even psychotic conditions)
- Diagnosis: determination of porphyrins and precursors in urine, faeces and / or red blood cells, red colored urine
- **Treatemnt** - avoiding of provoking factors, adminitsration of hemin (similar to heme complex, but containing Fe3+, supplemets the lack of heme + represses transcription of the gene for ALA-synthase)

Acute intermittent porphyria, hereditary coproporphyria and porphyria variegata

- So called. **liver porphyria**
- vomiting attacks with colony abdominal pain, neuropathy and psychiatric conditions
- all AD - enzyme activity is typically around 50% of normal values
- attacks are related to elevated δ-ALA acid level resulting from derepression of ALA-synthase transcription
- AIP - the most common form - **porfobilinogene deaminase (hydroxymethylbilan synthase)**
- **frequency of heterozygotes - 5-10 / 100** 000 (1/1000 in northern Sweden)
- **attacks often caused by drugs - ethanol, barbiturates or contraceptives**

2.31.4 X-linked recessive diseases

Haemophilia

- spectrum of diseases- only some of them in relarion to the X chromosome
- **defective synthesis or decreased functional activity of some coagulation factors**
- **Haemophilia A** (frequency 1/ 5 000 men): f. VIII (antihemophilic f.)
- **Haemophilia B** (frequency 1/ 35 000 men): f. IX (Christmas)
- **Haemophilia C** (rare): **AR diseases**, f. XI (PTA)
- Clinical symptms depend on the severity of the defect
- **Severe form of haemophilia** – decrease in plasma concentration of clotting factors below 1 %
- **Moderate form of haemophilia** – decline to 1-5% of normal levels
- Patients with severe form are in risk of spontaneous bleeding into joints (**haemarthrosis**), central nervous system, muscle and soft tissues, as well as life threatening bleeding after injury or medical procedures.
- Patients with moderate haemophilia suffer from similar symptoms but usually not from spontaneous bleeding
- **No causal treatment – patients have to get regular supplementation of missing factors**
- **Haemophilia A** is the most common type of haemophilia, in the Cz, about **1000 people with haemophilia,** , 80-85 % from which have haemophilia A
- o Haemophilia is called the Royal disease – due to the descendants of Queen Victoria of United Kingdom and Ireland haemophilia has spread to many other European royal families (Romanov dynasty, Bourbon-Anjou). It is believed that they suffered from Haemophilia B

2.32 NEWBORN SCREENING

prof. MUDr. Marie Černá, DrSc.

- Screening of **48-72 hours old** baby from one drop (from the heel) of his/her blood for genetic diseases
- **Active and nationwide** search for diseases in their early, preclinical stage so that these diseases can be diagnosed and treated before they can manifest themselves and cause irreversible health damage to the newbornví
- **Analyzation of dry blood on a filter paper, looking for specific markers - enzymes, amino acids, etc..**
- the sample = **newborn screening card**, taken under defined conditions from all newborns in the Czech Republic (48th–72nd hour); samples are shipped every working day to **2 laboratories**; parents give informed approval
- the number of tested diseases differs between countries (USA 2008: 4–54 by state; also varies across Europe)
- Only diseases that are frequent, can be diagnosed and **effectively treated to prevent them**
- **Since June 1, 2016, 18 genetic diseases have been investigated**
    - **update: since 1. 1. 2022, 20 diseases are tested in CZ** – the list newly includes **spinal muscular atrophy (SMA)** and **severe combined immunodeficiency (SCID)**
    - **Congenital hypothyroidism (CH)** - congenital decreased thyroid function
    - **Congenital adrenal hyperplasia (CAD)**
    - **Cystic fibrosis** - the only exception - cannot be avoided, but we can only alleviate symptoms
    - 8 amino acid metabolism disorders
        - **Phenylketonuria**
        - **Leucinosis (maple syrup syndrome)** - a disorder of BCAA metabolism
        - **Citrulinaemia type 1** - argininosuccinate synthetase deficiency
        - **Argininaemia**- arginase deficiency
        - **Homocystinuria from deficiency of cystathionin beta-synthase (CBS)**
        - **Homocystinuria from deficiency methylenetetrahydrofolate reductase (MTHFR)**
        - **Glutaric aciduria type I**
        - **Isovaleric aciduria**
    - 6 disorders of fatty acid metabolism
        - **Deficit medium chain acyl-CoA chain acyl-CoA dehydrogenase deficiency (MCAD deficiency)**
        - **Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD deficiency)**
        - **Very long chain acyl-CoA dehydrogenase deficiency (VLCAD deficiency)**
        - **Carnitine palmitoyl transferase I deficiency (CPT I deficiency)**
        - **Carnitine palmitoyl transferase II deficiency (CPT II deficiency)**
        - **Carnitine-acyl-carnitine translocase deficiency (CACT deficiency)**
    - hereditary disorders of vitamin transformation
        - **Biotinidase (BTD) deficiency**

Congenital hypothyroidism (CH)

- Is caused by a **disorder of the prenatal development of the thyroid gland** (**dysgenesis**, 80% of cases) or a **disorder of some step of thyroid hormone synthesis** (**dyshormonogenesis**, 20% of cases)
- manifests as fatigue, sleepiness, overweight, anemia, menstrual cycle disorder...

