Biochemistry of Liver

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General outline

More than 50% of dry residue – **proteins**

80% – hepatocytes

Kupffer cells – part of phagocyte mononuclear system

link between gastrointestinal tract and blood

Buffer between **portal vein** (70% of hepatic circulation) and **systemic circuit**

Water content may vary (65-80%) – role in **regulation of water homeostasis**

High content of protein (20%), glycogen (2-5%), lipids – up to 6%

high rate of energy metabolism

high circulation time (up to 25% of minute volume)

high rate of protein synthesis

Absorptive phase: synthetic pathways, accumulation of metabolic fuels

breakdown of amino acids and glucose

Post absorptive phase: providing other tissues with metabolic fuels

breakdown of fatty acids

Role in metabolism

- **Metabolism of carbohydrates**
  - Regulation of glycemia
    - **Increasing of glycemia:**
      - glycogenolysis
      - gluconeogenesis
        - from lactate and alanine (originated from muscles)
        - from glycerol (from adipose tissue)
        - from amino acids
    - **Decreasing of glycemia:**
      - glucokinase
        - K_m for glucose 10-fold higher than hexokinase
        - V_{max} 10-15-fold higher than hexokinase
        - not inhibited by G-6-P
      - transport protein with high K_M for glucose
      - synthesis of glycogen
        - G-6-P activates D-form of glycogen synthase
        - glucose binds to glycogen phosphorilase **a**, increasing its ability to dephosphorilation, *i.e. to inactivation*

Role in metabolism

- **Metabolism of carbohydrates**
  - Braking up glucose to precursors of:
    - other carbohydrates
    - fatty acids
    - cholesterol
    - ketone bodies
    - amino acids
  - Formation of NADPH
    - for reductive biosyntheses (fatty acids, cholesterol)
  - Formation of pentose phosphates
  - Formation of glucuronic acid and its active form
    - takes part in the synthesis of carbohydrate parts of glycolipids and glycoproteins, components of extracellular matrix
  - Metabolism of fructose and galactose
  - Gluconeogenesis

Role in metabolism

- **Metabolism of lipids**
  - **Synthesis of:**
    - TAG
    - phospholipids
    - cholesterol and its esters
      - cholesterol: free/esters 1 / 0.6
    - bile acids
  - **synthesis of fatty acids**
  - **β-oxidation of fatty acids**
    - from adipose tissue
    - of exogenous lipids transported by chylomicrons
    - own lipids (TAG) of liver
  - **ratio of synthesis and β-oxidation depends on ATP level**
  - **synthesis of ketone bodies** – "export metabolic fuel", *consumable in other tissues*

Role in metabolism

- **Metabolism of lipids**
  - **malonyl CoA level** in liver determines the fate of acyl-CoA:
    - excess malonyl CoA (normal ATP level)
      - inhibits carnithine-acyltransferase I – *inhibition of β-oxidation and ketogenesis.*
    - low malonyl CoA
      - activates β-oxidation and ketogenesis.
  - **transport of lipids:**
    - formation of transport forms of endogenous lipids (VLDL, HDL),
    - utilization of end products of metabolism of lipoproteins
      - uptake remnants of chylomicrons, VLDL, IDL, HDL.
  - **release of free fatty acids into blood**
    - for catabolism in other tissues (muscles)
    - for accumulation as TAG (adipose tissue)
  - **25-hydroxylation of vitamin D**
  - **metabolism and excretion of steroid hormones**

Role in metabolism

- **Metabolism of proteins and amino acids:**
  - **Synthesis of:**
    - liver proteins
    - most of proteins of blood plasma
    - serum enzymes (lipoproteinlipase, cholinesterase, blood coagulation factors)
  - **by synthesized proteins**:
    - maintaining osmotic pressure
    - regulation of blood pressure and circulation volume
    - blood clotting
    - metabolism of iron
  - **Regulation of amino acid content in blood plasma**, release of free amino acids into blood
  - **Degradation of protein and peptide hormones**
  - **Utilization of amino acids in synthesis of**
    - nucleotides
    - hormones
    - porphyrins
    - choline
    - creatine (2nd step: guanidine acetate → creatine)

