2. Pathobiochemistry

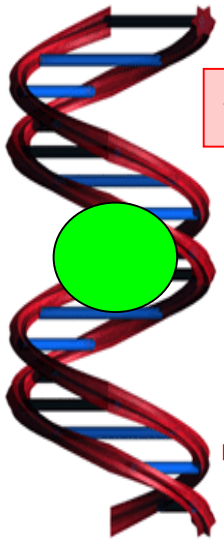
(MB)

2.1.Causes and kinds of disorders. Hereditary metabolic disorders (HMD).

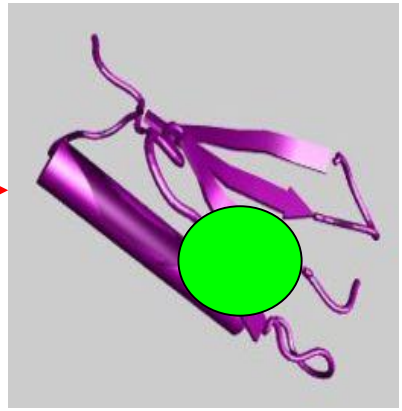
2.2. Enzymes, regulation of metabolism.

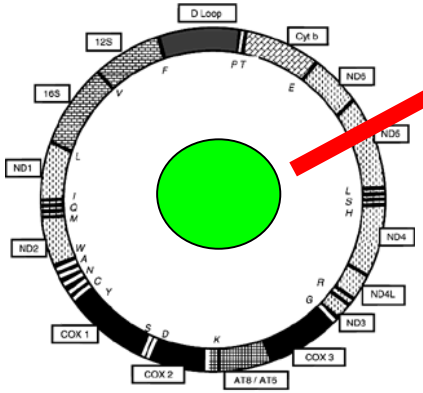
2.3. Causes of increased activity of cellular enzymes in plasma. Clinically important enzymes.

Hereditary metabolic disorders



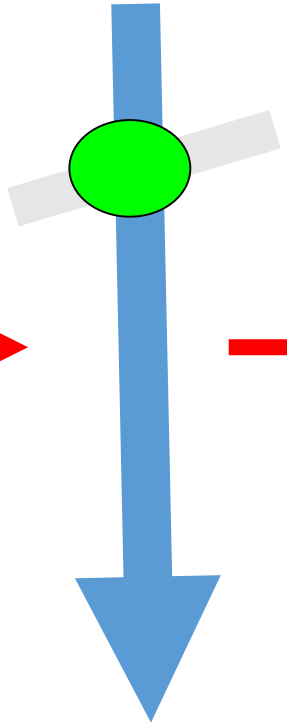
usually AR, GR



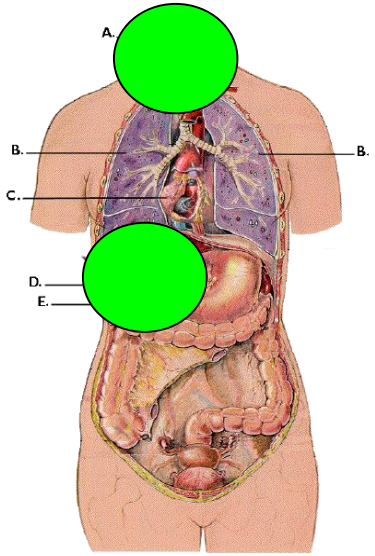


usually enzyme

substrate



product

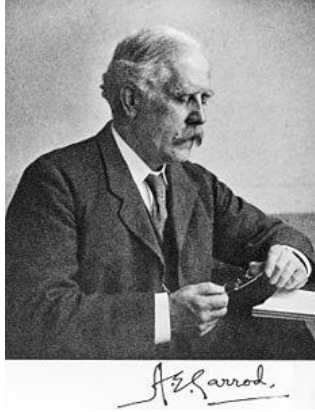


clinically variable

2.1. Causes and kinds of disorders. Hereditary metabolic disorders (HMD).

- Before: Inborn errors of metabolism
- Definition: diverse group of diseases whose common characteristic is a presence of genetically conditional protein change
- Beginning of 20. century - conception of HMD was formulated – sir Archibald Garrod – 4 HMD
- Today-HMD-more than 700 units

Sir Archibald Edward Garrod,



- Sir Archibald Edward Garrod, (25 November 1857 – 28 March 1936) was an English physician who pioneered the field of **inborn errors of metabolism. He also discovered alkaptonuria**, understanding its inheritance. He served as Regius Professor of Medicine at the University of Oxford from 1920 to 1927.[2]

- **History**
- Beginnings of a discovery of HMD are connected with name Archibald Garrod, who pointed to a connection between human diseases and Mendel´s principles of a heredity and formulated a concept of HMD (inborn errors of metabolism). Garrod engaged by a study of alkaptonuria and in 1902 published a book *The Incidence of Alkaptonuria: a Study in Chemical Individuality*, which is first record of human recessive hereditary. In 1923 next his book *Inborn Errors of Metabolism* was published, where we can find studies about alkaptonuriia, cystinuria, pentosuria and albinism.

Pathogenesis of HMD

- HMD are diseases which arise on a **molecular level**
- Causes of HMD is a change of genetic information (gene,DNA)→bad transcription into mRNA→bad synthesis of protein→protein with a changed structure
- Mutation→defective transcription→defective translation
- 1 gene encodes synhtesis of 1 protein molecule

Kinds of mutations: deletion, insertion, lost of a part or whole chromosome

Function of protein in intermediate metabolism

- Enzyme
- Transport protein
- Structural protein
- Regulatory protein

Most often-protein works like enzyme

$$\text{Substrate} \xrightarrow{\text{enzyme}} \text{product}$$

Example p53

- **Impact of mutation of a protein function**
- Lost of a function
- Amplification of a function – some of protein functions or intensity of a protein production amplificates by a mutation/accumulation
- Profit of a new function
- incorrect protein expression of (in a place and in time)

Impacts of mutations

- **Accumulation of a substrate** (small molecules-for example phenylalanine are difussaly scattered in body fluids, transfered across a filtering barrier of kidneys, excreted by urine. Big molecules-for example mucopolysaccharides accumulate in a place where they arise).

  Example = **PKU (phenylketonuria)** – a mutation of a gene for PAH (phenylalaninehydroxylase), enzymatic activity < 1% (2 alleles are affected). Low percentage of PKU is caused by a mutation of 1 allele or in a gene for a cofactor of PAH – tetrahydrobiopterin (milder form of PKU).

- **Lack of a product**
- **Accumulation of a defective enzyme**
- **Synthesis of an incorrect product** - block of a metabolic pathway
- **Lost of various enzymatic activities**

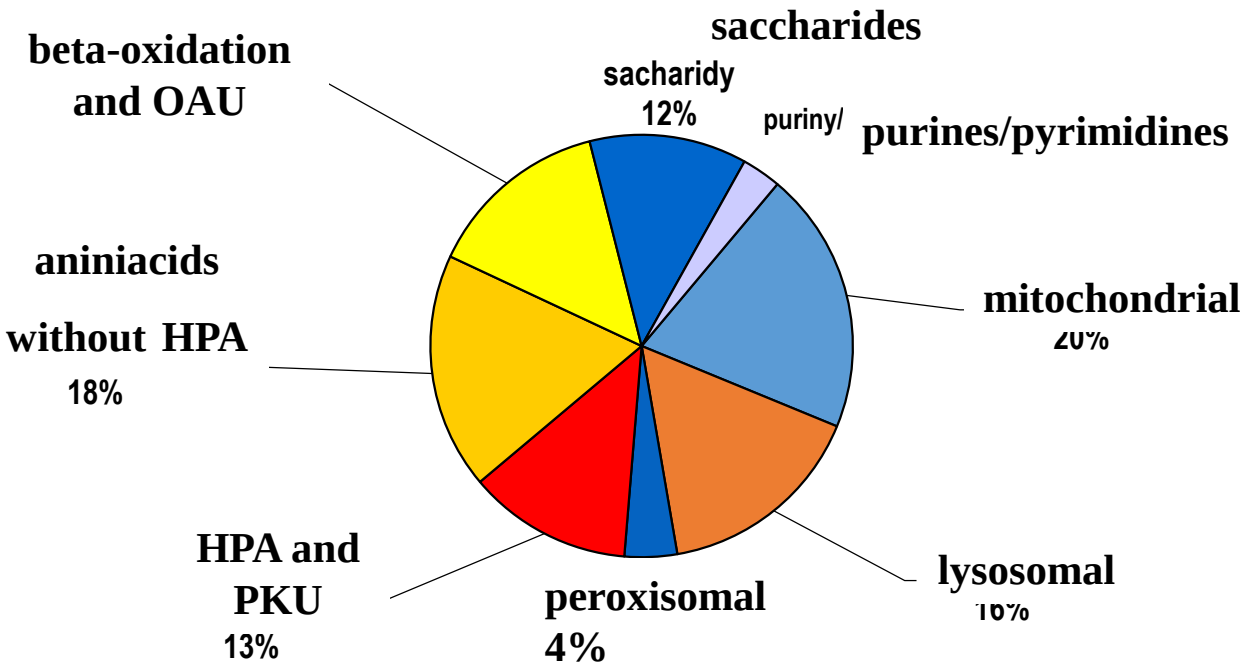
Incidence of HMD

- Individual incidence is relatively rare (1:15 000 – 200 000)
- Collective incidence is high (1: 1000)

Incidence of HMD

- Neonatal (newborn) screening 1:1000-1:4000
- select*i*ve screening at least 1:500-1:1000
- Frequency of heterozygots for HMD at least 1:15
- Representation differs according a population
    - Higher incidence in inbred populations (PKU Turkey, organic aciduria Middle East)
    - tyrosinemia I.type Quebec
    - aspartylglykosaminuria Finland
    - Lysosomal diseases Israel

Incidence of HMD in CR



CR , 2005, n=127

incidence for ČR ~ 1:1000
still ~150 various nosologically units

Ways of a HMD transfer

NUCLEAR DNA

- Autosomal recessive
- Autosomal dominant
- Gonosomal dominant
- Gonosomal recessive

EXTRANUCLEAR DNA

- Maternal type of a heredity (mitochondrial DNA)

Heredity AR (Autosomal recessive)

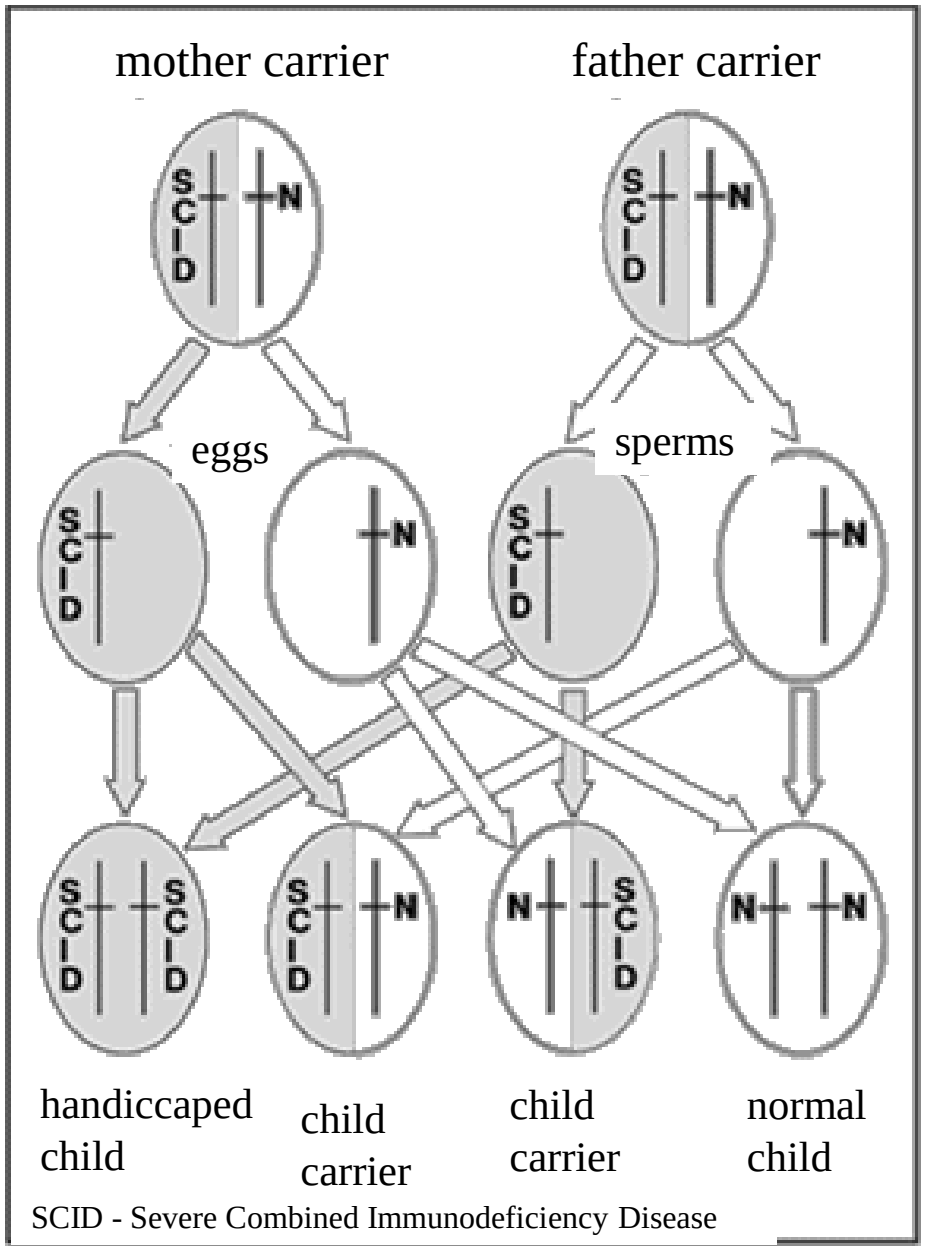
- The **vast majority HMD** - for example PKU
- The didease manifests only in a homozygot (it carries both defective alleles for the feature)
- Heterozygot is a clinically healthy individual, it carries a defective gene.

Heredity GR (Gonosomall recessive)

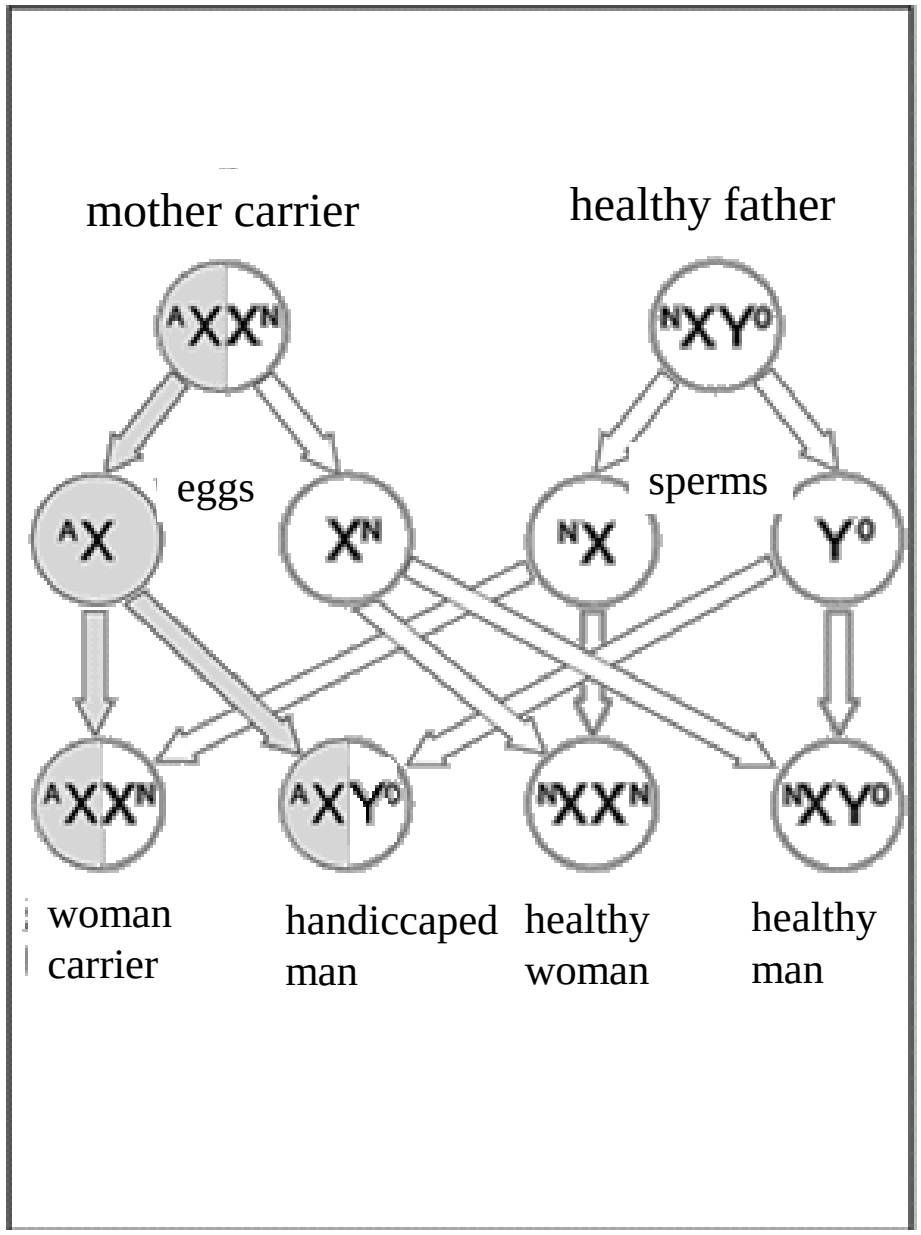
- An abnormal gene of a recessive type is bound to sexual chromosome X
- Clinically it manifests only in men (they have one X chromosome, women have XX)
- When one of parents is affects:
  - men are healthy or have the disease
  - women can be from 50% carriers

Examples: Hunter´s mucopolysaccharidosis, glycogenosis of VIII type

Graphic ilustration of AR and GR type of a heredity



Autosomal recessive heredity



Gonosomal recessive heredity

Maternal type of a heredity

- **Although mitochondrial DNA (mtDNA) has a negligible volume unlike nuclear DNA, the mutations in mtDNA can cause severe diseases.**
- **1) Every individual inherits all mitochondrias only after the mother (mitochondrias of zygote come from the egg, all mitochondrias of sperm vanish).**
  2) In every cell is about 1000 mitochondrias - 1 mitochondria with mutate mtDNA has no impact to the cell. If the mutation in mtDNA manifests in a cellular level or in a whole organism depends on how many percent of mitochondrias have mutate genetic information.

