ENZYMES - III

APPLICATION OF ENZYMES IN MEDICINE

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Application of enzymes

1. - for **diagnostic** (enzymes estimation in serum and body fluids for **diagnosis** and **prognosis**)
2. - **therapeutic** uses of enzymes (as a **drugs**)
3. - as an **laboratory** **(analytic)** reagents

The main principles for applying enzymes in diagnostic are based on the following positions:

- if **damaged cells** in the blood or other biological fluids (e.g. urine) increases the concentration of intracellular enzymes damaged cells;
- the **amount** of released enzyme enough to detect it;
- the activity of enzymes in biological fluids detected when cells are damaged, **stable** for quite a long time and is **different** from the normal values;
- there are differences in the intracellular localization of the enzyme;
- a number of enzymes takes precedence or absolute **localization** in certain organs (organ specific).

Organ-specific enzymes

Enzyme (isozyme)	Organ (system)
LDH_1, LDH_2	heart
LDH_3	lungs
LDH_4, LDH_5	liver, muscle
amylase	pancreas
ALT	liver
AST	heart
acid phosphatase	prostate
alkaline phosphatase	bone

Blood plasma enzymes

1. **Secretory enzymes -** own enzymes of plasma. They are formed in the liver, but exert their action in the blood (*prothrombin, proaccelerin, proconvertin, ceruloplasmin and cholinesterase*).
2. **Excretory enzymes –** enter to the blood from various secrets - duodenal juice, saliva, etc. (*amylase, lipase*).
3. **Cellular enzymes –** released into the blood when damaged or destroyed cells or tissues.

Blood plasma enzymes

The cell-derived enzymes enter the plasma in small amounts as a result of:

- ***owing to diffusion through undamaged cell membranes,*** or
- ***continuous normal ageing of the cells,***
- ***released into the blood when damaged or destroyed cells or tissues.***

Possible mechanisms responsible for abnormal levels

Serum level of a particular enzyme may be increased by **diseases** that provoke:

- **an increase in its rate of release;**
- **a decrease in rate of disposition or excretion.**

Increase Serum Level

Increased release -

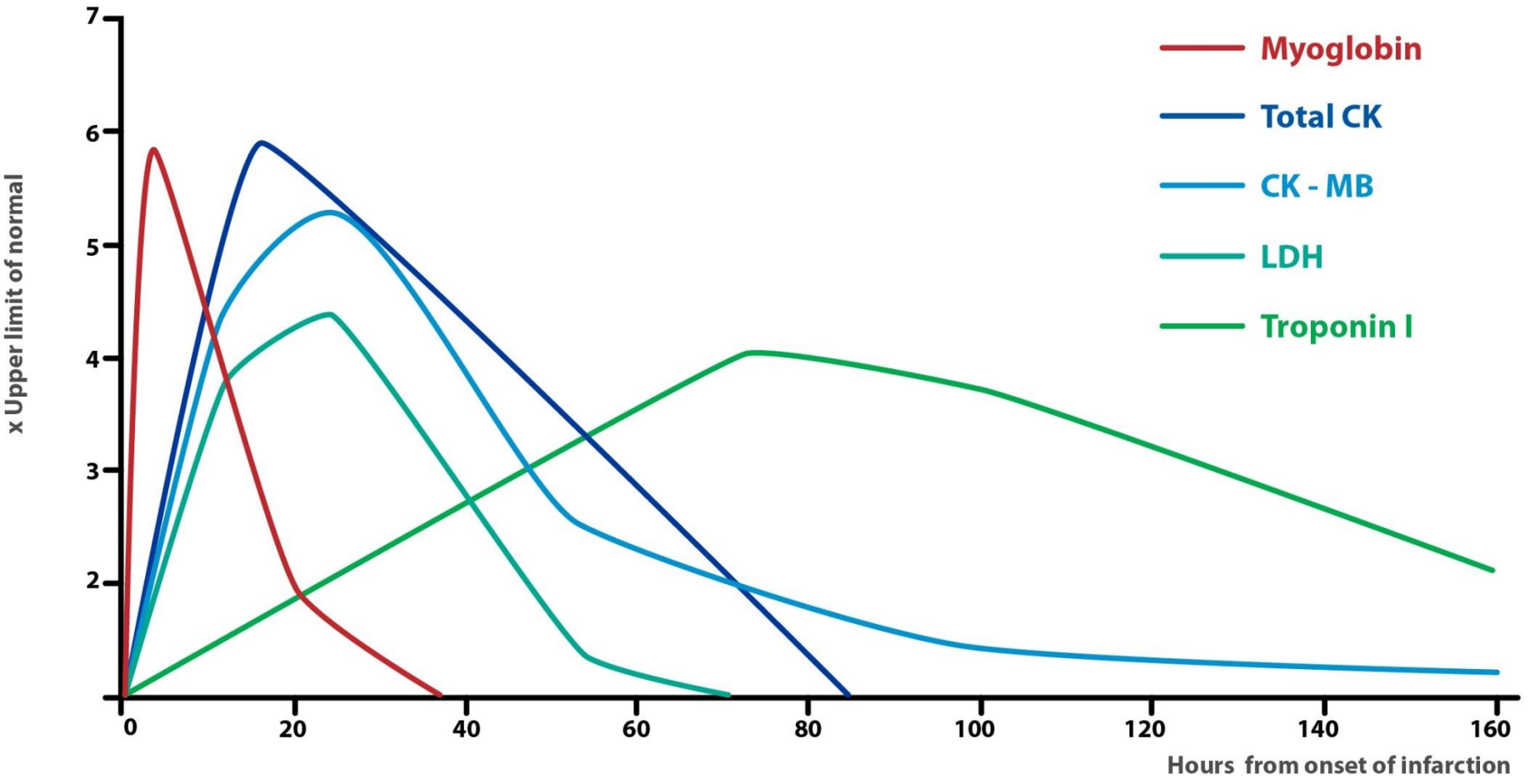
- Necrosis of cells;
- Increased permeability of cell membrane;
- Increased production of the enzyme within cell;
- An increase in tissue source of enzymes;
- Impaired disposition/excretion