Congenital adrenal hyperplasia (CAH)

- **loss of one of the five enzymes of steroid hormone biosynthesis (in most cases 21-hydroxylase is missing)**
- when there is no aldosterone and cortisol, massive salt loss occurs (untreated ends in death)
- **is associated with elevated levels of 17-hydroxyprogesterone in the blood**
- if there is no 21-hydroxylase, the common precursors are converted to androgens, which means **masculisation in women and premature puberty in men**
- since steroids are missing in the body, a large amount of ACTH is released into the body (the brain is trying to force the adrenal glands to produce more steroids), leading to excessive bilateral enlargement of the adrenal glands treated with mineralocorticoid and glucocorticoid substitution (= **cortisol and aldosterone**)

Cystic fibrosis

- **In CZ the most often mutation in CFTR gene is F508del,** which is the gene for a chloride ion channel in the cytoplasmic membrane
- **During analysis we search for Trypsinogen** (pancreatic proenzyme, a lot of trypsinogen means trouble)
- defective export of chlorides and water, leading to increased mucus density and viscosity, clogging the lower respiratory tract and possibly leading to death, either **by gradual loss of functional lung tissue** or by succumbing to some of **the frequent bacterial infections** (mean life expectancy is 37 years)
- in the Middle Ages heterozygotes had an evolutionary advantage in **resistance against certain dehydration diseases** (cholera)
- slides: CFTR gene on **chromosome 7q31** – a chloride channel; the protein has 5 domains: **2 MSD** (membrane-spanning), **2 NBD** (nucleotide/ATP-binding), **1 R** (regulatory, with phosphorylation sites)

- **the most common fatal AR disease of children in the white population**; incidence 1 : 6 700 (1 : 2 500), carriers 1 : 40 (1 : 25); gene discovered by **positional cloning in 1989**
- **F508del** = 70 % (45–88 %) of alleles; 50 % of patients are homozygotes, 40 % heterozygotes (F508del + other), 10 % other alleles; 7 mutations > 0.5 %, > 1 000 very rare
- symptoms: chronic obstructive lung disease, **insufficiency of the exocrine pancreas**, **meconium ileus** (10–20 % of newborns), **congenital bilateral absence of the vas deferens**
- screening: ↑ **immunoreactive trypsinogen** in blood; diagnosis: **sweat test** – Cl^- ≥ 60 mmol/l (Na^+ ≥ 70 mmol/l)
- mutations reflect settlement history: Celtic **G551D**, Nordic **F508del**, Slavic **CFTRdele2,3, 2143delT**, Mediterranean/Phoenician **G542X, N1303K**, Ashkenazi-Jewish **W1282X**
- heterozygote advantage: resistance to diarrhoeal diseases from bacterial enterotoxins (cholera) and to **salmonella** (CFTR as internalization receptor)

Evolution and civilization diseases (end of the lecture)

- primeval humans: close contact with nature, frequent change of dwelling, many infections, extreme physical activity, alternation of satiety and starvation
- modern "civilization" diseases: obesity, immunity disorders (allergy, autoimmunity), tumours (leukaemia)
- prevention: full **breastfeeding for 6 months**, flour side-dish from the 4th–6th month, abundant antigen stimulation, plenty of movement, optimal nutrition

Phenylketonuria

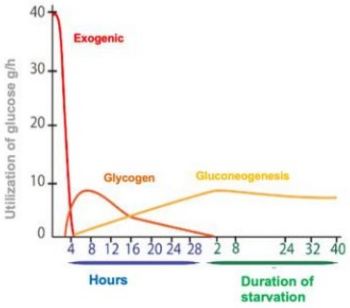
- deficiency of the **enzyme phenylalanine hydroxylase**, which converts phenylalanine into tyrosine
- phenylalanine accumulates in the blood and its metabolites damage the CNS nerve myelin sheath
- **a typical finding is elevated levels of phenylalanine and decreased levels of tyrosine**
- children have muscular hypertonicity and convulsions, rapidly developing **severe mental retardation**