Classification of HMD

1. According a speed of appearing of clinical signs
2. According of individual metabolic systems
3. Acccording a subcellular localization of changed protein
4. According an analytical methodics which are used for an evidence of HMD

1.According a speed of appearing of clinical signs – diseases:

- Akute metabolic
- With an intermitent development
- Chronically progressive

2. According of individual metabolic systems – disorders of metabolism

- **aminoacids**
- **carbohydrates**
- **lipids**
- **purines and pyrimidines**
- **High molecular weithgh compounds**
- **pigments etc.**

3. Acccording a subcellular localization of changed protein – HMD:

- **cytosolic**
- **mitochondrial**
- **lysosomal**
- **peroxisomal**
- **Golgi apparatus**
- **iont channels etc.**

Clinic of HMD

- **Exhibitions of DPM – in all age from the birth to an adulthood**
- **Manifestation – varied, from mild chronically developing forms to acute life threatening states**
- **A seriousness depends on a level of a disability of changed protein (for example an activity of enzyme 0-20%)**

Clinical signs of HMD

- Non-specific – most (PMR, disorders of muscle tension, disorders of behaviour, disorders of consciousness, convulsions, failure to thrive, vomiting, disorders of heart functions, muscles, liver, kidneys…
- Specific – for example typical abnormal bad smell of urine, sweat…,ectopia of a lens and tromboembolic incidents

Laboratory non-specific discoveries

- **Acidosis (for example lactic when it is a deficit of PDH)**
- **Alkalosis (for example deficit of OTC)**
- **Hypoglycaemia**
- **Hyperammonemia**
- **Hypoketosis (with hypoglycaemia – disorders of β-oxidation)**
- **Hyperketosis (some org. aciduria)**
- **Hypouricemia/hyperuricemia(disorder of met. purines)**
- **Hypocholesterolemia/hypercholesterolemia (deficit 7-dehydrocholesterol - Smith-Lemli-Opitz sy)**

1. Acute metabolic diseases

- Beggining: usually in early neonatal or early infant period
- Signs: respiratory failure, sepsis, convulsions, disorders of consciousness, protracted jaundice, development of RDS či DIC etc.
- Examples: disorders of metabolism of AMK, galactose, ureagenesis, organic acids, β-oxidation of fatty acids

2. Metabolic diseases with chronical development

- Characteristic: rotation of asymptomatic period with attacks, which typically appear after a load for example a change of nutrition (protein load), febrile period(increased energetic need of organism in developnent of catabolism)…
- Examples: late forms of OTC deficit, some disorders of β- oxidation of fatty acids

3. Chronically progressive metabolic diseases

- Characteristic: at the beginning normal psychomotoric development stops after the definite period, alternatively a regression comes
- Examples: storage diseases (mucopolysaccharidosis, neurodegenerative diseases…)

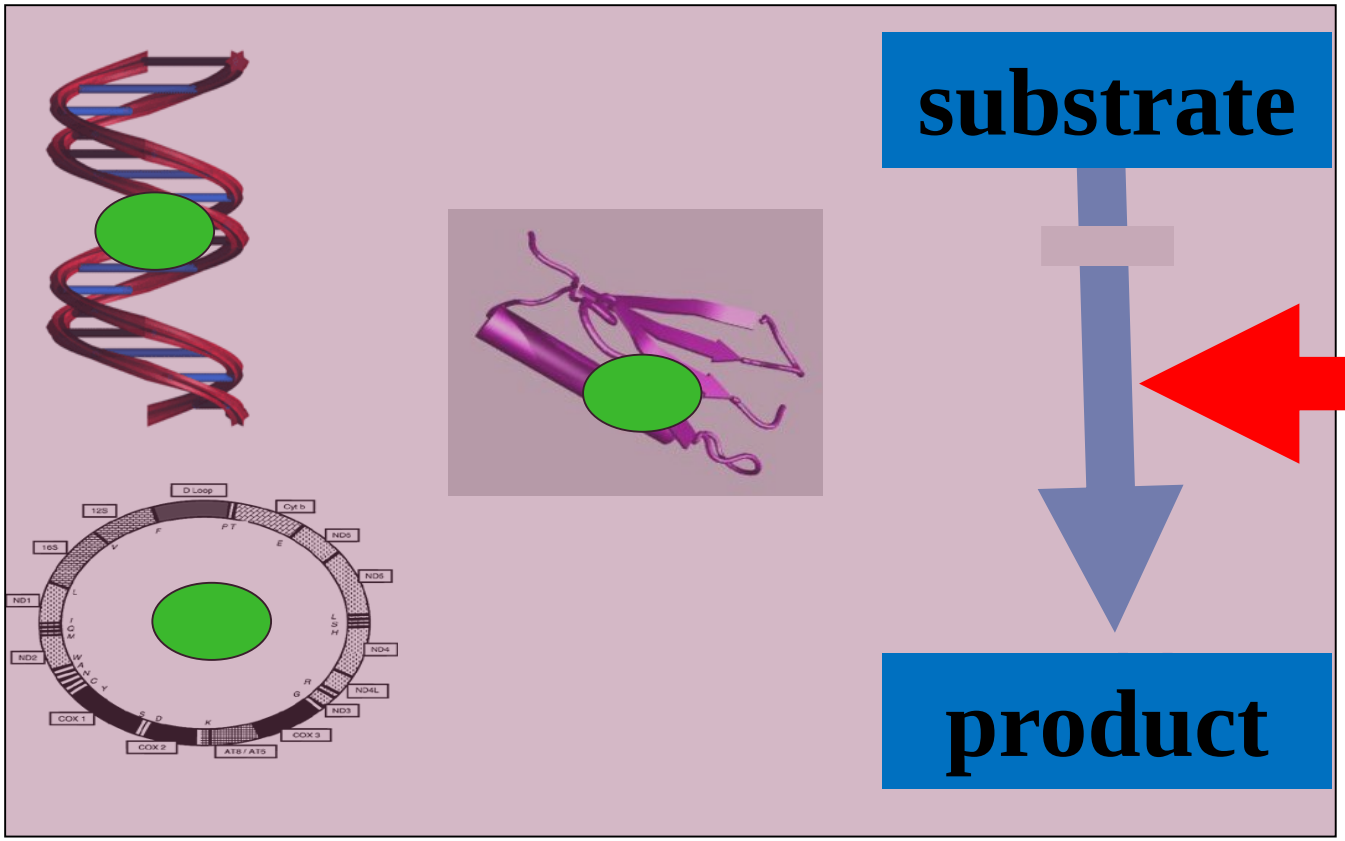
Examples of the most famous HMD

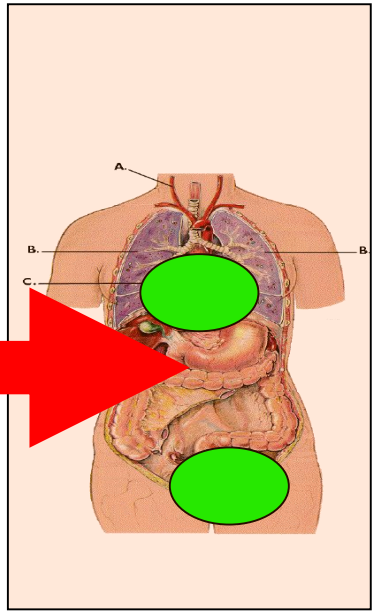
- Disorders of metabolism of aminoacids
- Organic acidurias
- Disorders of metabolism of saccharids
- Disorders of metabolism of lipoproteins
- Disorders of metabolism ofpurines and pyrimidines
- Disorders of metabolism of vysocomol. compounds

Laboratoroty diagnostics of HMD

1. **In a level of metabolites**
2. **In a level of enzymes**
3. **In a molecular level**

Diagnosis HMD





DNA/RNA | **Enzymology** | **Metabolites**

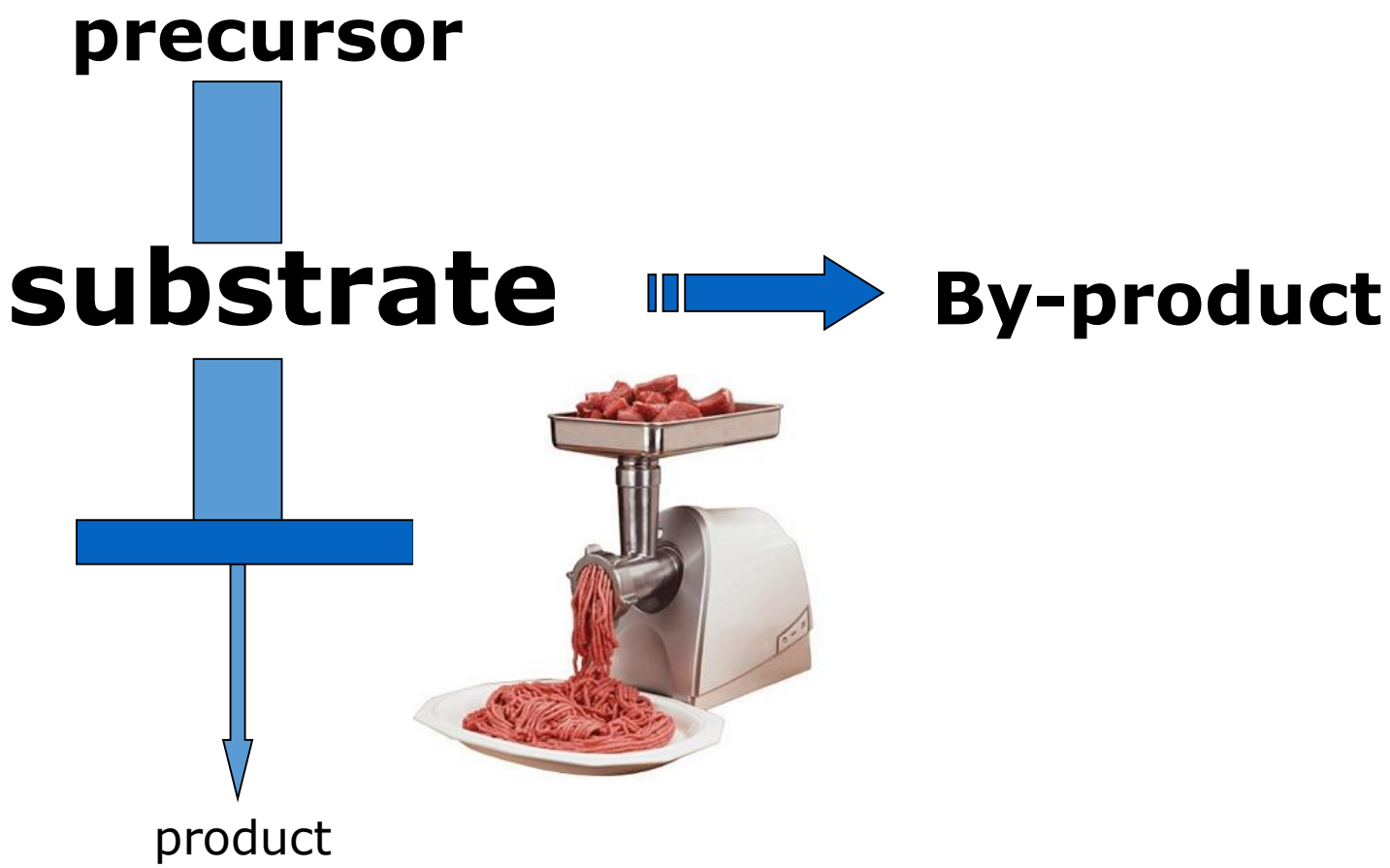
Symptoms

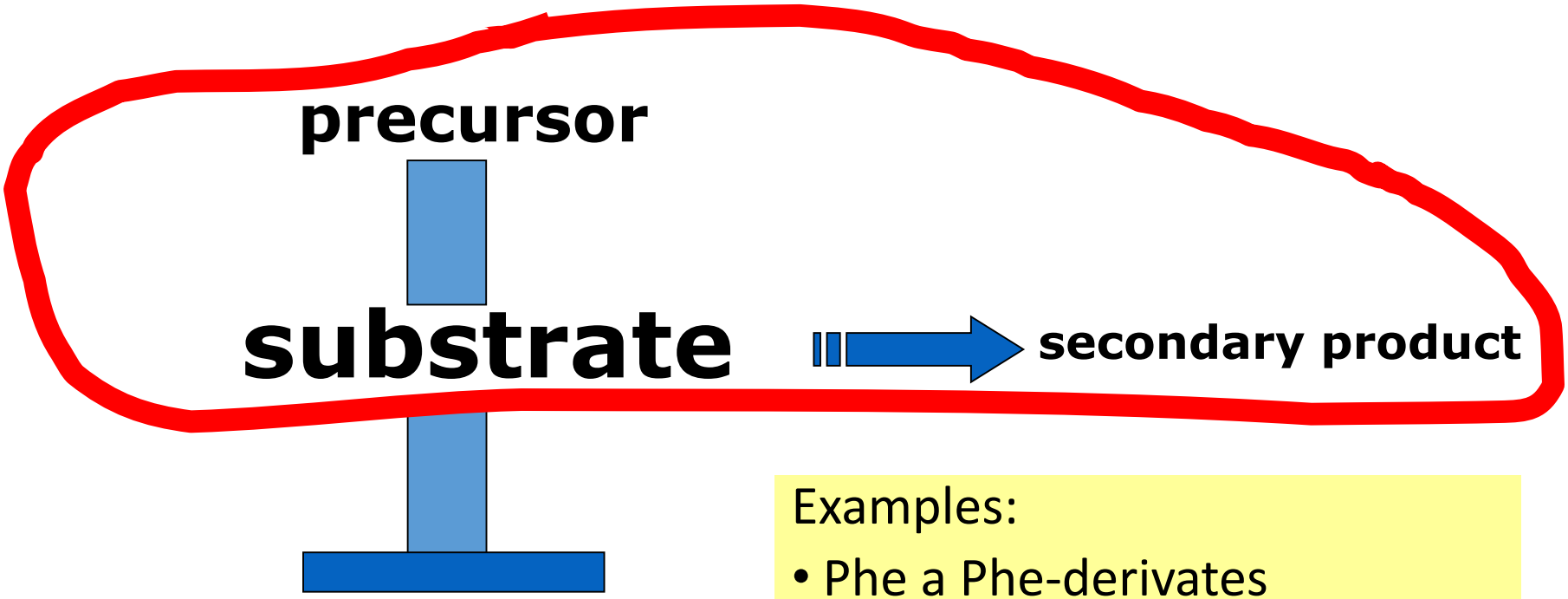
Specific – for example:.

- **odor**
- **urine color**

Nonspecific – for example:

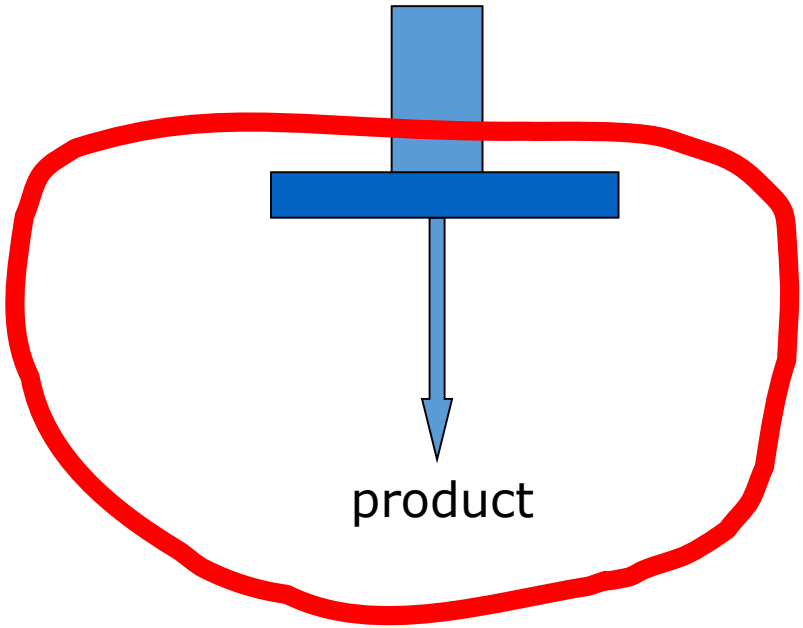
- **coma**
- **PMR**
- **dysmorphia**
- **hepatho/myopathies**
- **and so on**


precursor
substrate
By-product
product



Examples:

- Phe a Phe-derivates
- ammonia
- cystine in cystinosis
- cystine in cystinuria
- mucopolysaccharides



Examples:

- glucose in GSD
- ketone bodies in beta-oxidation disorders of fatty acids
- plasmalogenes in peroxisomal disorders
- cysteine in deficiency of CBS
- AdoMet in RM
- ATP in mitochondrial diseases

1.Diagnostics in a level of metabolites

- Characteristic: we prove a changed concentration of a metabolite(substrate, product, abnormal metabolit). The oldest simplest and most spread.
- Utilization: where an enzyme or a transport protein is a defective protein → in a place of metabolic block a substrate accumulates and a product misses, alternatively other metabolites are synthesized consequantly an activation of alternative metabolic pathways
- Material: serum or plasma, urine, liquor, whole blood in the form of dried blood spots on a filtering paper

Diagnosis HMD

Laboratory diagnosis HMD – on several levels:

- **Prenatal diagnosis** – examination to determine whether the fetus is affected by the HMD, which was demonstrated in the family – only justified cases (AFP, defect in the family)
- **Postnatal diagnosis** - *neonatal screening (PKU hypothyreosis etc.)*

Most of the HMD can be diagnosed prenatally - analyzing the enzyme activity or mutation in chorionic villi or amniocytes or by investigation of metabolites in amniotic fluid.