Decreased Serum Level

Decreased formation of the enzyme may be:

1. ***Genetic***
2. ***Acquired***
   - ***Enzyme inhibition***
   - ***Lack of cofactors***

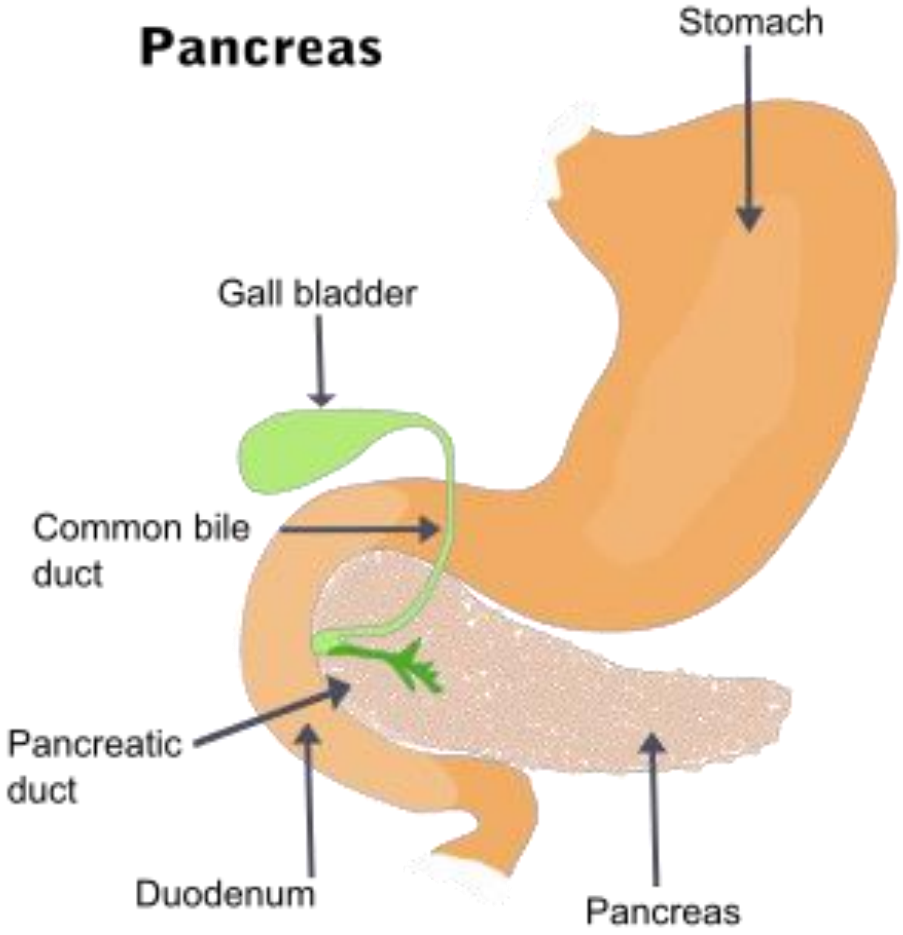
MI / heart attack.
Increase serum level of cardiac biomarkers



Acute pancreatitis

Elevated serum **amylase** and **lipase** levels, in combination with severe abdominal pain.