2.33 EXTREME SITUATIONS IN CELL

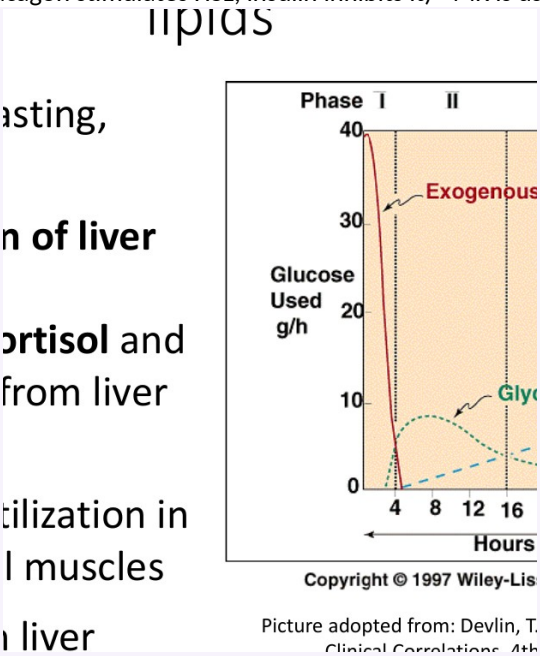
MUDr. Josef Fontana *Note. A case study is a part of this lecture. In QR code there is a link to the whole presentation including a case report.*

2.33.1 Starvation

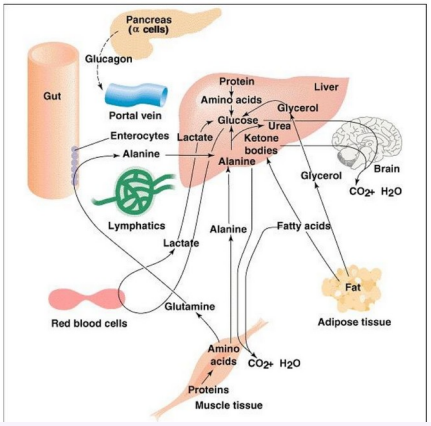
- The organism is in a state of short-term starvation after fasting overnight (before breakfast)
- **glycemia**- under physiological conditions it is fasted in the range of 3.9-5.6 mmol/l (after meals up to about 10mmol/l)
- Low **ratio of I/G** (low insulin / much glucagon) activates hepatic glucogenolysis and gluconeogenesis (↑ cortisol, growth hormone, glucagon), releasing glucose from the liver
  - ↑ ratio after meal
  - ↓ ration during fasting



- the main substrate- **lactate** -> lactate is produced in the muscles, which is transferred to the liver during the Cori cycle, where gluconeogenesis occurs (a smaller part of the lactate is used by the kidneys and the rest is metabolized by the myocardium...)
- slight activation of proteolysis in muscles → Ala is converted to pyruvate and gluconeogenesis can occur - gluconeogenesis and TAG mobilization are the primary energy source of the organism during starvation - of course proteins can be used, but we do not want that because protein loss = loss of cell function
  - The basis for survival is, for example, movement, so muscles can not be radically reduced in the long term - minimal use of proteins during prolonged starvation allows the synthesis of ketones, which after some time become the main energy source
- **lipolysis** is very important (glucagon stimulates HSL, insulin inhibits it) - MK is used in liver, heart and muscles



[slides] Glucose sources over time in starvation: exogenous → glycogen → gluconeogenesis



[slides] "Simple" starvation – early phase: interorgan fuel flows

Basic starvation

- 3 phases

Early phase
- lasts 2 to 3 days
- during this phase, glucagon, catecholamines and cortisol levels increase - this leads to gluconeogenesis for which muscle AMKs are used (ie, proteolysis in the muscles, resulting mainly in Ala and Gln)
- glutamine is an energy substrate for enterocytes
- ↑ lipolysis in adipocytes = release of MK and glycerol (glycerol on gluconeogenesis)
- many tissues are fed directly by MK (eg muscles and heart)
- MKs are processed in the liver to supply most of the energy for gluconeogenesis and ketogenesis (ketone bodies suppress proteolysis and oxidation of branched AMK in muscle and reduce Ala release into circulation)
- hepatic glycogenolysis is the primary source of blood glucose in an early fasting state

Adaptation phase
- the main energy substrates are MK and ketone bodies (the effort to conserve proteins, but it also gets to them, is the last backup from which energy can be taken)
- pyruvate, lactate, AK and glycerol are used for gluconeognesis

Terminal phase
- lasts 7 to 8 weeks
- at this stage, TAGs are already depleted and **proteocatabolism** is associated with progressive loss of muscle mass and significant suppression of the immune response, increased risk of infection