- Early diagnosis – **treatment, compensation**

1. Diagnosis at the level of metabolites - continuing

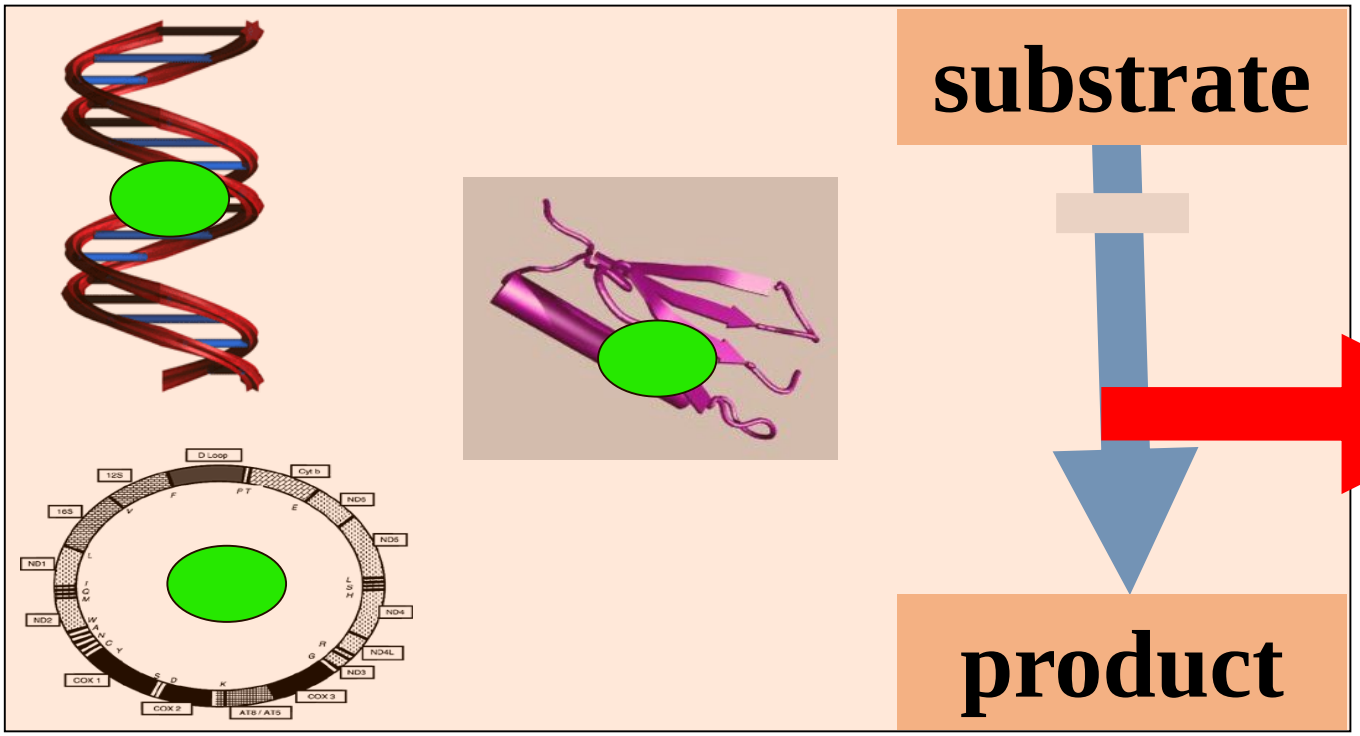
- Investigated metabolites: amino acids, carbohydrates, oligosaccharides, glycosaminoglycans, purines, pyrymidines,lipids, steroids etc.
- Used laboratory techniques:

chromatography - paper
- thin layer
- liquid (ion-exchange, high-performance HPLC)
- gas (mass spectrometry GC/MS)

electromigration techniques
- electrophoresis
- capillary electrophoresis

tandem mass spectrometry MS/MS

Presymptomatic diagnosis



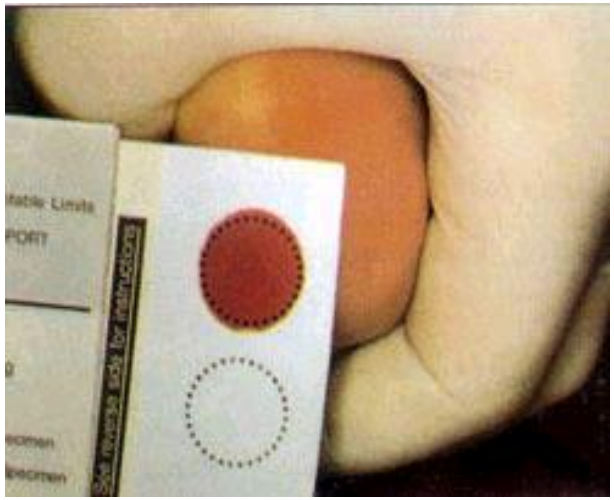
examination related risk HMD prenatal diagnosis

screening population segment

neonatal screening

one disease or group of diseases known in advance

Definition:



Neonatal screening (NS) = active nationwide search the disease in its preclinical stage.

The analysis of dried blood in filter paper collected by standard procedures from the footer of all neonates.

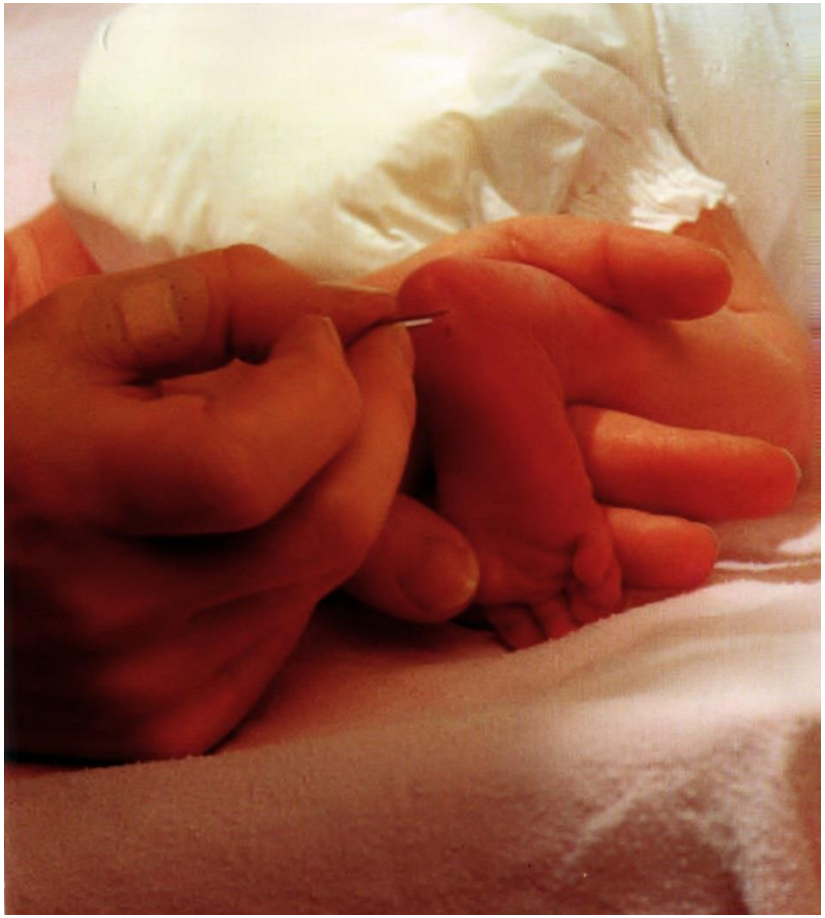
Hyperphenylalaninemia/ phenylketonuria

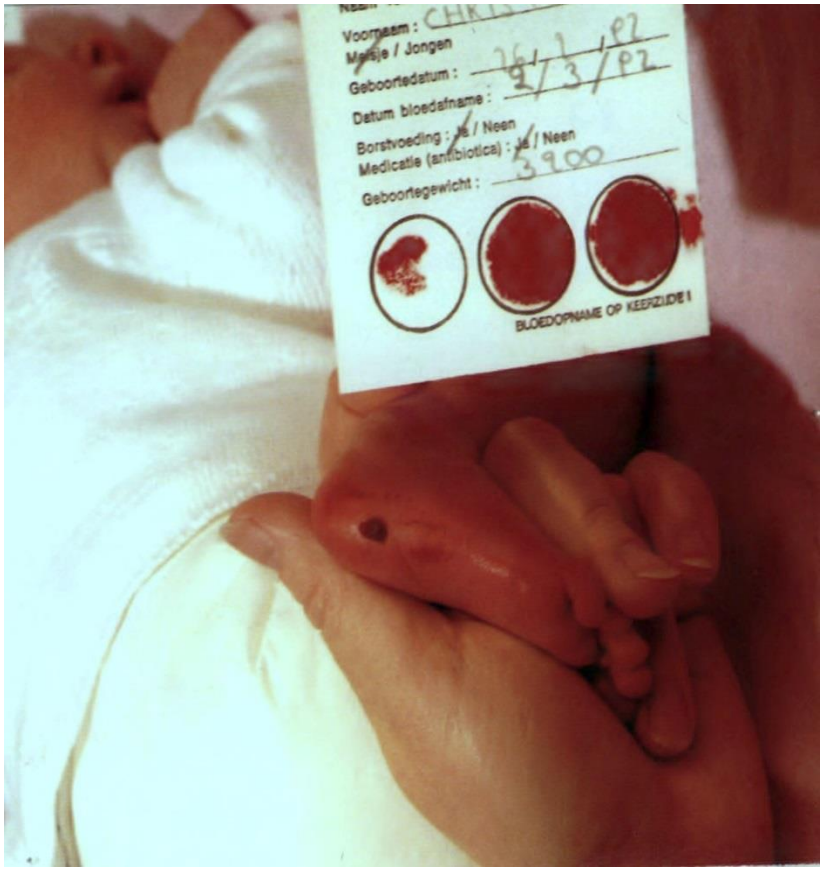
- Characteristics: insufficient conversion of Phe to Tyr
- Cause:

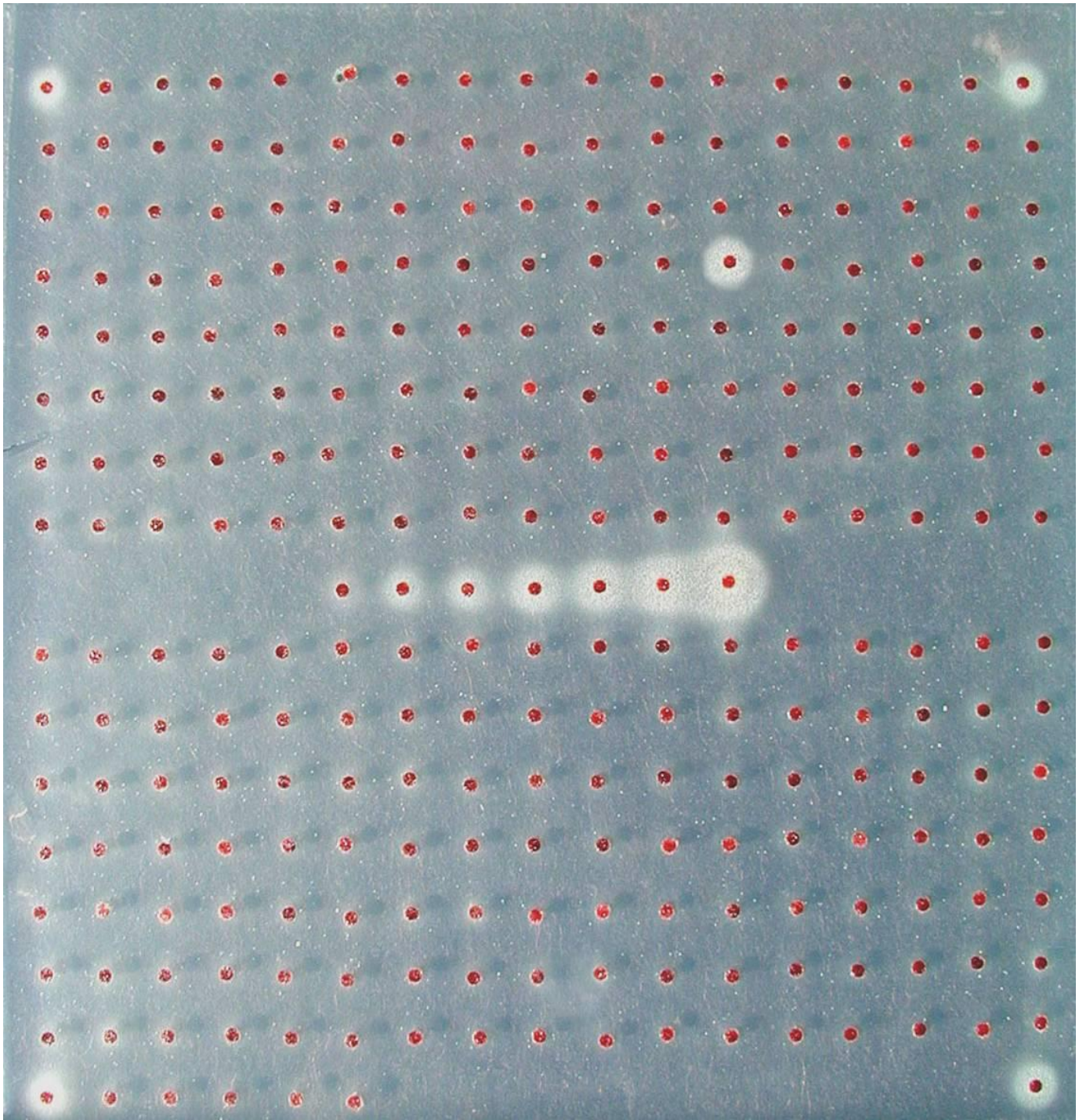
1) Deficiency phenylalaninhydroxylase
2) Disorder of the coenzyme tetrahydrobiopterin metabolism

- Occurrence: about 1:10 000, the most common DPM
- Neonatal screening- in Czech republic from 1975 nationwide – Guthrieho test

Blood collection from neonatal screening







Classical criterion for screening

- generally recognized screening test
- credibility of the scr. test: cut-off, fal.neg. Load the healthy population: recally, fal.poz.
- the company is able to secure NS and aftercare of patients retained the organization and economic

Classical criterion for screening

- Diagnostic costs and treatment should be economically balanced in the health care system
- NS is a positive contribution towards the cost of „benefit/cost“
- NS is a continuous process – efficiency must be constantly evaluated

Screening

- ***Screening*** = method for detecting early forms of disease or deviations from the norm in a given population through test
- is performed on all neonates born in the Czech republic
  - rapid diagnosis and early treatment mainly inherited metabolic disorders
  - **confirm / refute the disease** before its symptoms and damage to child

Method of sampling drops of blood from the footer to the neonatal screening card

The development of the neonatal screening

1962 - founder - prof. Robert Guthrie – introduced a bacterial test for the early detection and ***PKU and hyperphenylalaninemia*** in USA (using the strain of the Bacillus subtilis, they proliferate in the environment of high concentration of phenylalanine)

- **from 1969 Guthrieho method in Czech republic**, **allover screening up from 1975**
- from 1985 – the extension of the screening test for ***congenital hypothyroidism (CH)*** – *iodines deficit fetus, severe damage to the developing brain of a child*
- from 2006 – increasing testing of ***congenital adrenal hyperplasia*** *(CAH)* – previously called adrenogenital syndrome
- **2009** – changes and extensions of screening (according to the Bulletin of the Ministry of Health)

  - screening is expanded to include of the ***screening cystic fibrosis***

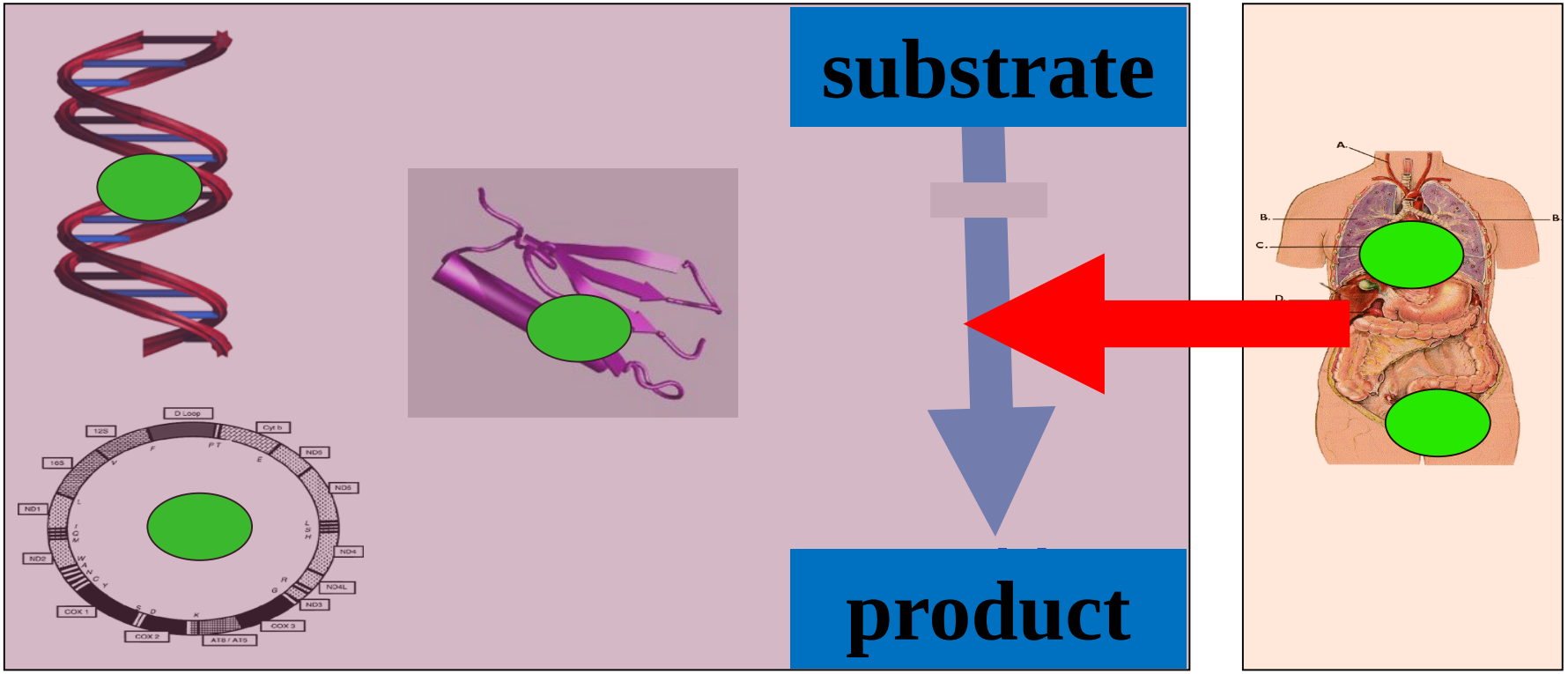
2. Diagnostics at the level of enzymes

- Characteristics: show reduced activity of the affected enzymes. Testing is difficult (economically costly, often greater burden for the patient – material removal).
- Use: in prenatal diagnosis, to confirm the appropriate DPM, normally precedes testing at the level of metabolites
- Material: leukocytes, erythrocytes and trombocytes isolated from peripheral blood, serum or plasma, culture of skin fibroblast, tissue from muscle or liver biopsy

3. Diagnostics on the molecular level

- Characteristics: diagnosis at the DNA level shows you the defective gene. Economically costly, indicate wisely
- Use: to definitively confirm the diagnosis, where it can be clearly do so on the basis testing of metabolites or enzymes, followed by genetic consulting
- Materiál: leukocytes from peripheral blood, cells from amniotic fluid obtained by amniocentesis, chorionic villus cells obtained by biopsy of the placenta