If the **lipase** level is about 2.5 to 3 times that of **amylase**, it is an indication of pancreatitis due to alcohol.



LABORATORY DIAGNOSIS

PANCREATIC ENZYMES

- Diagnosis of acute pancreatitis relies on at least a three-fold elevation of amylase or lipase in the blood.

- Serum Amylase(30-180 IU/L)
- It rises within 6 to 12 hours of onset (half-life, 10 hours).
- The serum amylase is usually increased on the first day of symptoms, and it remains elevated for three to five days in uncomplicated attacks.

- Sensitivity is greater than 85%, the serum amylase may be normal or minimally elevated in fatal pancreatitis, during a mild attack or an attack superimposed on chronic pancreatitis , or during recovery from acute pancreatitis also in hypertriglyceridemia-associated pancreatitis.

- Hyperamylasemia is not specific for pancreatitis because it occurs in many conditions other than acute pancreatitis.

Serum amylase in pregnancy

In a study of amylase activity in 200 pregnant women:

- Serum amylase rises gradually during pregnancy until the twenty-fifth week and thereafter falls slightly
- Serum amylase values in normal pregnant women during the second and third trimesters may exceed those in normal men and nonpregnant women

Kaiser R et al Am J Obstet Gynaecol. 1975 1;122(3):283-6

Enzymes as drugs

1. Enzyme **replacement therapy** (**ERT**)
2. Elements of the **complex therapy**
3. Drugs as **inhibitors** of enzymes

Enzyme replacement therapy (ERT)

- is a **medical treatment** **replacing** an ***enzyme*** in patients in whom that particular ***enzyme*** is deficient or absent.

ERT does not correct the underlying ***genetic defect***, but increases the concentration of ***enzyme*** in which the patient is deficient.

Usually this is done by giving the patient an **intravenous infusion** containing the ***enzyme***. Digestive ***enzymes*** can be replaced **orally**.

ERT is currently available for some:

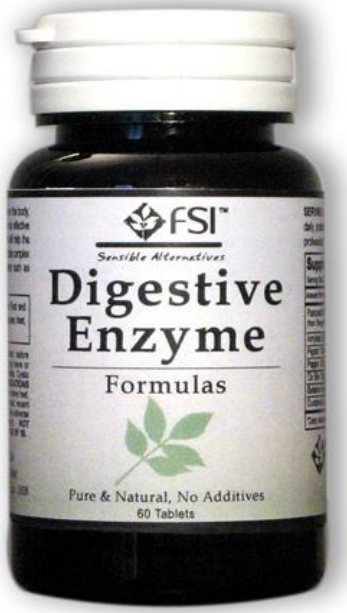
- **Lysosomal diseases**;
- **Mucopolysaccharidoses** (**MPS**);
- **Glycogen storage disease** (**GSD**)
- **Severe Combined Immunodeficiency** resulting from an ***adenosine deaminase*** deficiency (**ADA-SCID**) etc.

Enzymes
as drugs

1. Replacement therapy - the use of enzymes in the event of failure:

pepsin - ahilia, hypo-and an- acidic gastritis

festal, mezim – pancreatitis



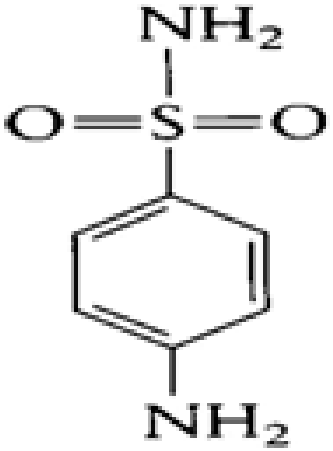
2. Elements of the complex therapy

- the use of enzymes in combination with other therapies:

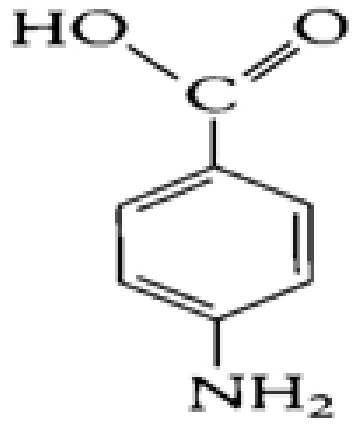
- **proteolytic** enzymes (local impact for the treatment of septic wounds) - ***trypsin, chymotrypsin;***
- **thrombosis** and **thromboembolism** – ***fibrinolisin, streptoliase, urokinase;***
- **scars resorption** after burns or operations - ***hyaluronidase (lidase);***
- **malignant lymphomas**, **acute leukaemia** - ***asparaginase***

3. Drugs as inhibitors of enzymes

- **sulfanilamide** - functions as competitive **inhibitor** of enzymatic reactions involving **para-aminobenzoic acid**. **PABA** is needed in enzymatic reactions that produce **folic acid (vit. B_9)**, which acts as a **coenzyme** in the synthesis of **purines** and **pyrimidines**.