Role in metabolism

- **Metabolism of proteins and amino acids:**
  - **Amino acid metabolism**
    - deamination
    - transamination
    - hydroxylation of amino acids
    - catabolism of amino acids to end products (acetyl-CoA and intermediates of TCA)
    - utilization of these products for syntheses
  - **Detoxification of ammonia**
    - urea synthesis
    - uric acid synthesis
      - high activity of xanthine oxidase
  - Detoxification of nitrogen and gluconeogenesis from **amino acids, originated from other tissues** (glucose-alanine cycle)

- **Storage of**
  - iron
  - vitamins A, B_{12}
  - glycogen

Detoxification in liver

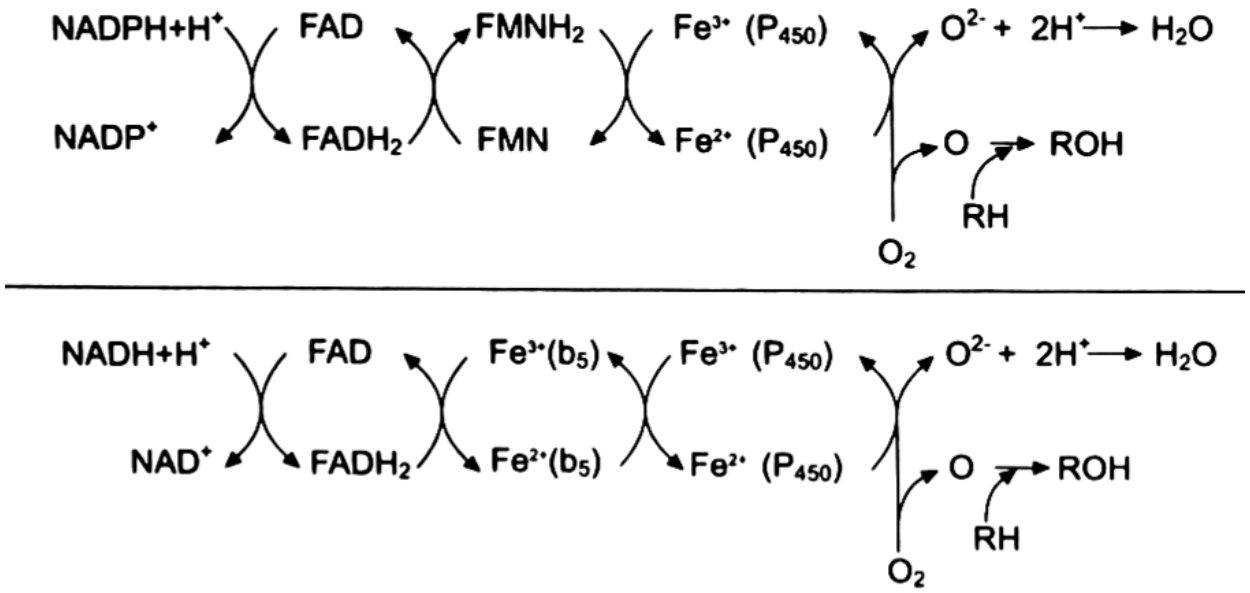
- **Compounds become**
  - more hydrophylic
  - suitable for excretion with urine
- **Hydrophobic or high-molecular-weight compounds**
  - excreted mostly with bile
- **Phases of detoxification**
  - biotransformation
    - hydroxylation — $RH \rightarrow ROH$
    - sulfo-oxidation — $R–S–R' \rightarrow R–S(=O)–R'$
    - oxidative deamination — $RNH_2 \rightarrow RH{=}O + NH_3$
    - dealkylation — $RNHCH_3 \rightarrow RNH_2 + H_2C{=}O$; $ROCH_3 \rightarrow ROH + H_2C{=}O$; $RSCH_3 \rightarrow RSH + H_2C{=}O$
    - formation of epoxides — $R–CH{=}CH–R' \rightarrow R–CH(O)–CH–R'$
  - conjugation
    - conjugation by transferases
    - hydrolysis of epoxides