Symptomatic diagnosis



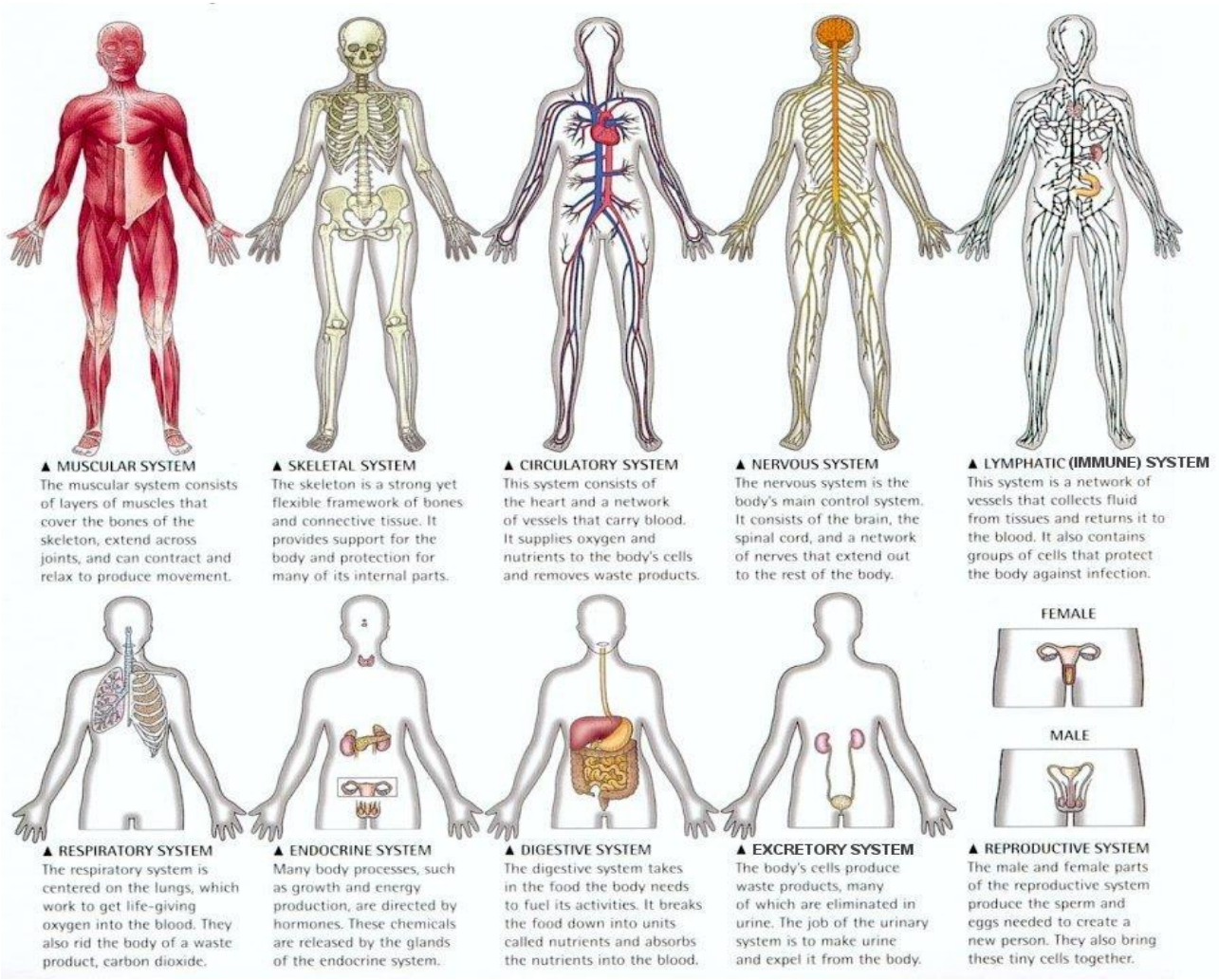
DNA/RNA
- screening
- scanning
- sequencing

Enzymology
- leuko/lymfo
- fibro
- muscle

Metabolites
- analyte
- profile of analytes
- urine/blood/liquor

Symptoms
- specific
- nonspecific
  - coma
  - PMR
  - dysmorphia
  - hepato/myopathies

The clinical picture of HMD - bodies



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The basic situation of the differential diagnosis of HMDT

- Small molecules
  - acutely ill newborn baby
  - (repeated) prolonged unconsciousness attack
  - failure to thrive infants
  - hypoglycaemia

- Large molecules
  - progressive disabilities of CNS and muscle
  - facial dysmorphia
  - organomegaly (liver, spleen, heart)

Abnormal smell and color of urine

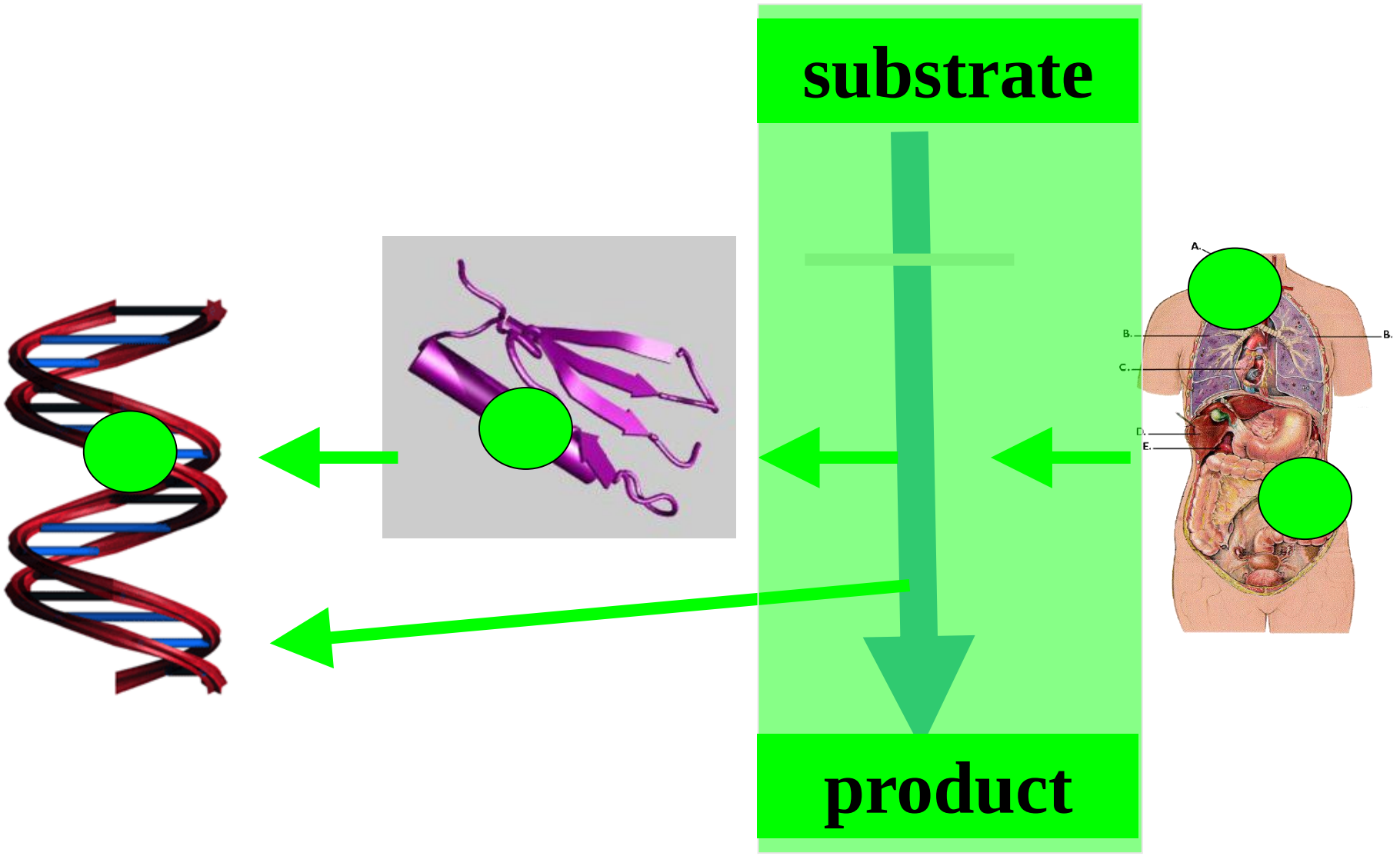
- smell (small volatile molecules):
  - sweaty feet - isovalerate
  - caramel/maple syrup - oxoacids
  - cooked cabbage - methionine oxide
  - fish smell - trimethylamine
  - black currant – some organic acids
  - mouse smell - phenylacetate
- coloring
  - red-orange - urate
  - black-brown in the oxidation – homogentisate
  - blue – indoxalid derivates
  - green - 4-OH-butyrate

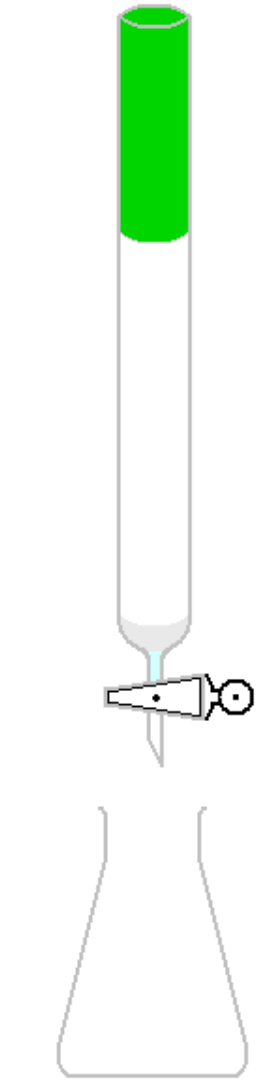
Common laboratory findings in HMD

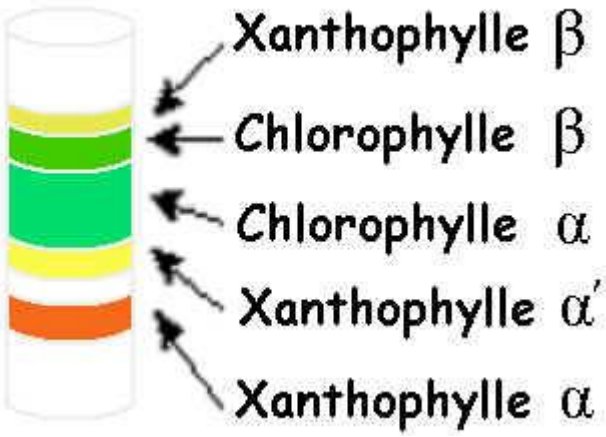
Blood
- glycemia
- cholesterol
- TG
- urine acid
- MAc
- hyperamonemia, RAlk
- ALT, AST
- CK
- anemia/pancytopenia

Urine
- ketones
- urine acid
- crystalluria
- myoglobinuria

HMD-diagnostic of metabolites







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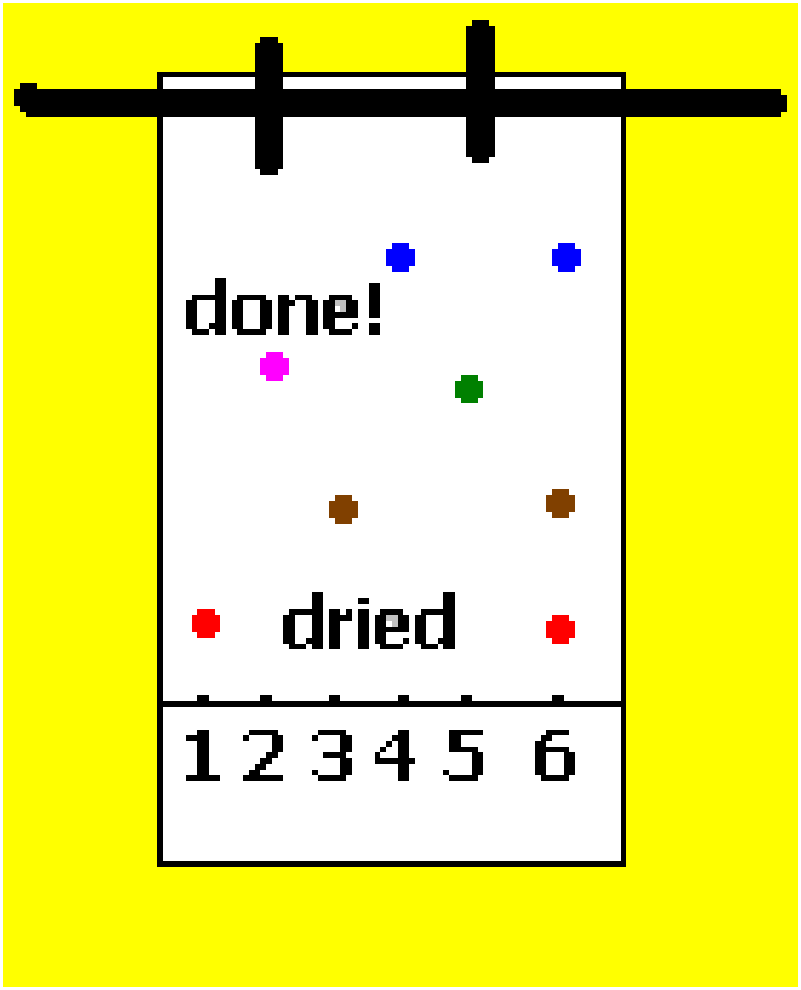
Principle of PC



Sensitivity of methods

- Alkaptonuria: 1-5 g homogentisate /day
- Cystinuria: 1-5 g cystine/day

Urine – liters for analysis

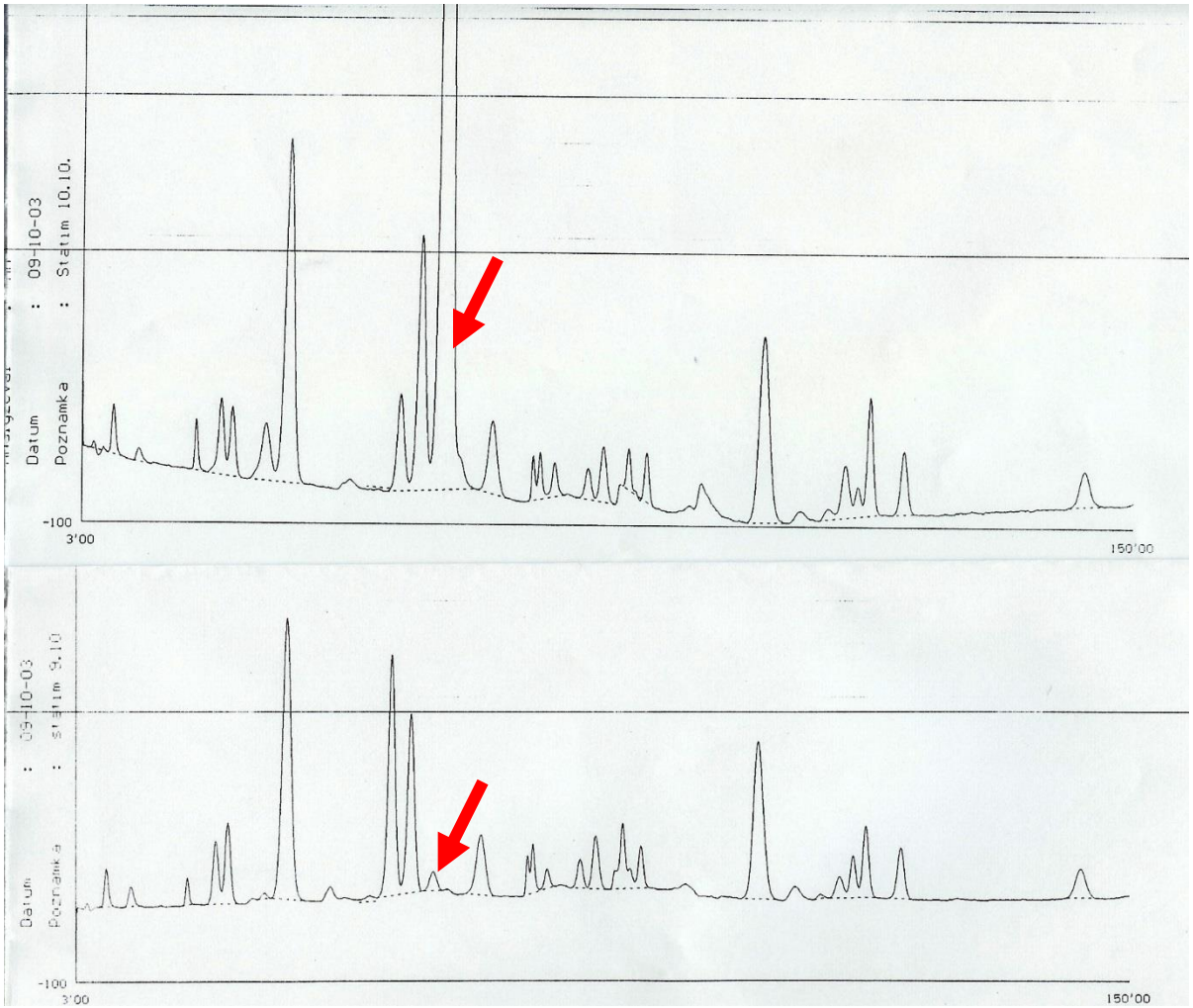
- Phenylketonuria: 0.1 g phenylalanine /l of blood