Sulfanilamide



PABA

Mammals do not synthesize their own **folic acid** so are unaffected by **PABA inhibitors**, which selectively **kill bacteria**.

3. Drugs as inhibitors of enzymes

- anticholinesterase drugs.

An ***acetylcholinesterase*** **inhibitor** (**AChEI**) is a **drug** that inhibits the ***acetylcholinesterase*** enzyme from breaking down **acetylcholine**, thereby increasing both the **level** and **duration** of action of the **neurotransmitter ACh**.

Are used medicinally to treat:

- **myasthenia gravis**
- **glaucoma**
- postural **tachycardia syndrome**
- **Alzheimer's** disease, and **Parkinson's** disease
- cognitive impairments in patients with **schizophrenia**
- as an **antidote** to anticholinergic **poisoning**.

3. Drugs as inhibitors of enzymes

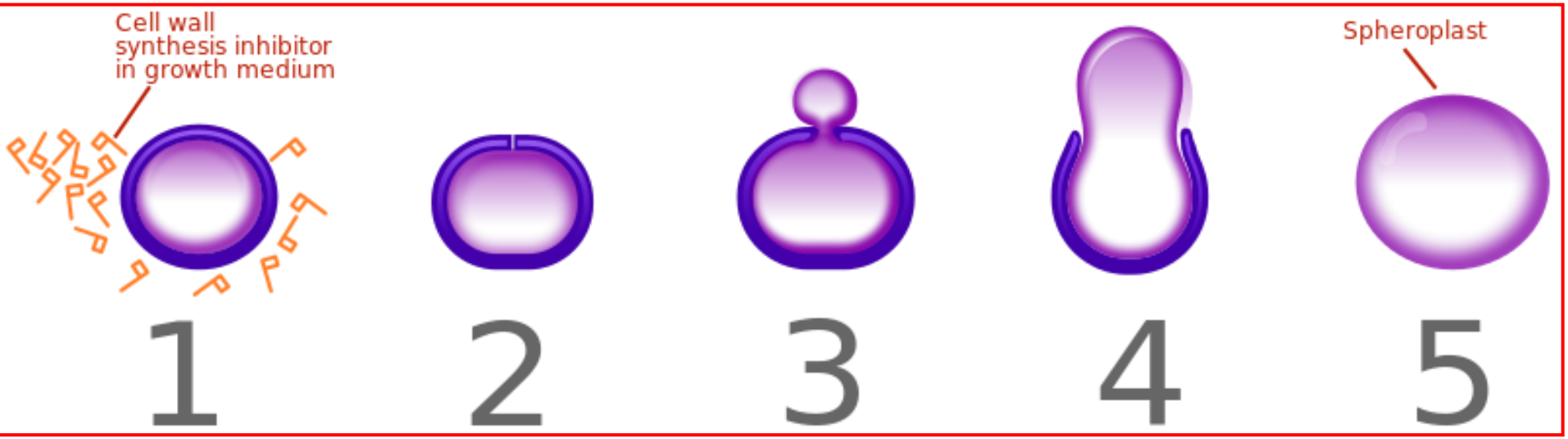
- Penicillin (β-Lactam antibiotics)

Bacteria constantly remodel their **peptidoglycan** cell walls, simultaneously building and breaking down portions of the cell wall as they grow and divide.

β-Lactam antibiotics inhibit the formation of peptidoglycan cross-links in the bacterial cell wall; this is achieved through binding of the four-membered **β-lactam ring** of **penicillin** to the enzyme ***DD-transpeptidase***.

As a consequence, ***DD-transpeptidase*** cannot catalyze formation of these cross-links, and an imbalance between cell wall production and degradation develops, causing the cell to rapidly **die**.

Bacteria that attempt to grow and divide in the presence of **penicillin** fail to do cross-links in the bacterial cell wall, and instead end up shedding their **cell walls**.



This **weakens** the **cell wall** of the **bacterium**, and **osmotic pressure** becomes increasingly **uncompensated** — eventually causing **cell death** (**cytolysis**).