Detoxification in liver

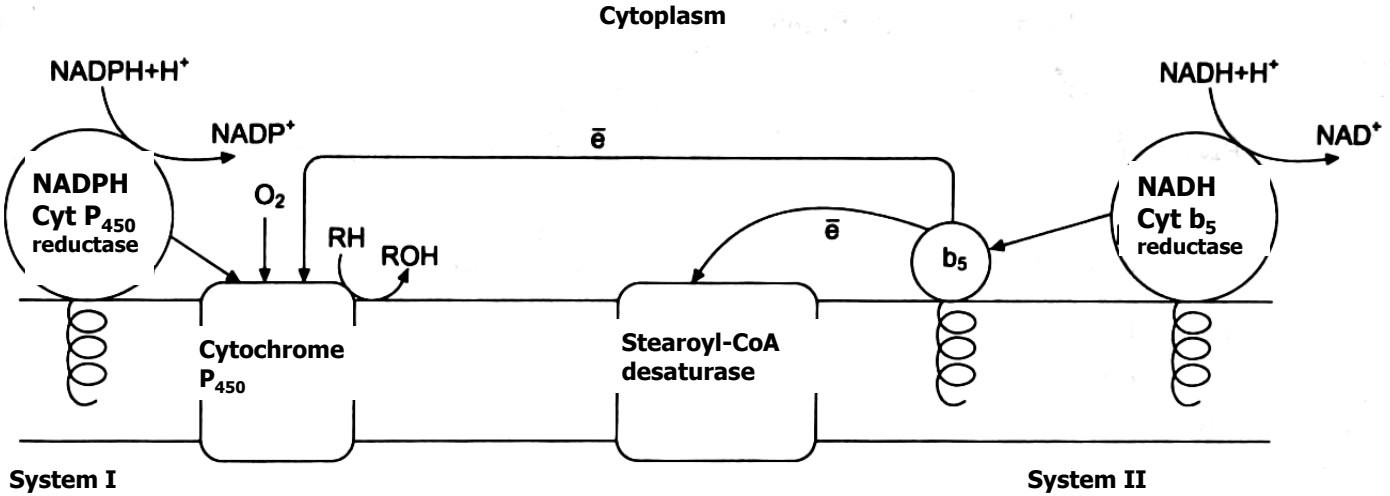
- Microsomal oxidation
  - wide substrate range (over 150 genes)
  - regulation of activity (induction)
    - on transcriptional level or
    - on post-transcriptional level

  over 250 inductors

  barbiturates, polyaromatic hydrocarbons, steroids, etc.



Detoxification in liver



- system I: **NADPH–cytochrome P_{450} reductase + cytochrome P_{450}**
- system II: **NADH–cytochrome b_5 reductase**
  - cytochrome b_5 can be a donor of electrons for
    - cytochrome P_{450}
    - stearoyl-CoA-desaturase
- Functioning of the systems:
  - formation of oxygen to singlet oxygen:

    $O_2 + 2e^- \rightarrow O^{2-} + \mathbf{O}$

    $O^{2-} + 2H^+ \rightarrow H_2O$

    $\mathbf{O} + RH \rightarrow ROH$
  - Result:
    - rise of hydrophilic properties,
    - increase of solubility
    - loss of biological activity of lipophilic compound RH.
    - In some cases
      - formation of new biologically active compound,
      - increase of toxicity (reactions of lethal synthesis; benz(a)pyrene)

Detoxification in liver

- Conjugation
  - **transferases** (formation of paired molecules)
    - UDP-glucuronosyltransferases

      glucuronic acid (active form – **UDP-glucuronate**) – **in ER**
      - bilirubin
      - products of conversion of amino acids in the intestine (phenol, cresol)
      - xenobiotics, drugs
    - sulfotransferases

      sulfate (active form – **PAPS**)
      - phenols
      - alcohols
      - amino acids
      - indole (indoxyl-3-sulphate – indican)
    - acetyltransferase

      acetate (active form – **acetyl-CoA**)
      - sulfonamides
    - methyltransferases

      methyl (active form – **SAM**)
      - methylation of P=O, NH_2, SH-groups
      - vitamin PP (formation of N-methylnikotinamide)