- MCAD: C8 acylcarnitine 0.0001 g / l of blood

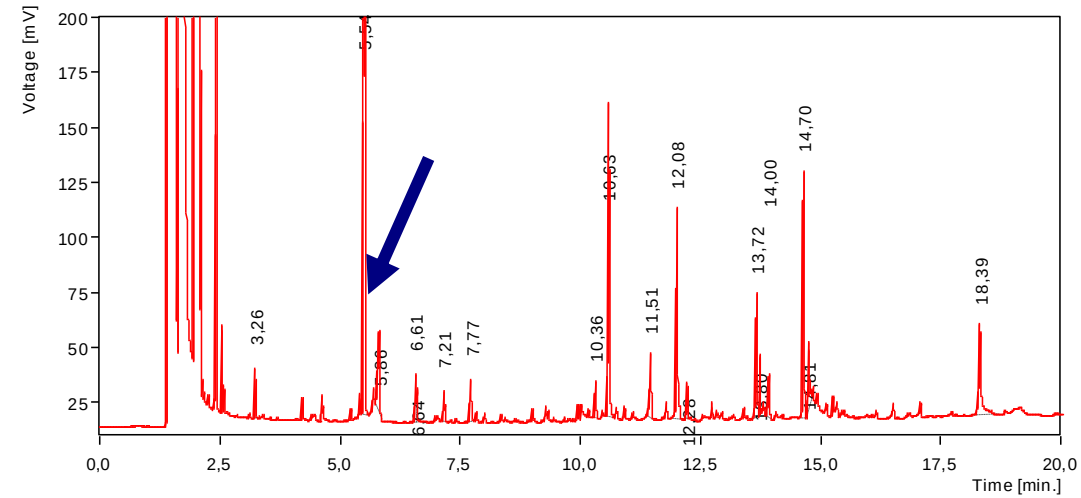
0.2 – 1 ml serum

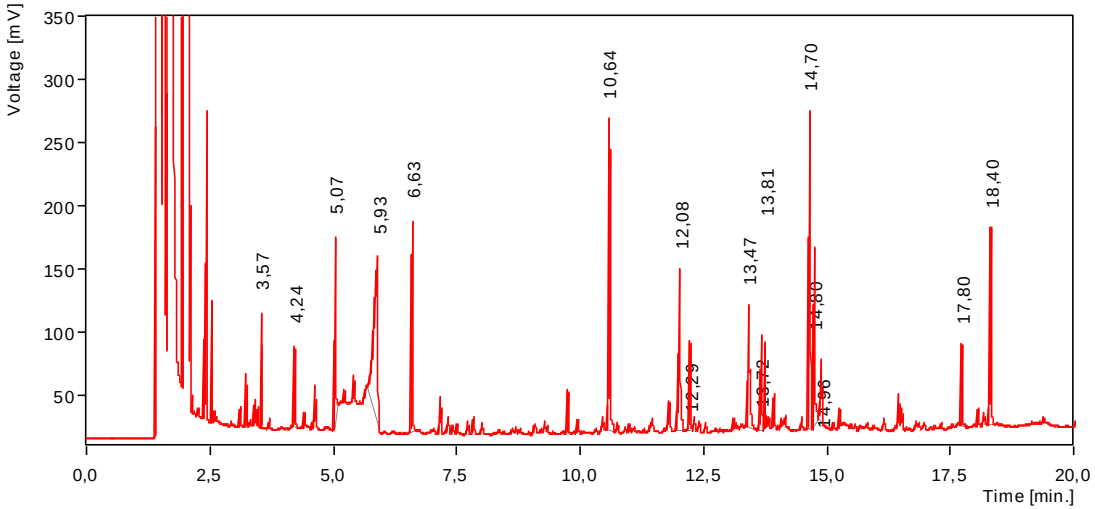
Blood paper about 0.05 ml of blood

Amino acids - citrulinemia

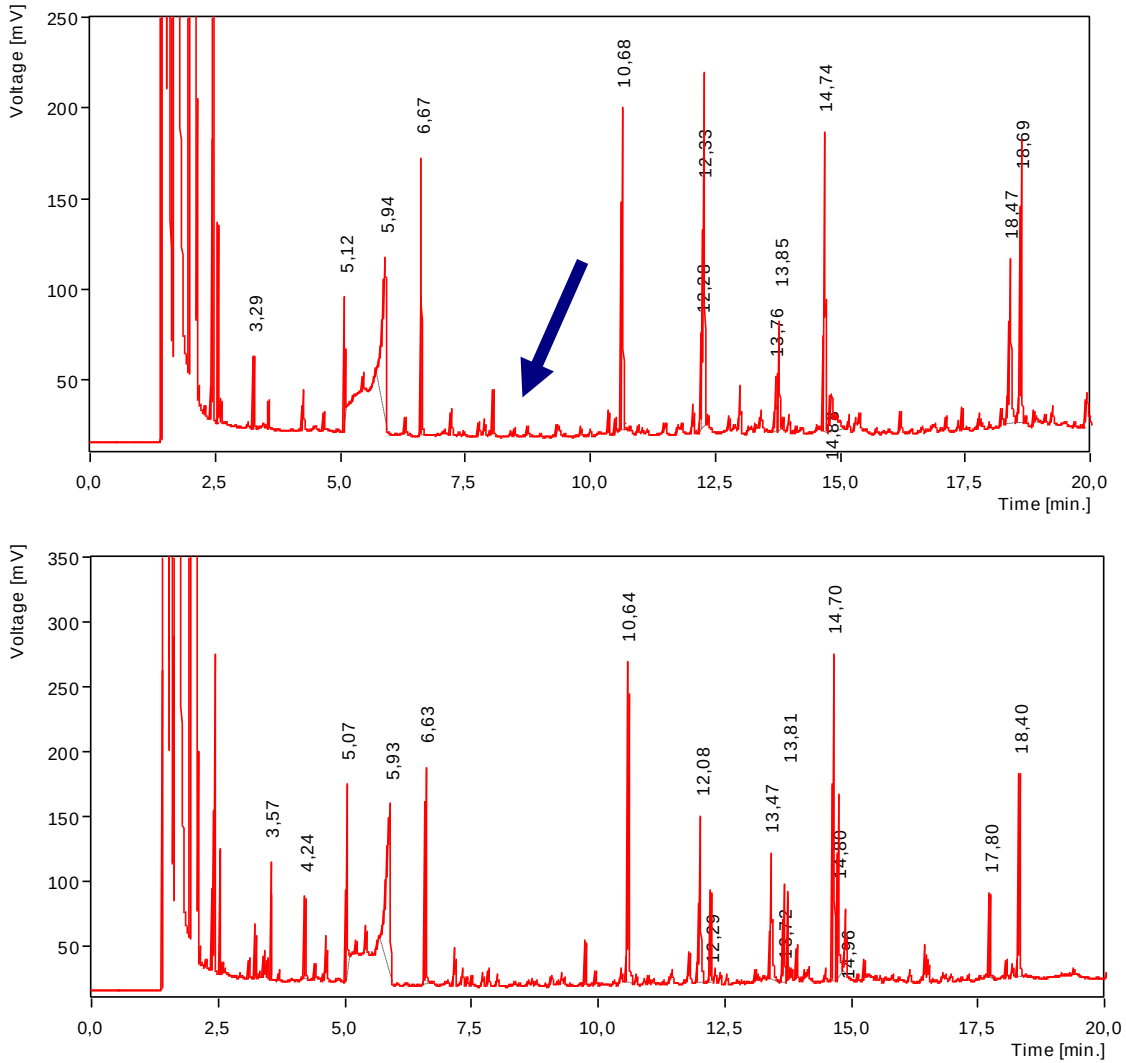


GC-MS: methylmalonic aciduria



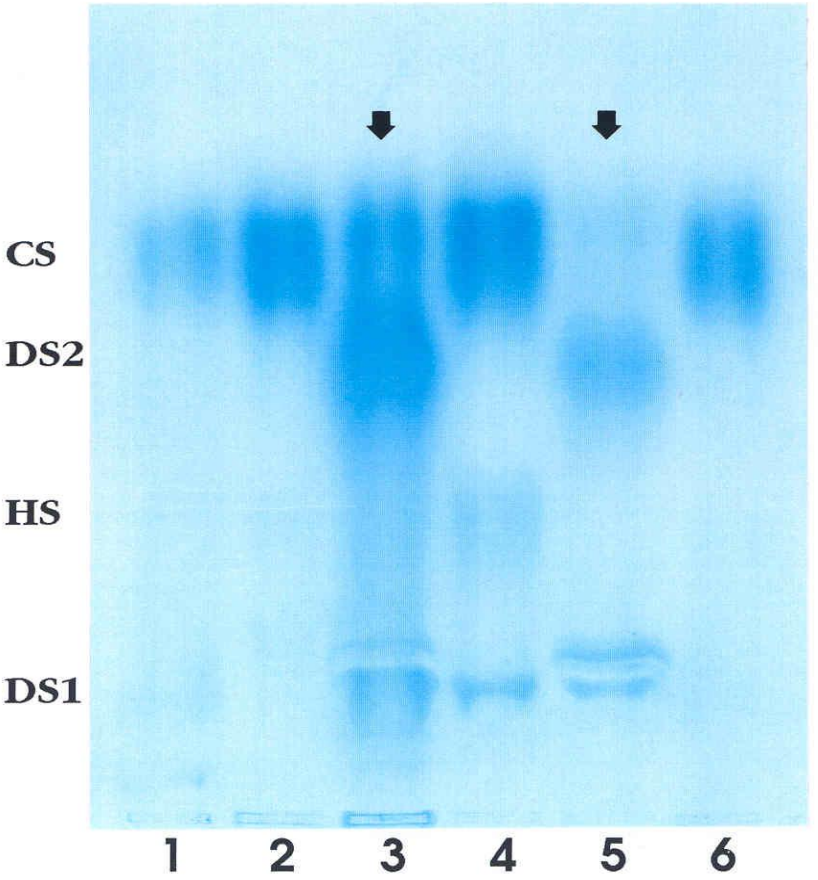


GC-MS: MCAD


Voltage [mV]
250
200
150
100
50
3,29
5,12
5,94
6,67
10,68
12,33
12,20
13,85
13,76
14,74
14,63
18,47
18,69
0,0
2,5
5,0
7,5
10,0
12,5
15,0
17,5
20,0
Time [min.]
Voltage [mV]
350
300
250
200
150
100
50
3,57
4,24
5,07
5,93
6,63
10,64
12,08
12,29
13,47
13,81
14,70
14,80
14,96
17,80
18,40
0,0
2,5
5,0
7,5
10,0
12,5
15,0
17,5
20,0
Time [min.]

MPS I – Hurler disease (deficiency of α –iduronidase)

MPS I in a 6-year-old girl

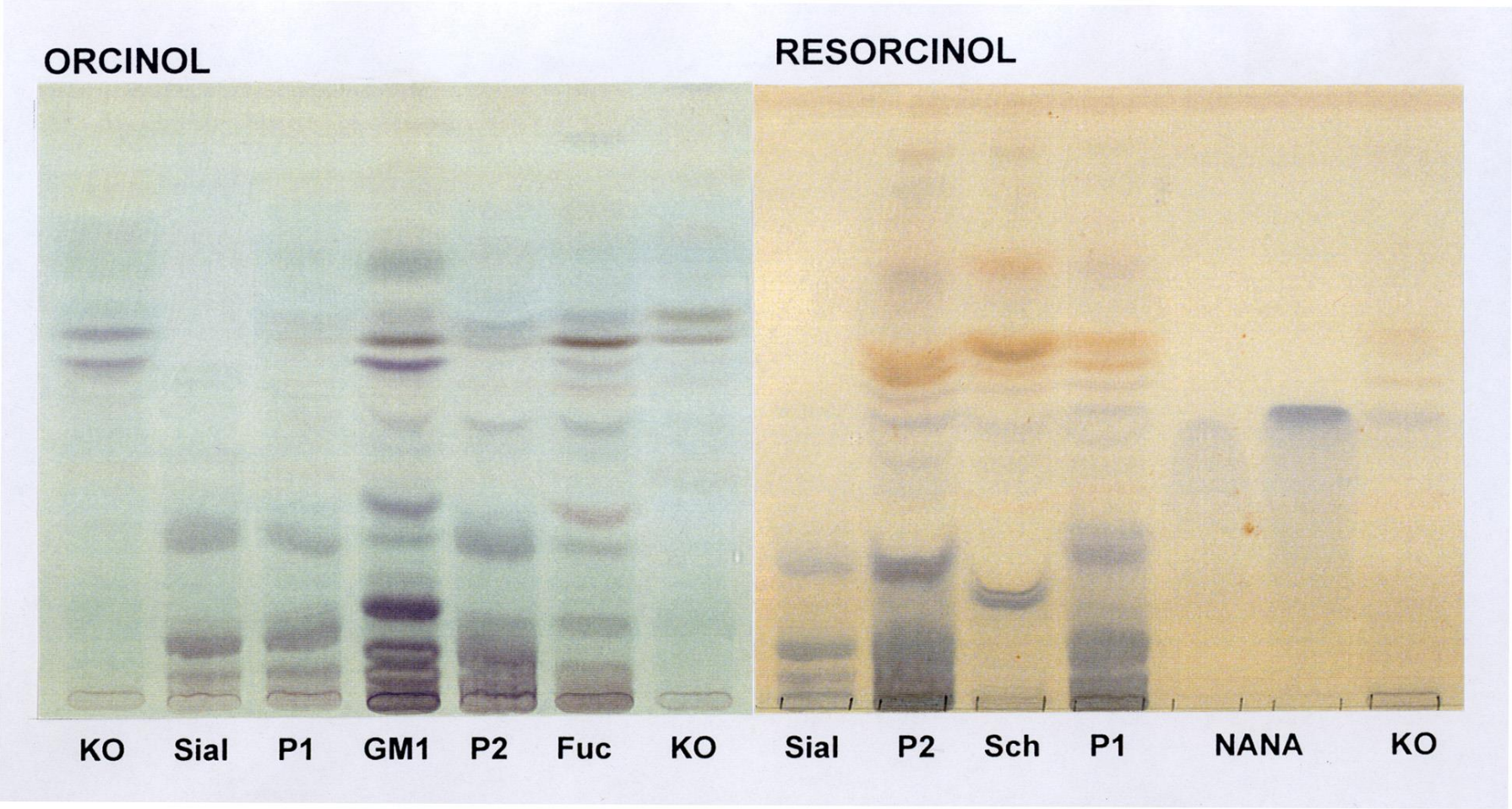


Electrophoresis of urinary GAGs

(excretion of dermatan sulphate/DS and heparan sulphate/HS)

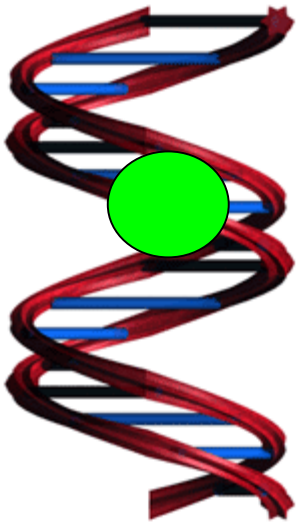
lent by Dr.Ledvinová

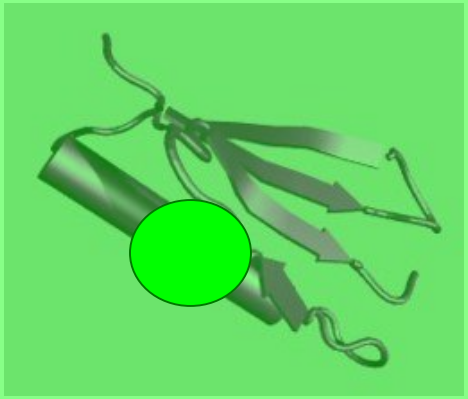
Glycoproteinosas – HPTLC oligosaccharides in urine



lent by Dr.Ledvinová

Diagnostics of HMD

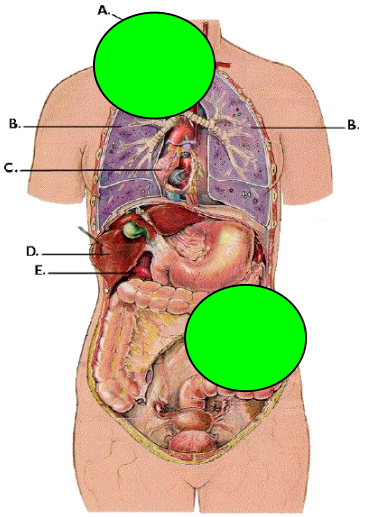




Substrate

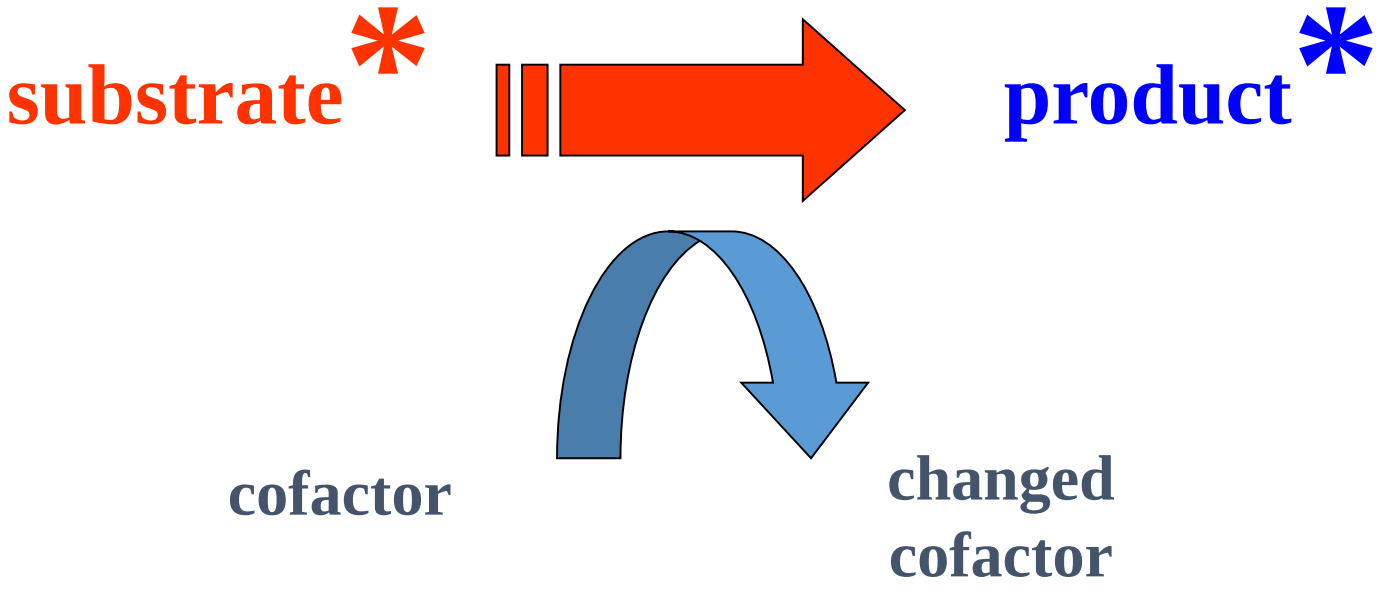
enzyme

product



Principles od enzymatology examination

- **Separation of substrate and product**
- **Quantification of gain or loss**



- **Quantification of gain or loss**

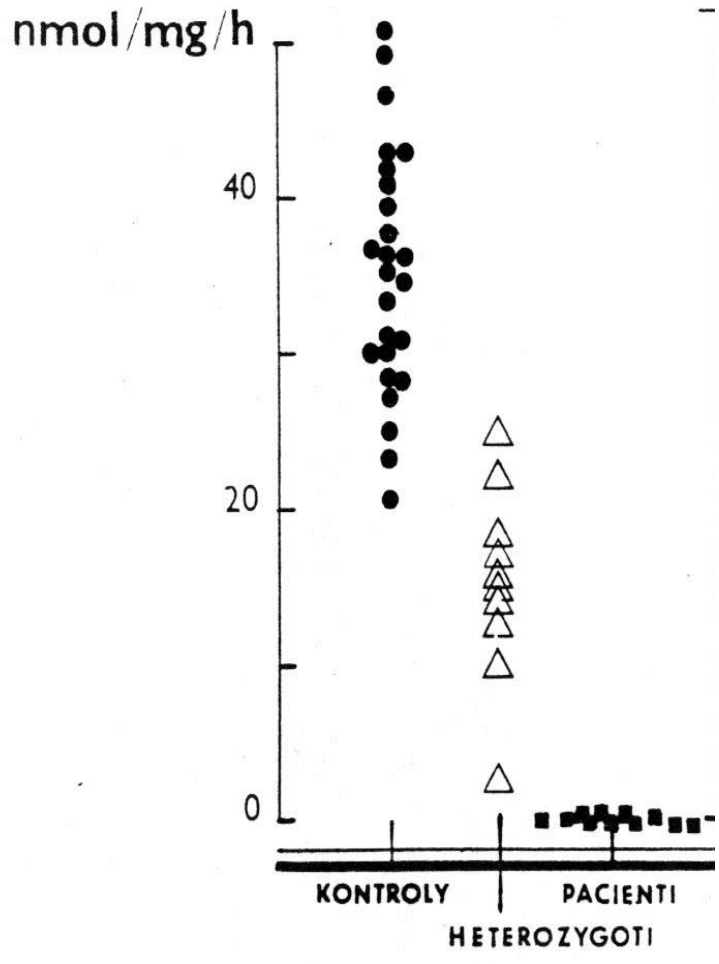
Assessment of enzymes in HMD

- **Cells are usually necessary**
- **Leukocytes, fibroblasts**
- **Fetal tissues and fetal (germ) layers**
- **Fluorimetric and radiometric techniques (eventually fotometric)**
- **Measured parameter: the loss of substrate or the product formation**