3. Drugs as inhibitors of enzymes

- **An Angiotensin-Converting-Enzyme inhibitor (ACE inhibitor) .**
- is a pharmaceutical drug used primarily for the treatment of **hypertension** (elevated blood pressure) and **congestive heart failure**.

This group of drugs cause relaxation of blood vessels. They **inhibit** the ***angiotensin-converting enzyme*** **(ACE)**, an important component of the **renin–angiotensin–aldosterone system (RAAS)**.

Frequently prescribed **ACE inhibitors** include – **captopril**, **enalapril**, **lisinopril**, and etc.

3. Drugs as inhibitors of enzymes

- Renin inhibitors

are a group of pharmaceutical drugs used primarily in treatment of **essential hypertension**. These drugs inhibit the first and rate-limiting step of the **renin–angiotensin–aldosterone system (RAAS)**, namely the conversion of ***angiotensinogen*** to ***angiotensin I***. This leads to a totality in absence of ***Angiotensin II.***

Renin inhibitors bind to the **active site** of ***renin*** and inhibit the binding of ***renin*** to ***angiotensinogen***, which is the rate-determining step of the **RAAS** cascade.

Aliskiren, an orally active non-peptide ***renin*** **inhibitor**, was the first drug in its class on the market (2007).

The key to the discovery of **aliskiren** was **crystallography** and **molecular modeling** techniques.

3. Drugs as inhibitors of enzymes

- Nonsteroidal anti-inflammatory drugs (NSAIDs).

Most NSAIDs inhibit the activity of ***cyclooxygenase-1*** (COX-1) and ***cyclooxygenase-2*** (COX-2), and thereby, the synthesis of **prostaglandins** and **thromboxanes**. It is thought that inhibiting COX leads to the **anti-inflammatory**, **analgesic** and **antipyretic** effects

3. Drugs as inhibitors of enzymes

- **Statins** also known as ***HMG-CoA reductase* inhibitors**, are a class of **lipid-lowering medications**.

Statins have been found to reduce cardiovascular disease.

Statins act by competitively inhibiting ***HMG-CoA reductase***, the first enzyme of the **cholesterol** pathway.

Because **statins** are similar in structure to **HMG-CoA**, they will fit into the enzyme's **active site** and compete with the native substrate (**HMG-CoA**). This competition reduces the rate by which ***HMG-CoA reductase*** is able to produce **mevalonate**, the next molecule in the cascade that produces **cholesterol**.

A variety of natural **statins** are produced by **Penicillium** and **Aspergillus fungi** as secondary metabolites.

3. Drugs as inhibitors of enzymes

- **Methotrexate** is a **chemotherapy** agent and immune system **suppressant**.
- competitively inhibits ***dihydrofolate reductase*** (**DHFR**), an enzyme that participates in the **tetrahydrofolate** synthesis

The affinity of **methotrexate** for **DHFR** is about 1000-fold that of **folate**. **DHFR** catalyses the conversion of **dihydrofolate** to the active **tetrahydrofolate**.

Folic acid (B_9) is needed for the *de novo* synthesis of the nucleoside **thymidine**, required for **DNA** synthesis, is essential for **purine** and **pyrimidine** base biosynthesis.

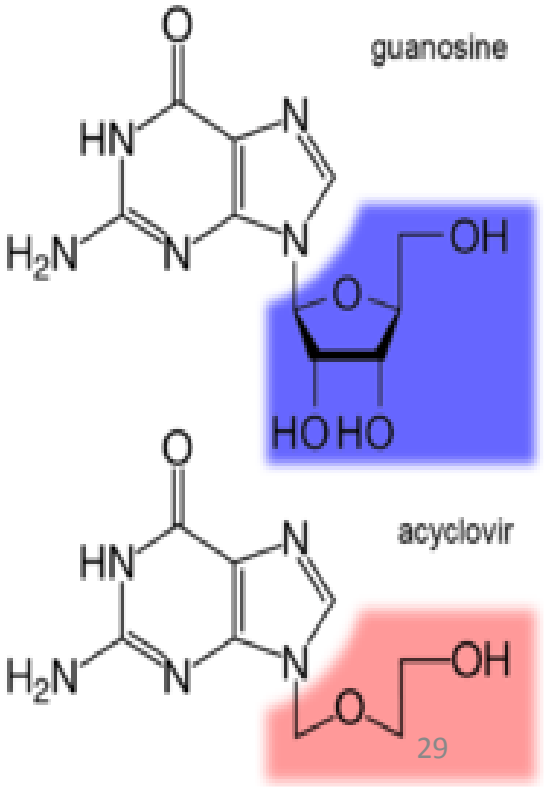
Methotrexate, therefore, inhibits the synthesis of **DNA**, **RNA**, and **proteins**.