Detoxification in liver

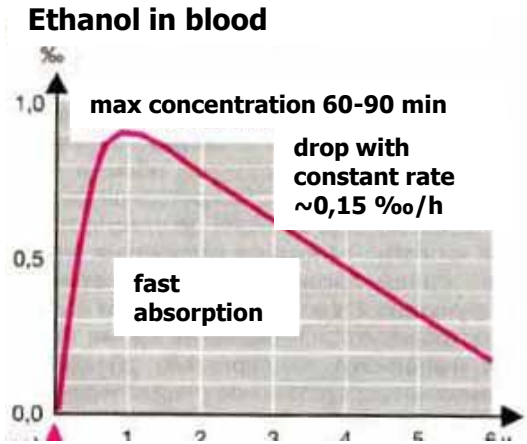
- Conjugation
  - **transferases**
    - glutathione transferases – **in cytosol**

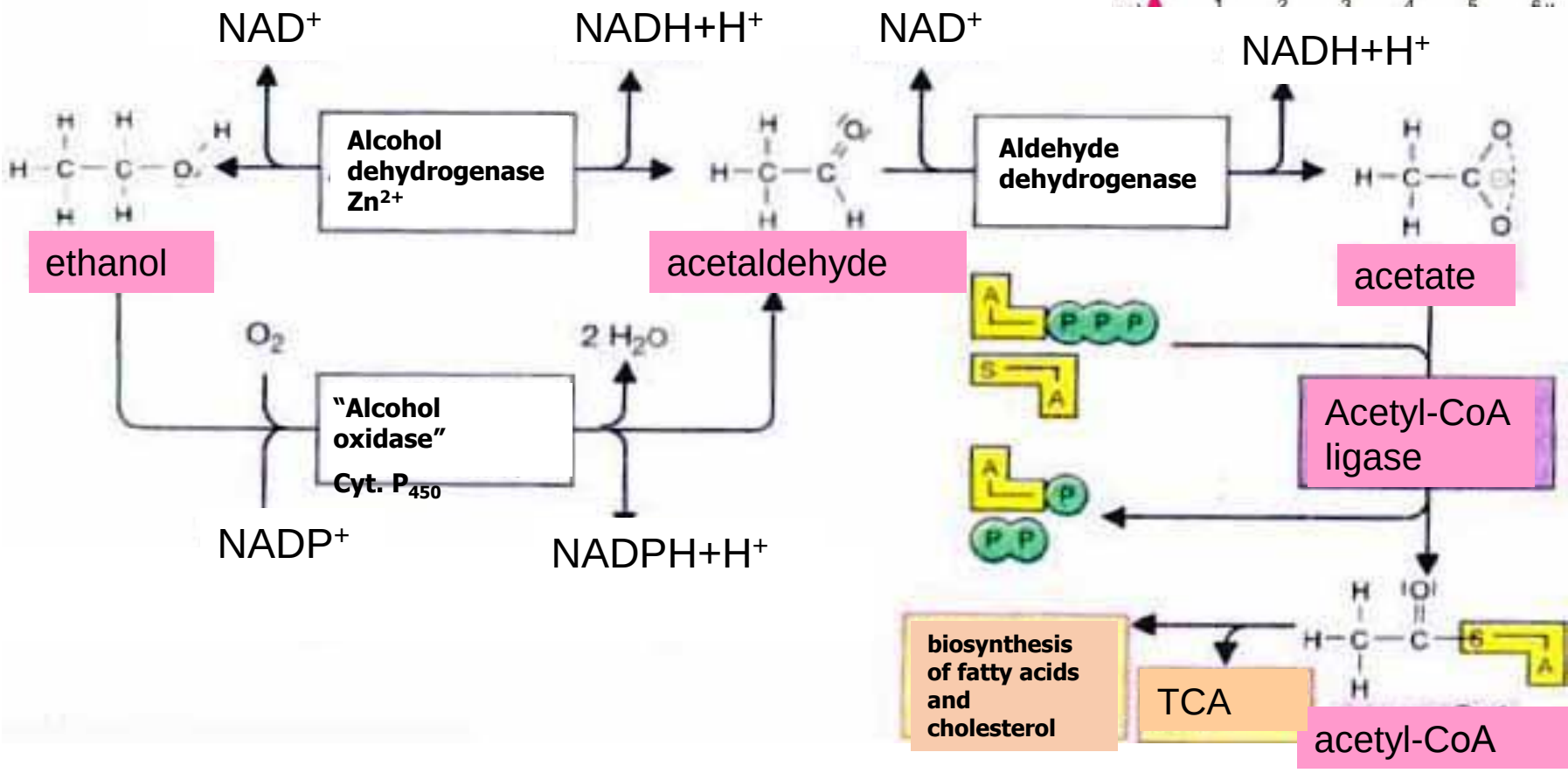
      GSH
      - steroids
      - prostaglandins
      - bile acids
      - products of lipid peroxidation
    - Action of glutathione transferases: 3 ways
      - Conjugation R+GSH → GSRH
      - Nucleophilic substitution RX + GSH → GSR + HX (formation of thioesters)
      - reduction of hydroperoxides R–HCOOH + GSH → R–HC–OH + GSSG + H_2O