- **ÚHMD: 46 enzymes**

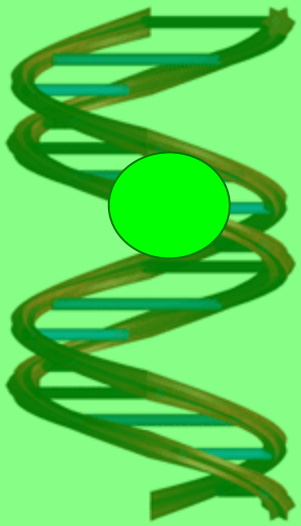
Typical results of enzymology

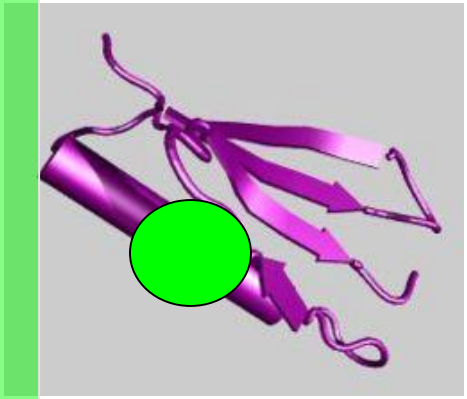
- Afflicted homozygotes clearly deficient
- Heterozygotes: overlay
- Healthy homozygotes: usually normal distribution of activity in population



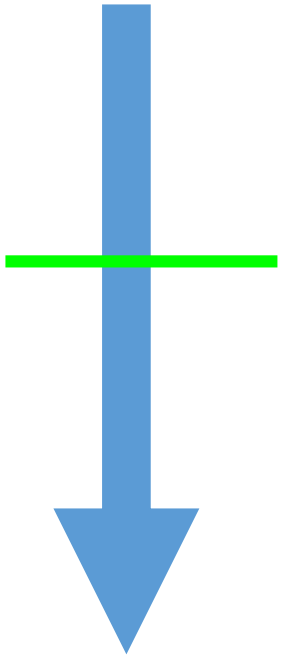
Diagnostics of HMD

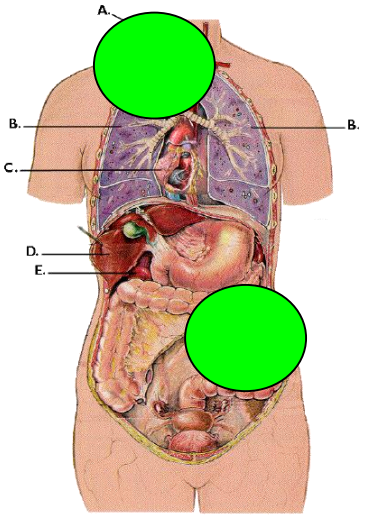
DNA





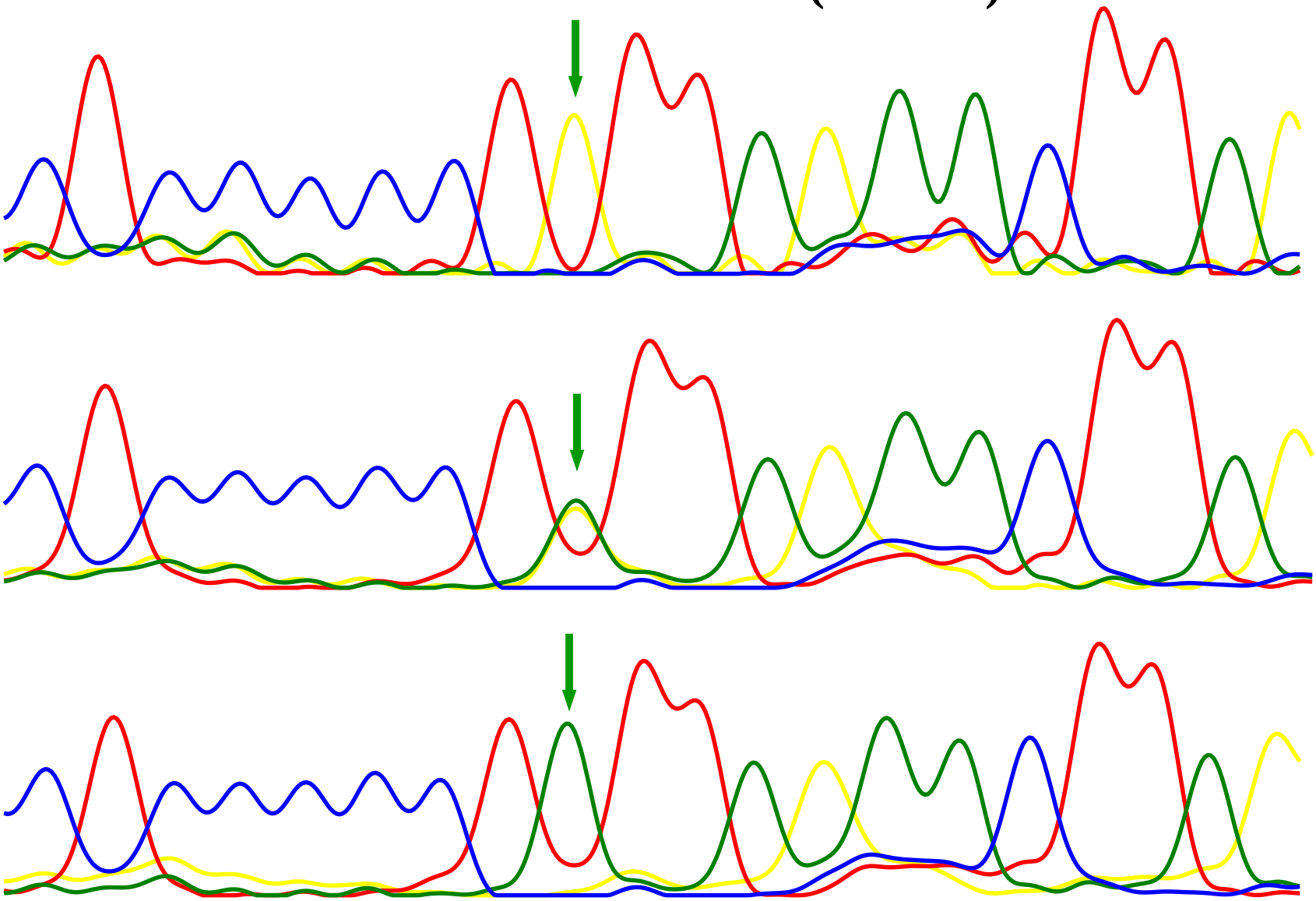
Substrate





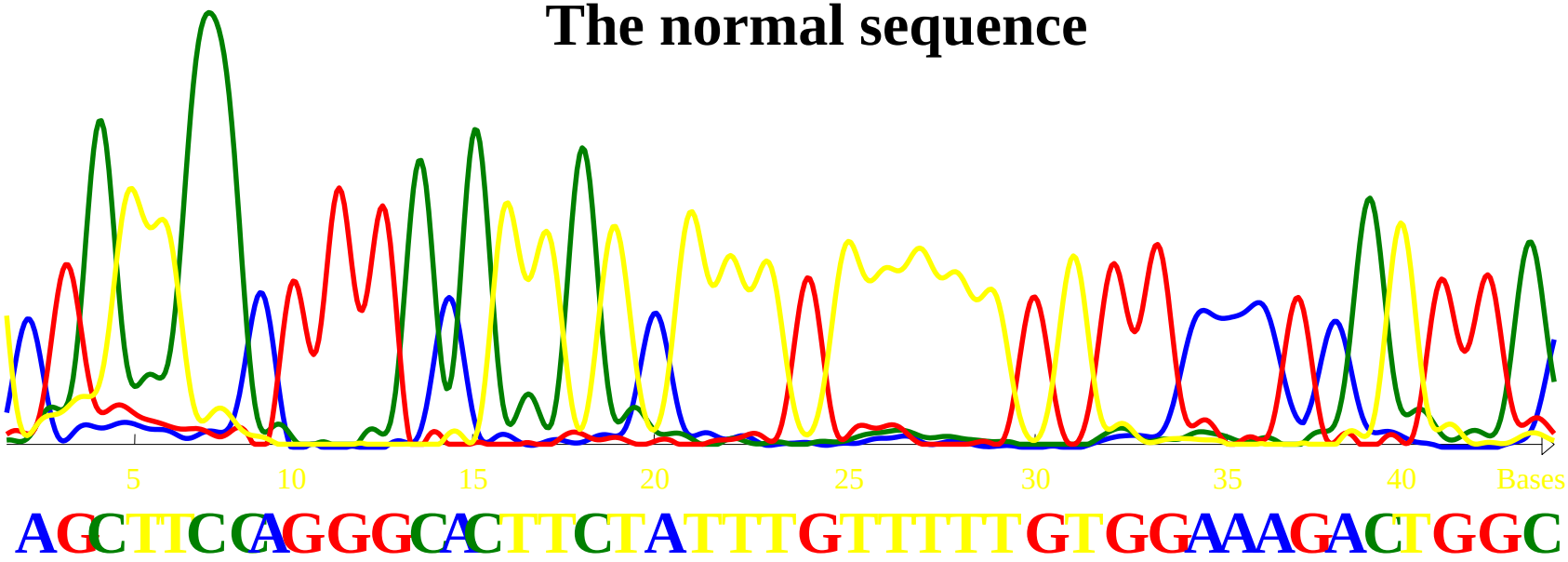
product

OTC: Mutation c. 829 C>T (R277W)

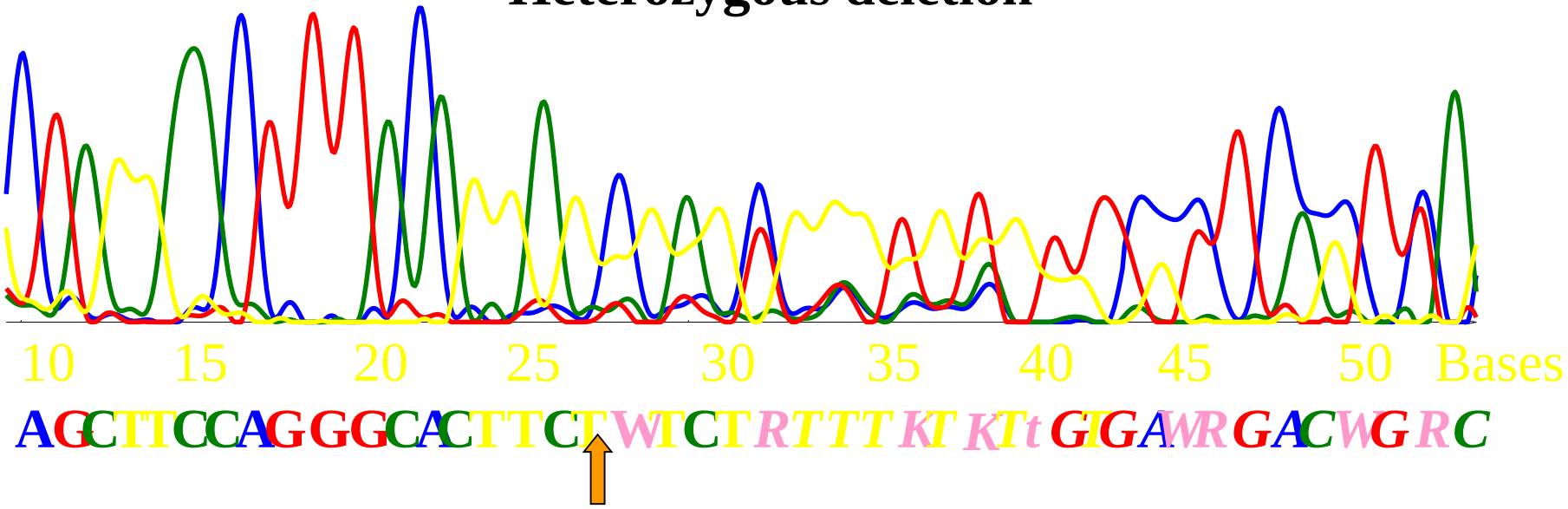


A G A A A A A G T/C G G C T C C A G G C T

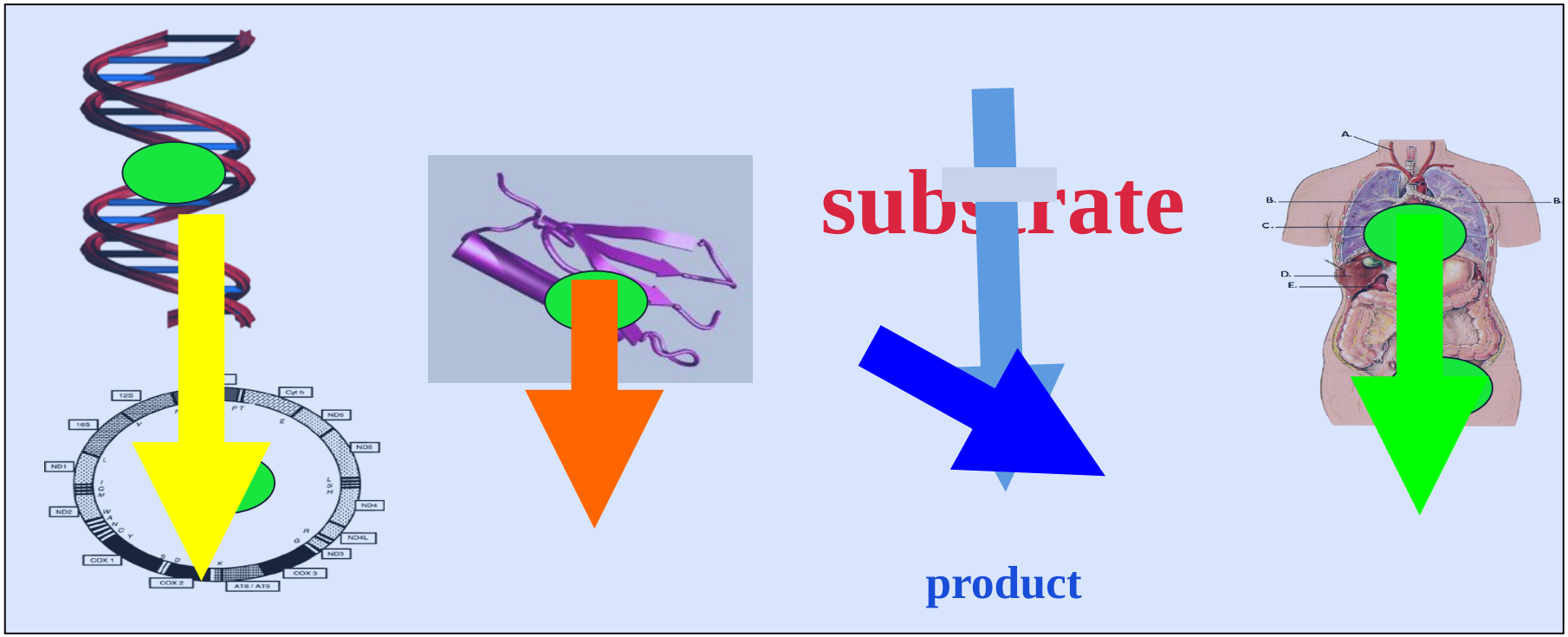
The normal sequence



Heterozygous deletion



Treatment of HMD



Treatment od HMD

1. **At the metabolite level**
2. **At the enzymatic level**
3. **At the cell level**

- The only causal treatment– at the cell level.
- Symptomatic and supportive treatment– mitigates syntomps, not removing the cause.