3. Drugs as inhibitors of enzymes

Aciclovir – is an **antiviral** medication.

- is converted by viral ***thymidine kinase*** to **aciclovir monophosphate**, which is then converted by host cell ***kinases*** to **aciclovir triphosphate** (**ACV-TP**).

ACV-TP competitively **inhibits** and inactivates HSV-specified ***DNA polymerases*** further viral **DNA** synthesis without affecting the normal cellular processes.



It is on the **W**orld **H**ealth **O**rganization's List of Essential Medicines, the **most important medications** needed in a basic health system. (NP in 1988)

Enzymes as an analytic reagents

determination of *cholesterol, glucose, uric acid, urea*, *etc.*

Glucose oxidase - is used for estimation of ***glucose*** in blood and body fluids.

Uricase - is used for estimation of serum **uric acid**.

Urease - is used for estimation of **urea** in blood and body fluids.

Enzymes as an analytic reagents

Enzymopathies

fermentopathy

- pathological change of activity of enzymes.

Hereditary diseases in which, owing to change of activity of enzymes violated during the relevant biochemical reactions in the body and developed a metabolic disease.

There are approximately **500** types of **enzymopathies**.

1) enzyme **not synthesized** at all, because there is no nucleic acid — matrix;
2) the **incorrect sequence** of amino acids in the enzyme molecule;
3) it incorrectly synthesized **coenzyme**;
4) activity enzyme changed due to **anomalies** in other **enzyme systems**;
5) blockade may be caused genetically predetermined synthesis of **substances** that **inactivate** the enzyme.

An **enzymopathy** may be

1. **primary** (hereditary) and
2. **secondary**.

An **enzymopathy** may be caused by the

- **absence** of an enzyme,
- a **decrease** in an enzyme's activity, or
- the **absence** or incorrect synthesis of a **coenzyme**

In results of **enzymopathy -**

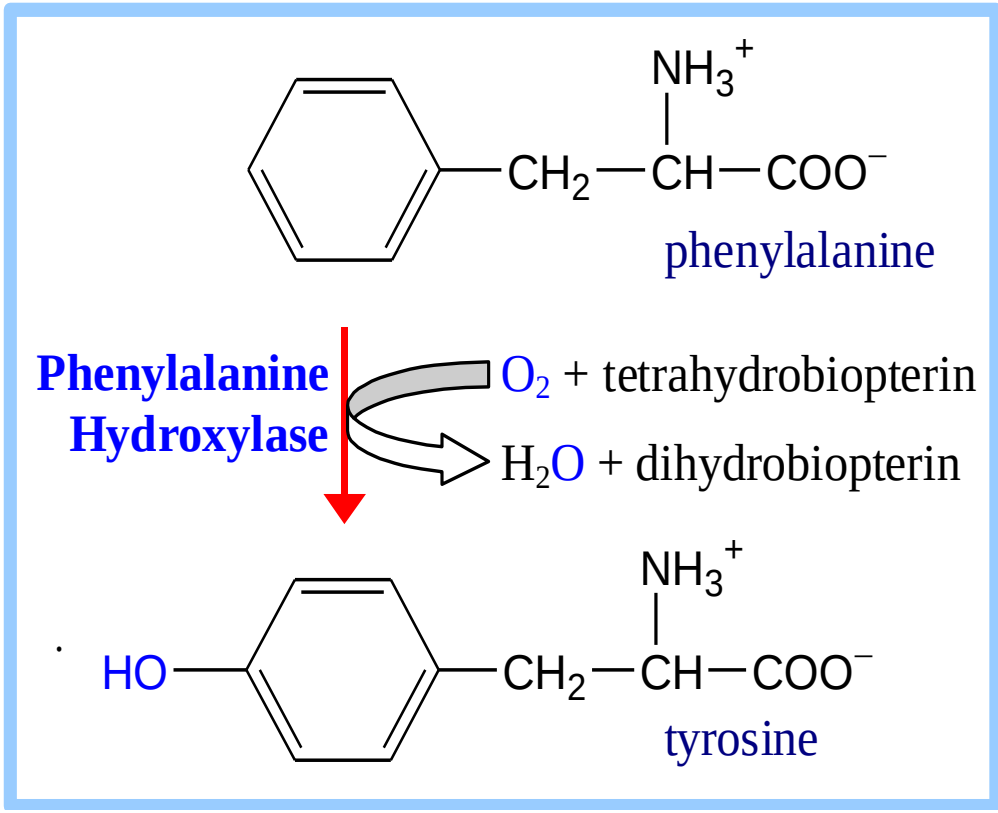
- ✓ **Accumulation of precursor substrates.**
- ✓ **Disturbances of formation of the final products.**

Primary (hereditary) enzymopathy

Genetic deficiency of ***phenylalanine hydroxylase*** leads to the disease

phenylketonuria.