      - for action of glutathione transferases **a hydrophobic part** in the moiety of substrate **must present**
      - detoxification of xenobiotics is possible by:
        - **hydrophobic interactions**
        - **covalent binding** to glutathione transferase.
    - Glycine transferase
      - bile acids
      - benzoic acid
        - Quick's test : – 4,5 g Na-benzoate, in 4 h not less than 70% of benzoate must be excreted
  - **epoxide hydrolases**
    - formation of diols

Detoxification in liver

- Oxidation in peroxysomes
  - urate oxidase
  - lactooxidase
  - oxidases of amino acids
  - catalase
- Protective syntheses (urea, uric acid)
- metallothionein (SH-groups bind to heavy metal ions)
- Role in **ethanol** metabolism





Pigment metabolism

- Degradation of RBC – in **Mononuclear phagocyte** (reticuloendothelial) **system**
  - endocytosis, breaking up globin by lysosomal proteases
  - heme oxygenase (in EPR) is induced by heme
    - splitting – between rings I and II (containing vinyl groups)

  heme → (verdoglobin) → biliverdin (yellow) + Fe^{3+}, $NADP^{+}$
  - biliverdin reductase:

  biliverdin → bilirubin (35 mg from 1 g Hb, 250–350 mg/day)
- Bilirubin transport: bound to albumin
  - 1 molecule of albumin – up to 3 molecules of bilirubin
- In acidosis
  - binding capacity of albumin decreases
  - bilirubin binds to collagen and membrane lipids
- Bilirubin competes with drugs/xenobiotics for binding sites on albumin
- uptake of albumin-bilirubin complex by hepatocyte membranes,
  - facilitated diffusion of bilirubin into hepatocytes:
    - glutathione-S-transferase **(ligandin)** - main carrier
    - protein Z

Pigment metabolism

- Conjugation of bilirubin in smooth EPR → diglucuronid
  diglucuronid is water-soluble (direct bilirubin)
  - Secreted into bile against concentration gradient
- **inductor** of synthesis od UDP-glucuronosyltransferase and **activator of transport** of direct bilirubin into bile – barbiturates
- **Inhibitors** of bilirubin conjugation – free fatty acids, estrogens

In intestine:

β-glucuronidases release bilirubin

bilirubin → mesobilinogen → urobilinogens (colorless tetrapyrrols) → **stercobilinogen**

- absorption in large intestine (main part – 250 mg/day)
- excretion with feces → urobilin **(stercobilin)**

- urobilin formed by microflora

- **excretion with urine** of urobilinogen absorbed to **inferior vena cava** – up to 4 mg/day
- **breakup** of the rest of urobilinogen absorbed into **portal vein** in liver to di- and tripyrrols and their excretion with urine.
  - small amount of urobilinogen is sectered to bile

Hyperbilibubinemias

- conjugated
- non-conjugated
- with covalent bond between bilirubin-glucuronid and albumin **(delta-bilirubin)** (prolonged conjugated hyperbilirubinemia) – hepatocellular failure

Jaundice: when bilirubin concentration exceeds 50 mcM (diffusion of bilirubin into tissues)

- **Encephalopathy (Kernicterus):** with **unconjugated** bilirubin only (>340 mcM)
- **Bilirubin in urine:** only **conjugated**