1. Treatment at the metabolite level

a) Restriction of the gain or the formation of toxic metabolites (eg. diet in PKU, galaktosemia, prevention of catabolism in aminoacidopathies, organic aciduries)
b) Removal of toxic metabolites(peritoneal dialysis, hemodialysis, exchange transfusion) and the use of alternative metabolic pathways(eg. benzoate administration in hyperammonemia)
c) Administration of metabolic inhibitors(eg. allopurinol in hyperuricemia)
d) Replacement of deficient products(eg. arginine in disorders of the urea cycle, tyrosine in PKU)

2. Treatment at the enzymatic level

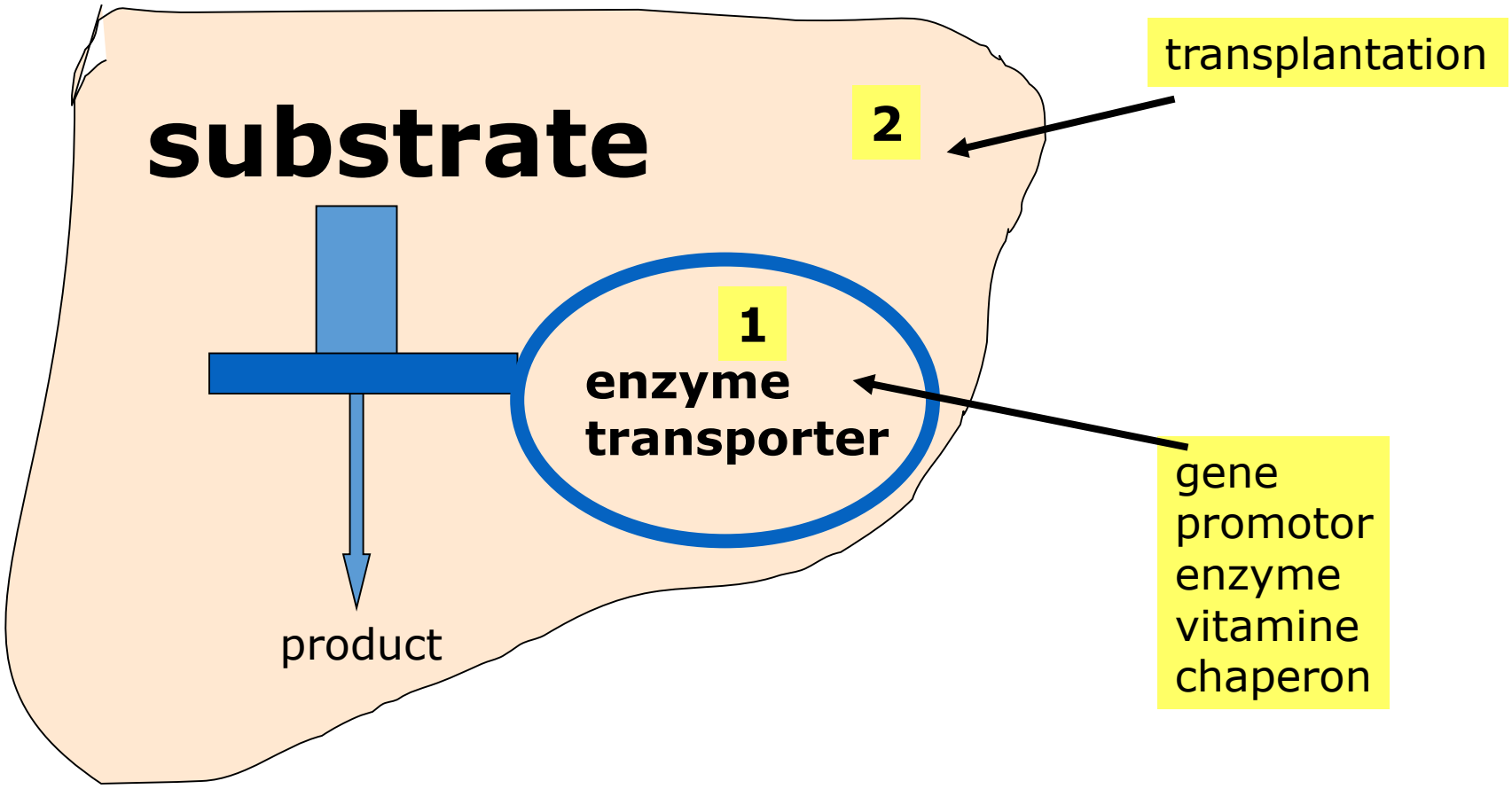
a) Activation of enzyme by coenzymes delivery at pharmacological doses(eg. pyridoxine in deficiency of cystathionine β-synázy)

b) Delivery of the deficient enzyme directly - enzyme (eg. in Gaucher and Fabry disease, some types mukopolysachyridoses or glycogenoses)

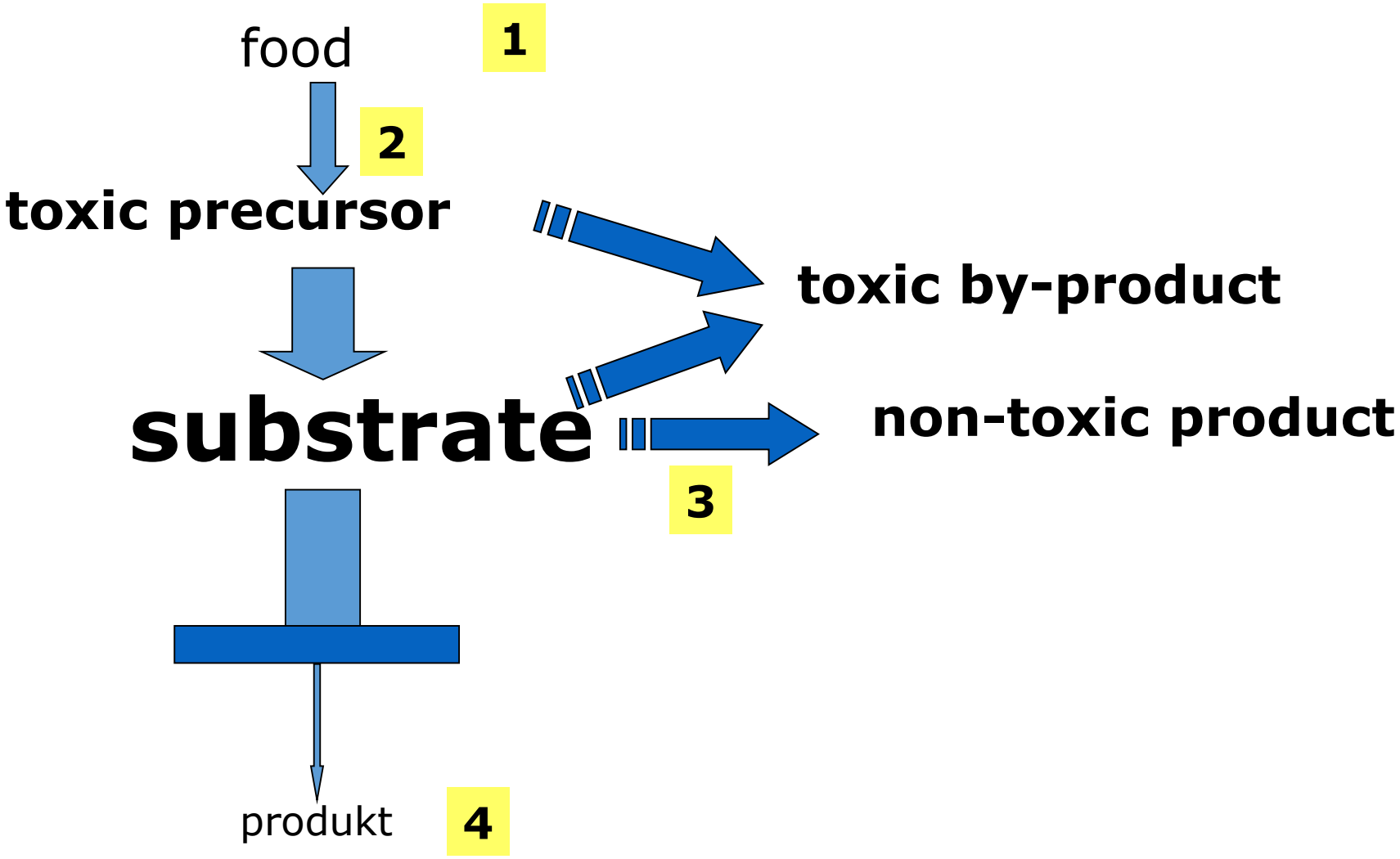
3. Treatment at the cell level

- Gene therapy with viral or non-viral vectors (yet with no DPM is not used routinely, has its pitfalls
- A special place in the treatment takes transplantation of organs and tissues (eg. liver in tyrosinemia , kidney in cystinosis, bone marrow in adrenaleukodystrofia)

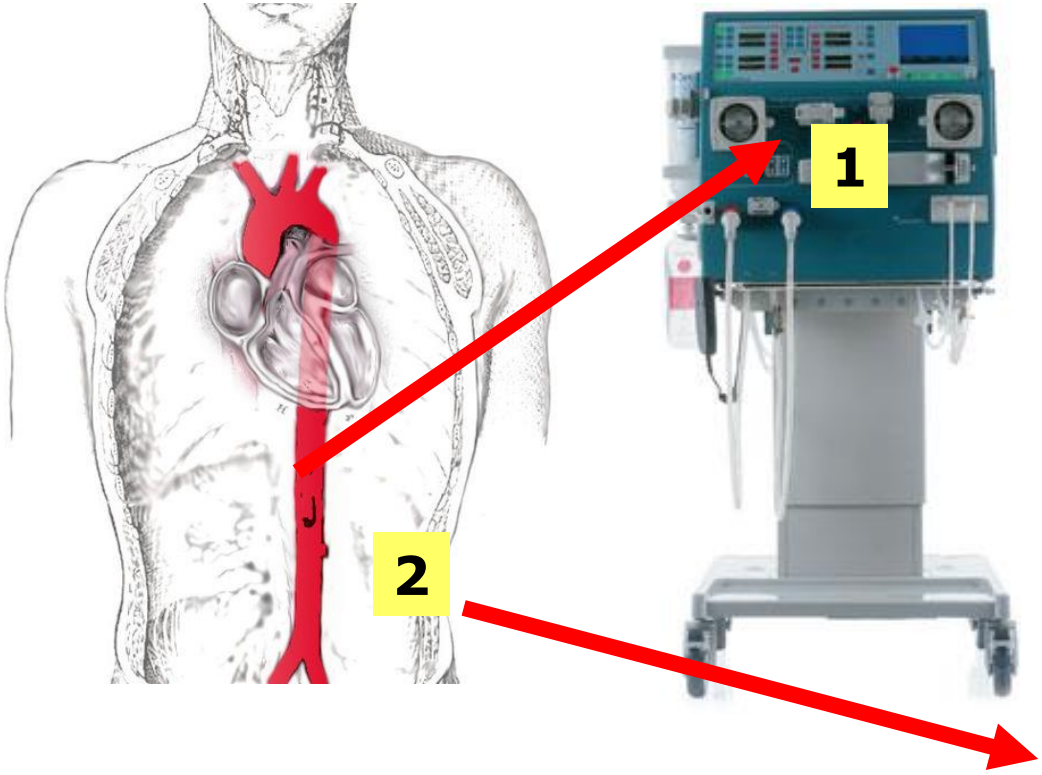
Treatment 1- causal



Treatment 2- influence the path



Treatment 3- systematic



Elimination of toxins
hemodialysis
Hemadsorption
Exchange transfusion

General treatment
Energy
E
Hydration
Treatment of infections
Etc.

ENZYMES

Enzymes - proteins - speed up biochemical reactions

1 molecule is transformed noncatalyzed in comparison to106-1014 molecules transformed via catalysis by enzyme

The mechanism of action of enzymes consists of reducing the activation energy for the reaction (enable course of metabolic processes at relatively low temperatures (37 ° C) and at pH 6.5 to 7.5 in an aqueous medium)

There are from 1000 to 4000 different types of enzymes in animal cell

- protein part - apoenzyme, nonproteinic – coenzyme
- reactant - substrates, the substances formed –products
- Reaction: The substrate binds to the specific binding site on the enzyme molecule (binding site is wedged into the recess enzyme called. Active center)

The enzymatic reaction-principle

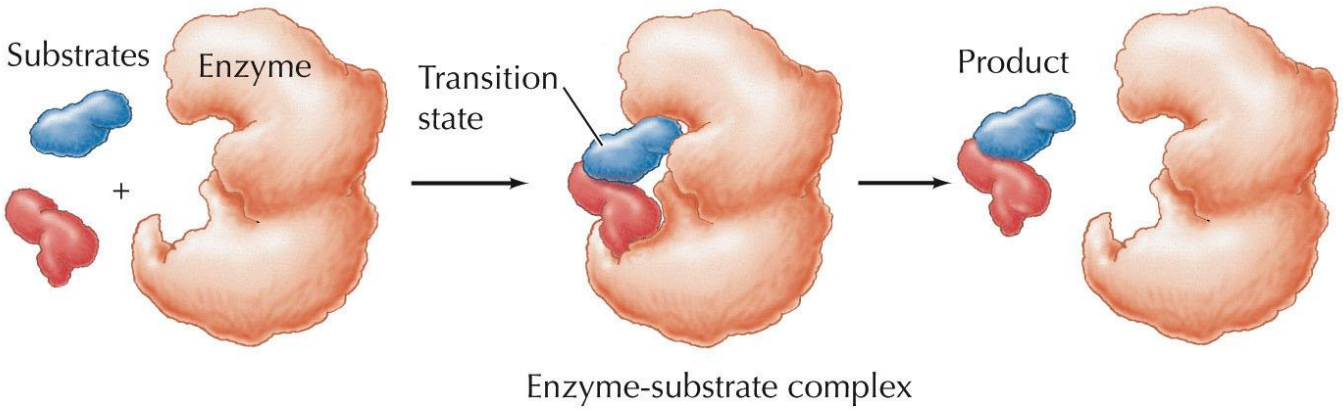
Binding of substrate - enzyme:

- by **non-covalent ionic bond**
- **hydrogen and hydrophobic bridges**
- **van der Waals interactions**

- On the **functional groups of aminoacids residues in active site of enzyme** are linked **coenzymes,** eventually metal atoms (metaloenzymes), participate in the catalyzed reaction

- Functional groups **activate the substrate and reduce** the energy which is required to produce high-energy intermediate stage

Binding of the enzyme-substrate



THE CELL, Fourth Edition, **Figure 3.2** © 2006 ASM Press and Sinauer Associates, Inc.

http://www.google.cz/url?sa=i&rct=j&q=&esrc=s&source=images&cd=&cad=rja&uact=8&ved=0CAcQjRw&url=http%3A%2F%2Ffaculty.samford.edu%2F~djohnso2%2F44962w%2F405%2Fmetabolism.html&ei=meLyVPKHJcf5UqDMgMgP&bvm=bv.87269000,d.d24&psig=AFQjCNFd4SZ50y4rttbYAjGKliZh-KU7fA&ust=1425290217916178

Pathobiochemistry of regulation enzyme activity

Inhibition of enzyme activity

The effect of **multiple drugs or toxins** resides in its ability to inhibit the enzyme.

Strongest inhibitors - bind **covalently** to functional groups of the active site, eventually **substrate analogs** - forming enzyme complexes

The rate of enzyme reaction :

a)Concentration of **substrate, product**

b)Concentration of **activators**, concentration od **inhibitors**

The relationship between the rate of the enzyme reaction and the substrate concentration - given by Michaelis-Menten equation $E + S \leftarrow \rightarrow ES \rightarrow P + E$

Products and physiological inhibitors may compete with the substrate for **binding to the active center** of the enzyme and thus slow the rate of reaction

Physiological regulation of metabolic pathways– the ability to alter the rate of progression of metabolic reactions in the pathway via activation of enzymes that catalyze **the slowest part**

Enzymes have so called **allosteric activators or inhibitors** i.e. compounds which bind to a different part of the enzyme molecule than the active site thus affecting the conformation of the enzyme molecule

The regulation also – by modulatory protein or phosphorylation

Isoenzymes: enzymes having a different amino acid sequence in the peptide chain, but **catalyzing the same reaction**

Mechanisms of regulation of the enzyme activity - INHIBITION

Reversible inhibition of the active center

The enzyme inhibitor is a compound that reduces the rate of response by **binding to the enzyme**. Reversible inhibitors – there is not a covalent bond, can be detached from the enzyme. Products - are reversible inhibitors for their own reaction.

Competitive inhibition

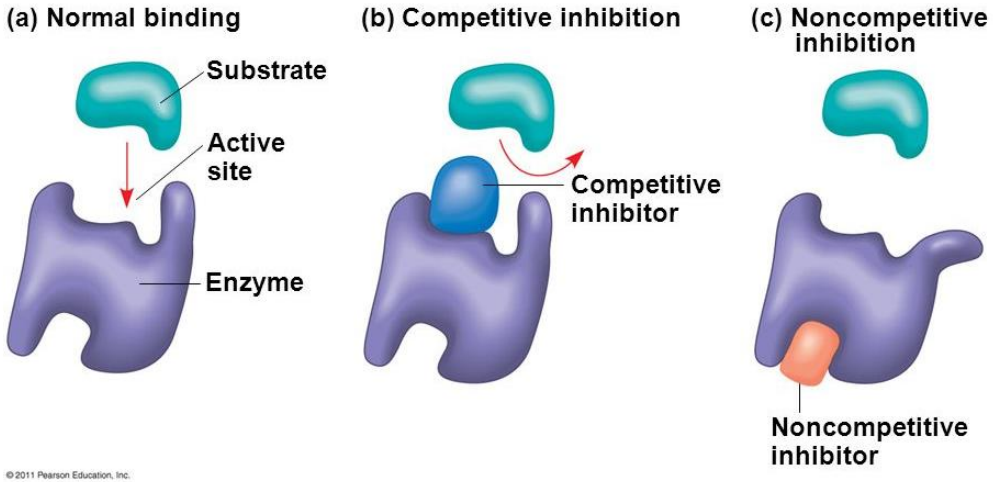
Reversible inhibitor may compete for binding to the active center with the substrate, producing an enzyme complex that may be dissociated into free enzyme and inhibitor.

Non-competitive inhibition

This inhibitor does not compete for the binding site, but its binding to the enzyme reduces the concentration of active enzyme, i.e. always decreasing Vmax

Scheme of inhibition

In **competitive inhibition** of the binding site for the substrate A competes with structurally very similar other substrate.
In non-competitive inhibition substrate A gets to its binging site, but at the position on the binding site of its partner i.e. substrate B occupies a non-competitive inhibitor (to substrate A), but that is competitive with to B.
In **non-competitive** inhibition the inhibitor binds to the enzyme-substrate complex.



http://images.slideplayer.com/1/277615/slides/slide_53.jpg

Exaplex of inhibition

Non-competitive inhibition

- **Non-competitive inhibitor** binds only to the enzyme-substrate complex (when the enzyme binds substrates cotrollably):
- the first substrate molecule induces a change in the conformation of the enzyme molecule, the binding site for either the co-substrate or inhibitor openes. Non-competitive inhibitor reduces Km and Vmax, as well.