Phe & **phenylpyruvate** (the product of **Phe** deamination via ***transaminase***) accumulate in blood & urine.



Phe is a large, neutral amino acid (LNAA). LNAAs compete for transport across the **blood–brain barrier** via the large neutral amino acid transporter (**LNAAT**). If **Phe** is in excess in the blood, it will saturate the transporter. Excessive levels of **Phe** tend to decrease the levels of other **LNAAs** in the brain. As these amino acids are necessary for protein and neurotransmitter synthesis, **Phe** buildup hinders the development of the brain, causing intellectual disability.

Mental retardation results unless treatment begins immediately after birth.

Treatment consists of -

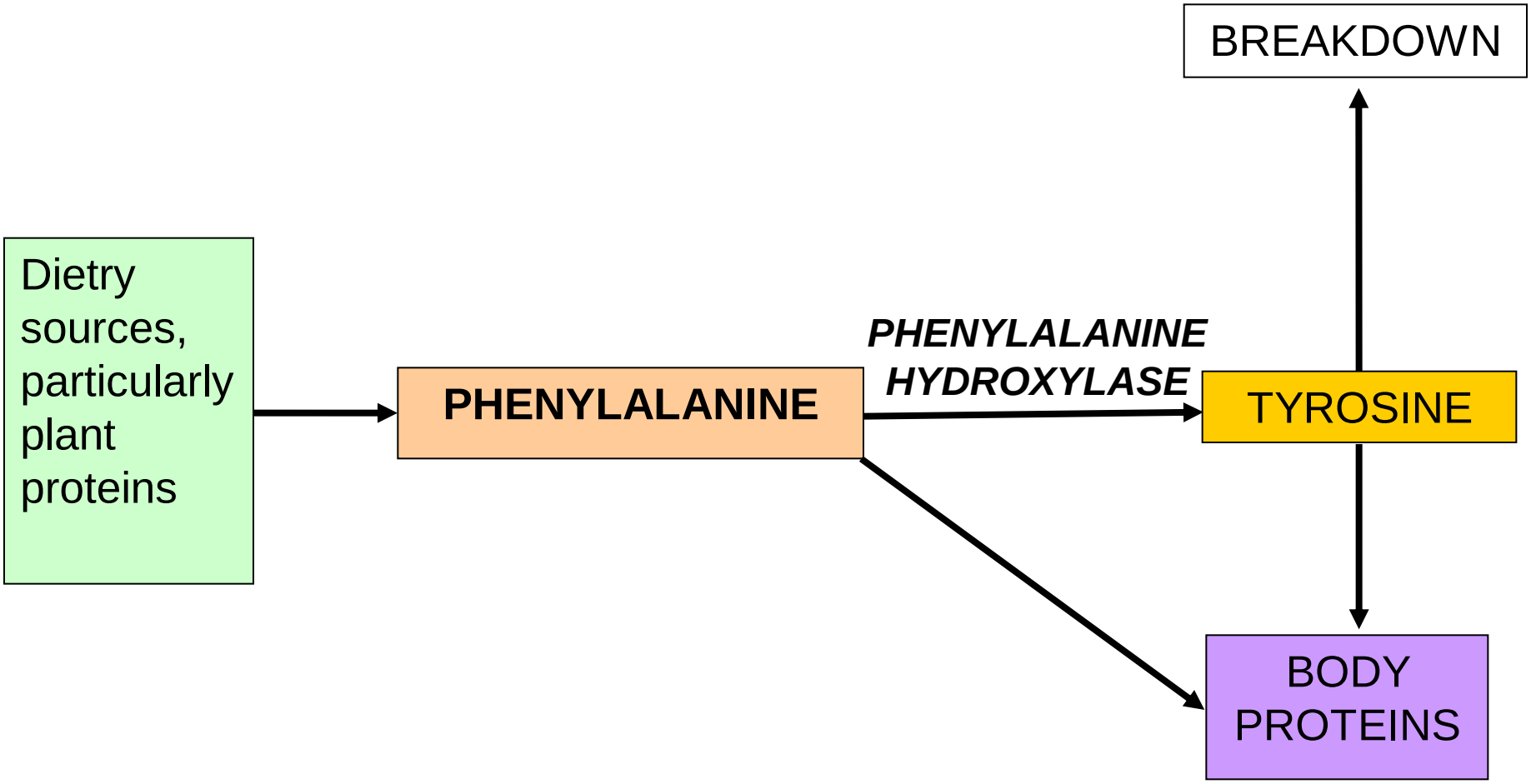
1. **limiting Phe intake** to levels barely adequate to support growth.