- **Inpaired conjugation** (**neonatal jaundice**):
  - deficiency of transport proteins
    - Decreased uptake of indirect bilirubin
  - deficiency of UDP-glucuronosyltransferase
  - insufficient synthesis of UDP-glucuronate
  - family hyperbilirubinemia of infants – presence of free fatty acids and estrogens in breast milk
  - disorders of active transport of direct bilirubin
    - In blood and urine – increased level of direct bilirubin

Impaired conjugation

- **Gilbert's syndrome**
  - decreased activity of the **bilirubin UDP-glucuronosyltransferase**, harmless
  - Typically inherited, autosomal recessive
- **Crigler-Najjar syndrome**
  - Type I – complete loss of activity of the **bilirubin UDP-glucuronosyltransferase**, fatal
  - Type II – some activity of the **bilirubin UDP-glucuronosyltransferase** remains, large doses of phenobarbital may be effective

Toxic Hyperbilirubinemia

CCl_3, CCl_4, acetaminophen, Amanita pallida

Hemolytic (pre-hepatic) jaundice

- Increased formation of bilirubin (more than 3-4-fold): hemolysis
  - sepsis
  - radiation syndrome
  - deficient glucose-6-P-dehydrogenase of RBC
  - thalassemia
  - transfusion of incompatible blood
  - poisoning with hemolytic substances

- Increased formation of direct bilirubin and its excretion with bile
- **Increased** absorption of **urobilinogen** and **stercobilinogen**, their level in blood and urine (direct bilirubin is absent in urine)

- Main symptom – increased **indirect** bilirubin in the blood

- Toxicity of indirect bilirubin:
  - uncouples electron transport and oxidative phosphorylation,
  - inhibits protein synthesis,
  - decreased permeability of K^+-channels.
- **Kernicterus** – bilirubin encephalopathy.

Hepatic (hepatocellular) jaundice

- damage of hepatocytes and bile capillaries
  - retention of bilirubin in the liver,
  - disturbance of active transport of direct bilirubin into the bile
  - Formation of large amount of monoglucuronids
- **direct bilirubin** comes to blood, decreased levels of bilirubin and urobilinogen in the intestine
- In urine: more direct bilirubin, **urobilinogen** (not detoxified by liver)
  - High direct bilirubin: **cholestatic hepatitis**
  - Low urobilinogen: **cholestatic hepatitis**
- In blood: increased levels of both **direct and indirect** bilirubin

Post-Hepatic / cholestatic (mechanical) jaundice

- obstruction
- compression, stenosis
- birth defects

- Rate-limiting stage in the metabolism of bilirubin – excretion of direct bilirubin with bile
- in blood the level of direct bilirubin increases
- in the intestine – no bilirubin & urobilinogen
- disruption of enterohepatic recirculation
- in the urine – high content of direct bilirubin

Micro-obstruction: by damaged hepatocytes, oedema

Dubin-Johnson syndrome

Mutation of gene of **direct bilirubin transporter**

Biochemical diagnosis of liver diseases

Fatty infiltration of liver

- TAG content in the liver over 2%
  - Disturbed synthesis of phospholipids – lack of lipotropic factors (methionine and choline)
- occurred in
  - chronic intoxications (alcohol),
  - poisoning by phosphoro-organic substances, xenobiotics, some drugs,
  - lack of carbohydrates, diabetes mellitus
  - insufficient protein synthesis in liver

Liver failure

- State of the patients is most affected by decrease of
  - detoxification in liver (high ammonia, bilirubin)
  - protein synthesis (coagulopathies)
  - release of the intermediates of metabolism into blood, intoxication by these substances (influence on the CNS – hepatic encephalopathy)
- Ratio A/G
  - Decrease – lower albumin content
- Colloid resistance tests
  - Positive in hepatic jaundice
  - Negative in post-hepatic jaundice
- Fractions of residual nitrogen (urea nitrogen must comprise = 50%)
- Increased activity of hepatic enzymes: F-1-P-aldolase, AlAT, LDH_5, sorbitol dehydrogenase
  - Cholestasis: alkaline phosphatase, leucine aminopeptidase, gamma-glutamyl transpeptidase
  - Severe hepatocellular insufficiency: increased: GDH, **mitochondrial AsAT**
- Decreased: cholinesterase, LCAT