2.33.2 Diabetes mellitus I
- **Insulin deficiency due to destruction of pancreatic β-cells**
- **hyperglycaemia** is a hallmark of this disease - glucose is not transmitted to skeletal muscle, because GLUT4 channels that transport glucose into cells are opened by insulin that is lacking in these patients
  - no β-cells = no insulin = GLUT4 carriers closed = blood glucose = hyperglycaemia
  - insulin also inhibits gluconeogenesis, so in its absence gluconeogenesis is not inhibited = hyperglycaemia
- exceeding the renal threshold for glc leads to **glucosuria** (= urinary excretion of glucose), which is accompanied by **polyuria** (increased urination) → dehydration → **polypsidia** (increased fluid consumption)
  - when the body works properly we should not get above 10 mM due to glucokinase
- Lipolysis (HSL) is not controlled in adipose tissue → increased plasma MK → increased β-oxidation → increased production of ketone bodies in the liver → ketoacidosis
- **Hyperlipoproteinemia**- insulin does not activate lipoprotein lipase (= an enzyme that releases MK from lipoproteins) and the liver, in excess of MK, forms VLDL (very low-density lipoproteins)
- **Proteocatabolism** in muscle

2.33.3 Nutrient metabolism in excess
- increased activity of GIT, liver and pancreas
- rapid glucose and AMK resorption through the vena portae (collecting blood from unpaired organs)
- increased insulin secretion and glucagon decrease = ↑ I / G ratio (= glycogen synthesis, glycolysis, lipogenesis, proteosynthesis)
- Chylomicrons and VLDL - maximum concentration of ~ 2 mM reached 3-5 hours after a meal
- **what's happening in the liver?**
  - ↑ AK leads to proteosynthesis, branched AK are into muscles (BCAA)
  - ↑ glycemia leads to glycogen synthesis, glycolysis and conversion of glc to MK and TAG
  - stimulation of lipogenesis = VLDL formation
- **adipose tissue**
  - Large amounts of insulin **activate LPL** (= lipoprotein lipase), resynthesis of TAG in adipocytes and **inhibition HSL**
- **muscles**
  - increased glycogen synthesis, conversion of Glc to lactate
- proteosynthesis
- do not replenish the plasma of Glc-6-P when the liver is re-established

Metabolism in obesity
- Obesity is a risk factor for **diabetes mellitus II, hypertension and cardiovascular disease**
- the body remains in a state of satiety
  - the liver produces TAG, but it is not used during fasting

- Long-term satiety = a lot of insulin for a long time → reduction of the number of receptors to insulin (signal strength leads to a decrease of receptors) => **insulin resistance** - hyperinsulinemia = the cornerstone of metabolic syndrome - obesity, hypertension, ↑ TAG, ↑ LDL and ↓ HDLL
- as long as β-cells can overcome insulin resistance, glycemia and lipoproteinemia are relatively normal but may progressively progress in T2DM (= diabetes mellitus II)

Diabetes mellitus II

- 90% of diabetics in the Czech Republic suffer from T2DM
- frequent consequence of obesity - **insulin resistance** → hyperinsulinemia
- **hyperglycaemia and hyperlipoproteinemia**
  - reduced entry and use of Glc in tissues (muscles, adipose tissue)
  - ↑ synthesis FA, TAG VLDL in liver
  - ↑ LPL activity – do not produce chylomicra, unlike diabetes mellitus I)
  - glucosuria
  - ketone levels are usually normal because insulin inhibits HSLL

Metabolism of ethanol

- During detoxification, ethanol is converted to acetate while converting 2 NAD to 2 NADH
  - NADH is re-oxidized in the respiratory chain, but NADH accumulates when consumed significantly
  - inhibition of gluconeogenesis and β-oxidation and reduction of KC rate, occurs during NADH overproduction
    - conditional on lack of oxidized cofactors (NAD +) and on shifting the balance of some reactions
    - oxaloacetate is converted to malate and pyruvate is converted to lactate
- risk of hypoglycaemia due to insufficient carbohydrate intake
- the accumulating lactate causes **lactic acidosis**
- accumulating **acetaldehyde** causes nausea and vomiting
- ethanol contains a high amount of energy: 29,4 kJ / g
  - from the energy point of view, we could live on ethanol, but we would lack essential AA, minerals...
    - one of the most common problems of alcoholics is lack of vitamins - they eat very little food because they consume a lot of energy from alcohol, which gives their body a feeling of satiety
- high levels of NADH and AcCoA inhibit the activity of KC in the liver and stimulate the synthesis of TAG and cholesterol
- increased concentration of NADH in the liver after alcohol consumption may lead to fatty liver due to inhibition of β-oxidation
- alcoholics may develop cirrhosis of the liver