Irreversible competitive inhibition

Molecules of inhibitor - structurally similar to the substrate that covalently bind so tightly in the active center that this binding can not be displaced. This way - a common mechanism of action of drugs or antimetabolites

Examples of irreversible competitive inhibition

Inhibitor	**Enzyme**	**Effect**
Aspirine	cyclooxygenase	Anti inflammatory
Allopurinol	xanthinoxidase	Treatment of gout
5-Fluorouracil	thymidilatesynthase	cancerostatic
Penicilin	transpeptidase	antibiotics
Sarin	cholinesterase	Nerve gas
ß-aminopropionitrile	lysyloxidase	lathyrism

Mechanism of action of irreversible inhibitors

- **Affinity changes of binding site** – the substrate analog has a reactive group that is not the natural substrate and which permanently blocks the active site for substrate (covalent bond with an amino acid residue)

!!! resistance to antibiotics

Excessive and prolonged use of antibiotics also in agriculture, veterinary practice… have made some bacterial strains - *Pseudomonas, Streptococcus,Staphylococcus, Mycobacterium tuberculosis*… resistant to some antibiotics

The mechanism of this resistance may be due to induction of the synthesis of enzymes, which modify the antibiotic molecule so that it becomes analog of the transitional stage.

Example of resistance

Induction of ß-lactamases are altered so called ß-lactam antibiotics :

- **penicillins, cephalosporins, carbapenems**

A similar effect may have the induction of:

- **acetyltransferase, fosfotransferase or nukleotidyltransferase on aminoglykosides**
- **acetyltransferase on chloramphenicol**

Other regulation mechanisms of enzyme activity

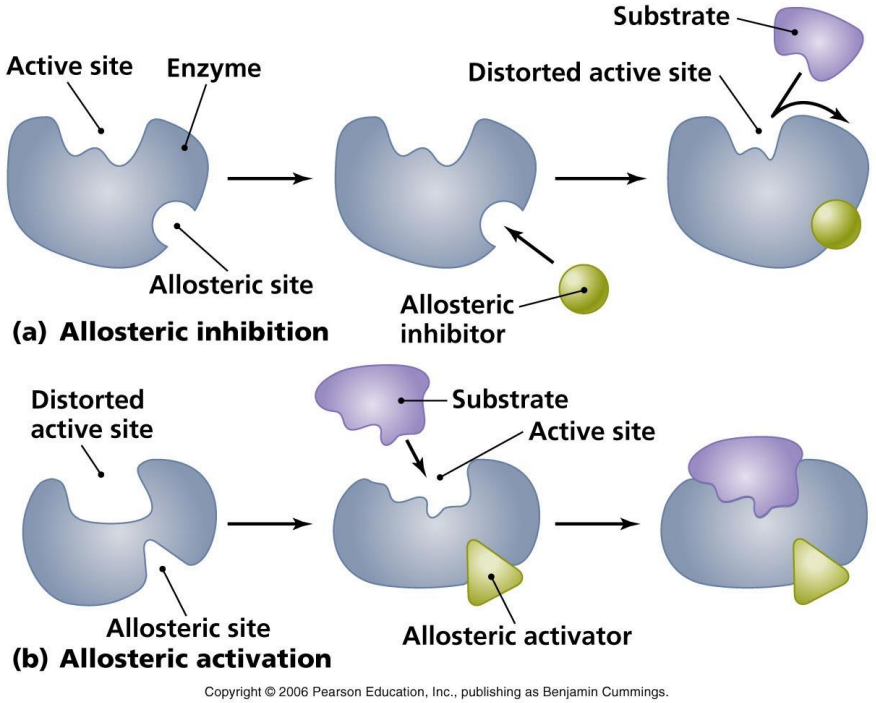
Allosteric regulation

Allosteric effector reversibily binds to another than binding site of the enzyme.

This bond induces: <u>conformational change of the active site</u>, so it may **activate** or

inhibit the enzyme

Scheme of allosteric activation and inhibition



http://academic.pgcc.edu/~kroberts/Lecture/Chapter%205/05-11_AllostericControl_L.jpg

Other regulation mechanisms of enzyme activity

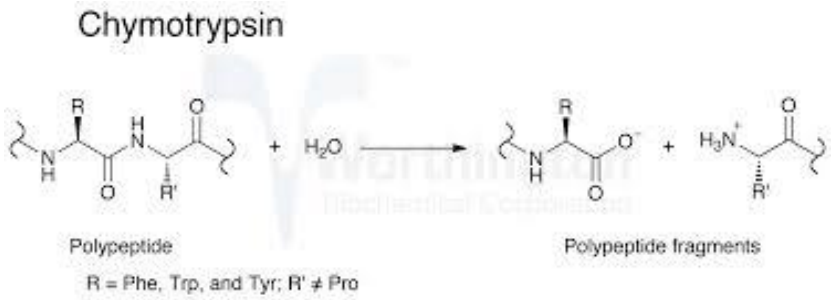
Covalent modifications – phosphorylation of hydroxyl group by phosphorylasakinase. Phosphorylation of enzyme **activates the active site** of enzyme → activ conformatin, dephosphorylatin → inaktivation.

Limited proteolysis - aktivation induced by cleaving the short polypeptide chain from the peptide, e.i. proenzyme or enzymogene

✓leads to change in the conformation of the active site that can bing the substrate in this form.

Examples:**chymotrypsinogen → chymotrypsin**

prothrombin → thrombin



Note: - proteolytic pancreatic enzymes, which are formed in the pancreas as prodrugs, are activated in the gut lumen. Otherwise, they would digeste own pancreatic tissue.

http://www.worthington-biochem.com/chy/images/reaction.jpg

Other regulation mechanisms of enzyme activity

➢Induction of enzyme synthesis

the amount of enzyme in the cell depends on the rate of its synthesis or degradation (lasts several hours or days, previous methods of control activities last only)

➢Suppression synthesis of the enzyme

➢**Example: by this mechanism acts e.g. omeprazole – suppresses protein synthesis e.i. proton pump in the gastric mucosa, thus preventing the production of HCl. It is used to reduce the acidity of gastric secretion, thereby limiting its aggressive action (treatment of peptic ulcer disease).**

➢feedback regulation

final product regulates the speed of its own synthesis, the final product of a metabolic pathway may inhibit or related metabolites may activate regulatory key) enzyme

final product may regulate the speed of its own synthesis - action on gene transcription key enzyme in the metabolic pathway – much more slower process than regulation by allosteric mechanism.

Classification and nomenclature of enzymes

Classes of enzymes:

1. oxidoreduktases
2. transferases
3. hydrolases
4. lyases
5. isomerases
6. ligases

The ending of enzyme names: **substrate + -ase**

e.g. lactate dehydrogenase, amylase, alcohol dehydrogenase, aspartate transaminase…

The causes of increased activity, enzyme concentration in plasma

1. **Patological ↑ conc. of enzymes in plasma** – the result of the increased permeability of the cell membrane - damaged by chemicals, anoxia, hypoxia, viruses, inflammation. May lead to cell degradation.

Cell degradation→ Increased phospholipase activity, the degradation of cytoplasmic membrane phospholipids → perforation→ release of contents + enzymes into extracellular space → plasma

2. **Incerased synthesis E** – is not pathological, but is associated with a condition in organism :

e.g. bone growth ↑ osteoblasts activity↑ **alkaline phosphatase** in blood

In children ALP 3x-7x higher than in adults

3. Drugs, alcohol ↑ enzyme activity (liver) – **ALP, GMT etc.**

The causes of increased enzyme concentration in plasma

3. **Release from cells not associated with cell death or increased synthesis –**

E.g. ethanol releases expression of mitochondrial AST in hepatocytes, its transport to the surface hepatoctů → release into blood.

E.g. Food intake→ intestinal ALP →into lymph, ↑ in blood.

E.g. Liver enzymes are bound to the surface of hepatocytes ....

4. **Some cases of increased concentration** – inadequate removal from circulation.

E.g. Small enzymes– amylase, lipase – removed from circulation by glomerular filtration.

Renal impairment**, renal failure** ↑ their concentration in the blood.

Formation of complexes E-Ab, macroenzyme, half-life Ig-3 weaks

Time course of the increase and decrease of enzyme activity in plasma

Time course – influenced by many factors:

- **During apoptosis the cell membrane defects deepen with time.** Result – from cells are first released small molecules of enzymes and later large enzymes.

E.g. myocardial infarction (IM) – **at first in plasma AST and CK** (small molecules), **later LD** (bigger). In IM – concentration of CK in plasma depends on the size of the bearing affected by IM.

- When the **cause of cell damage disappear** enzyme **concentration persists for some time, then decreases**.

E.g. **acute hepatitis** can be distinguished from **toxic** liver damage. At virus hepatitis – imunological cell damage, longer persistance of increased enzyme concentration, toxic damage – faster return to normal levels of the enzyme (GGT,AST, ALT).

The activity of enzymes in plasma

- Enzyme concentration gradient between the cell and plasma– inside hepatocytes in cytoplasm = **higher concentration of AST** than ALT , LD minimum there.
- E.g. Hepatocyte damage- fastest-growing AST, but LD at least.
- E.g. Myocardium – hogh concentration of CK, low of LD, damage → hogh increase of CK in plasma

Enzyme concentration in plasma is determined by **the rate of its removal.**

- **low molecular weight -glomerul. filtration**
- **others (the majority) - inactivated in plasma, removed by cells of the RES via receptor, endocytosis**

Definition - the half-life of enzyme

The period during which the enzyme is increased in plasma is determined by its own half-life.

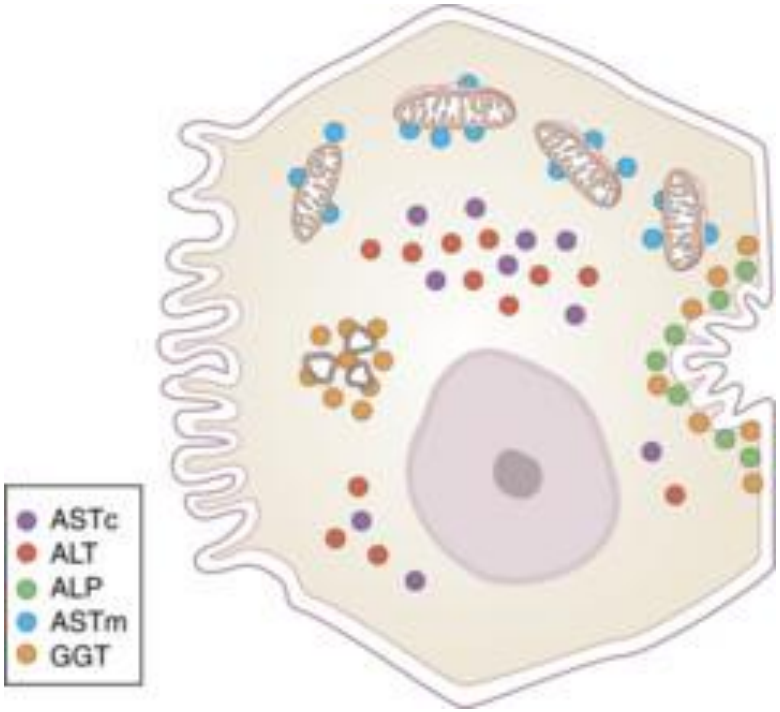
The biological half-life of enzyme – the period during which the amount of enzyme decreases half, unless added from tissues.

Enzyme	Half-life
ALP	3-7 days
AMS	9-18 hours
ALT	2 days
AST	12-14 hours
CHS	10 days
CK	10 hours
GMT	3-4 days
LDH1(HHHH)	4-5 days
LDH5(MMMM)	10 hours

The use of enzymes in clinical diagnosis

- **Detection of tissue damage**
- **Identification of the beginning of tissue damage**
- **Determining the extent of damage**
- **Estimation of the severity of cell damage**
- **Diagnosis of basic disease**
- **Specification diagnosis within the damaged organ**

- The main diagnostic hepatocellular enzymes are localized in different areas of hepatocyte. ALT and cytoplazmatic isoenzyme AST are placed in cytoplasm.



http://o.quizlet.com/i/eHnZCzz2IZU1GLwWW5FTwg_m.jpg

- When is the membrane damaged (e.g. viral or chemical), these enzymes are released and entered the sinusoid. The result is an increase in plasma.
- Mitochondrial AST is primarily released during mitochondrial damage, egg. when exposed to alcohol.
- ALP and GGT are located on the canalicular surface of hepatocytes and released in cholestasis, particularly due to the effect of bile acids on the membrane.
- GGT is also located in the microsomes, where is induced by certain drugs. The administration of these drugs increases the plasma levels of GGT.

Specification of diagnosis

- Information suitable for precise diagnosis is obtained :
- - from values of the catalytic enzyme concentration in a body fluid (a direct correlation between the degree of organ damage and increased activity of enzymes in blood).
- - spectrum of enzymes present in the blood (e.g. in severe liver damage accompanied by cell necrosis is increased activity of enzymes in the blood subsequently : LD> AST> ALT).
- - calculating the ratio of the enzyme activities (e.g. according to the ratio of AST / ALT in serum can be distinguished initial obstructive jaundice (AST / ALT <1) from chronic active hepatitis (AST / ALT> 1). For acute disease can be from the ratio of enzyme activities with short and long biological half-life to determine the stage of disease or predict the course of the disease, e.g. in acute hepatitis decrease in the ratio of AST (half-life 12 h) / ALT (half-life of 2 days) helps to qualify the type of hepatitis.
- - monitoring the enzyme activity (mechanism of enzyme release into the blood from damaged tissues and their clearance is characteristic curves of typical kinetic activity of which can be derived during the period of time during which the disease may be present or to determine the stage of disease)
- - determination of isoenzymes.

Macroenzymes

- Macroenzymes are complexes formed by enzyme linked immunoglobulin, a lipoprotein, protein, or cell membrane fragments.
- Generally makroenzymes have higher molecular weight and longer half-life in blood. Makroenzyme´s presence in serum may affect its analytical determination or cause misinterpretations of results.
- Makroamylasemia is a well-known example when the amylase forms a complex with other macromolecules and therefore is not filterable into urine. As a result, accumulates in the blood. The level of amylase in the blood is then increased without causing its increased release from damaged tissue (about 1-3% of patients with elevated serum amylase).
- Because makroamylase does not penetrate into the urine, urine amylase level is normal or reduced in this case. Unlike the situation during e.g. pancreatitis when elevated levels of serum amylase is accompanied by an increase of amylase in urine.

Tissue distribution of diagnostically important enzymes

- Tissue damage can be diagnostically prooved indirectly either by determining the activity of tissue-specific enzymes or by isoenzymes in the blood.
- Tissue-specific enzymes are found preferentially in a particular tissue or have high activity in that tissue. Exemples of tissue-specific enzymes are listed in the following table.
- Expressionof isoenzymes is mostly determined genetically for each tissue . Therefore, determination of isoenzymes in the blood enables to identify damaged tissue which they come from (e.g. pancreatic lipase, CK-MB, LD1).

Organ	AST	ALT	LD	LD_1	CK	GGT	ALP	ACP	AMS	LPS	CHS
Liver	x	xx	x			xxx	x				xxx
Myocardium	x	x	x	xx	xx						
Muscle	x	x	x		xx						
Bile duct							xx				
Kidneys	x		x	x		x	x				
Bones							xx	x			
Erythrocytes	x		x	x				xx			
Prostate								xxx			
Pancreas	x					x			xx	xxx	
Parotid gland									xx		

TEST

Clinically significant enzymes

- **Causes of increased activity in serum**
- **AST** *aspartate aminotransferase* myocardial infarction; hepatopathia; blood diseases; muscle damage
- **ALT** *alanine aminotransferase* hepatic dysfunction, heart disease, AST / ALT ratio> 1 alcoholic liver disease, myocardial infarction, AST / ALT <1 viral hepatitis
- **LD** *lactate dehydrogenase* LD1,2 - myocardial infarction, hemolytic anemia; LD3 - pulmonary embolism; LD4,5 - hepatopathy diseases of skeletal muscle
- HBD *hydroxybutyrátdehydrogenase* activity subunit H (LD1,2), myocardial infarction
- **GGT** *gamma-glutamyltransferase* hepatopathia (inflammation, alcohol, drugs); test of chronic alcohol consumption; cholestasis
- **ALP** *isoenzyme of alkaline liver phosphatase* - diseases of the biliary tract, bone isoenzyme - bone disease (Paget's disease, rachitis, tumors), *physiologically increased during growth*
- **ACP** *acid phosphatase prostatic isoenzyme* - prostate tumors, bone isoenzyme - tumor metastasis to bone, osteoporosis marker
- **CK** *creatine kinase*, CK-MB - particularly myocardial infarction, but also in the regeneration of skeletal muscles, chronic muscular diseases and acute renal failure
- CK-MM - diseases of skeletal muscle, intramuscular injections, physical activity
- **AMS** *amylase* (Mr ~ 50,000) pancreatic isoenzyme - acute pancreatitis, salivary isoenzyme – parotiditis
- **LPS** lipase of *acute pancreatitis*, acute reversal of chronic pancreatitis
- **PSA** *prostate specific antigen* in prostate cancer
- **Causes of decreased activity in serum**
- **CHE** *cholinesterase* chronic hepatopathy, alcoholic-toxic hepatitis (organophosphate intoxication); indicator of hepatic protein synthesis

clinically important enzymes

Aminotransferases

- provide the conversion of amino acids and keto acids α-amino transfer, the donor and acceptor amino group is a 2-oxoglutarate / L-glutamate
- **Alanine aminotransferase, ALT** – donor -NH2 Ala to form pyruvate, marker- liver (viral hepatitis, alcohol, hepatopathy, ...)
- **Aspartate aminotransferase, AST** – donor – NH_2 Asp to form oxaloacetate, a marker of damage - liver, heart, myocardial infarction, muscular damage

Clinically important enzymes

α-amylase, AMS

- **synthesized in the pancreas, cleaves α-1,4-glycosidic linkage in starch and glycogen to produce maltose and maltotriose, endoglycosidase, marker - acute pancreatitis**

- **alkaline phosphatase, ALP**

- **hydrolysis of monoesters of phosphoric acid with alcohols, phenols, Glycine - nonspecific - cleaves POC, POP, PS, PN, has several isoforms according to the synthesis of tissue - bone, liver, placenta, intestinal, optimum in the alkaline environment, marker - bone damage, liver**

- **acid phosphatase, ACP**

- properties ALP, optimum in the acidic region, marker- prostate

Other clinically important enzymes

creatine kinase , CK

- catalyzes the reversible phosphorylation of creatine to phosphocreatine for ATP consumption, a marker of muscle damage, heart

lactate dehydrogenase, LDH

- isoforms (tissue) of the heart, liver, catalyzes the reaction:

  Lactate + NAD + ↔ pyruvate + NADH + H + (reversible) is non-specific tissue damage according isoforms - electrophoretically (LDH3 pulmonary embolism, myocardial infarction LDH1,2, hepatopathy + disease koster.svalstva LD4,5).