2. **Tyr**, an essential nutrient for individuals with **phenylketonuria**, must be supplied in the diet.

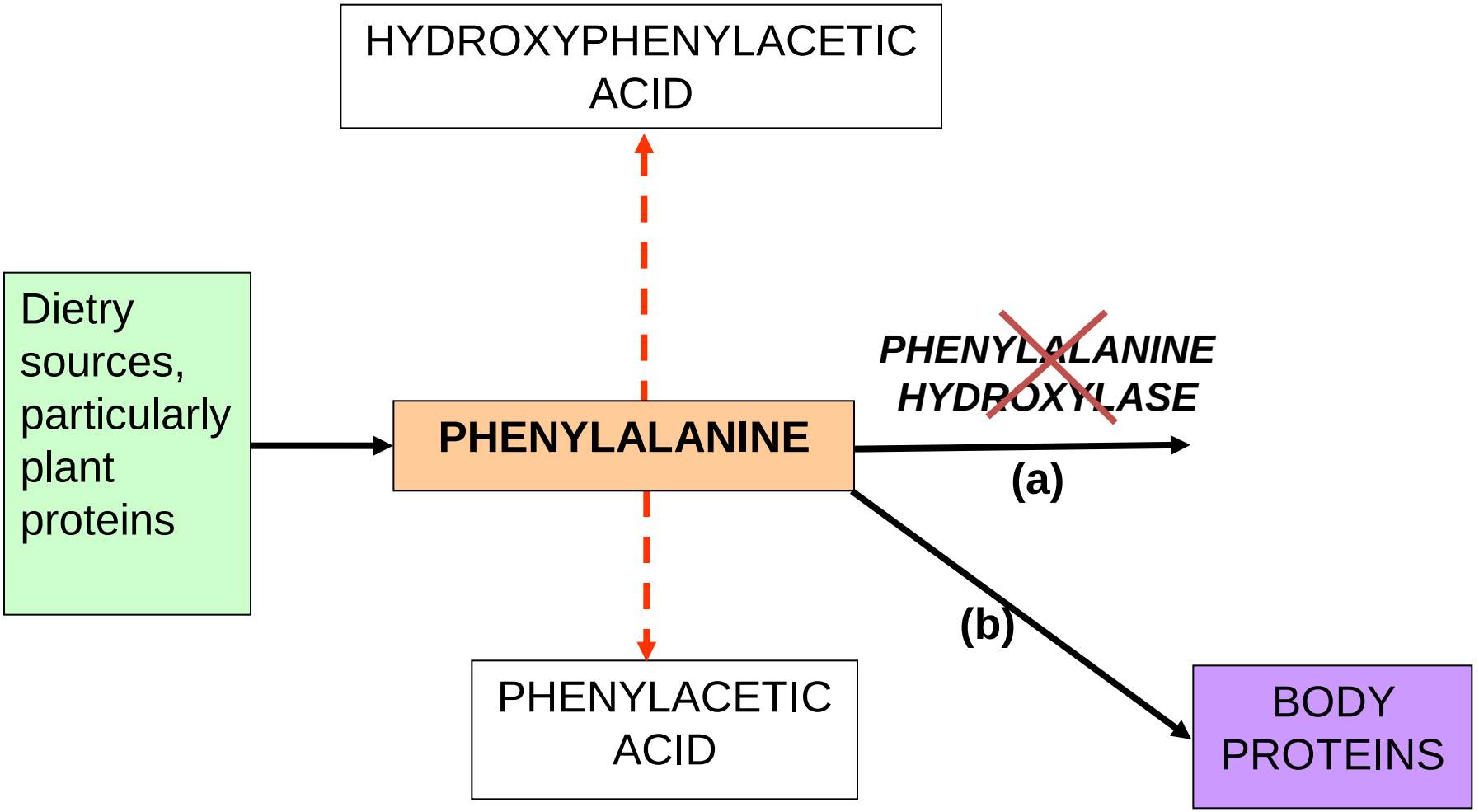
Causes

1. A single **mutant** recessive allele of the ***phenylalanine hydroxylase*** gene Locus.
2. Dietary excess of plant proteins which results in the exhaustion of a protein cofactor (**pterin**) needed by the enzyme.

The **normal** metabolism of phenylalanine



The **abnormal** metabolism in **phenylketonuric subjects**



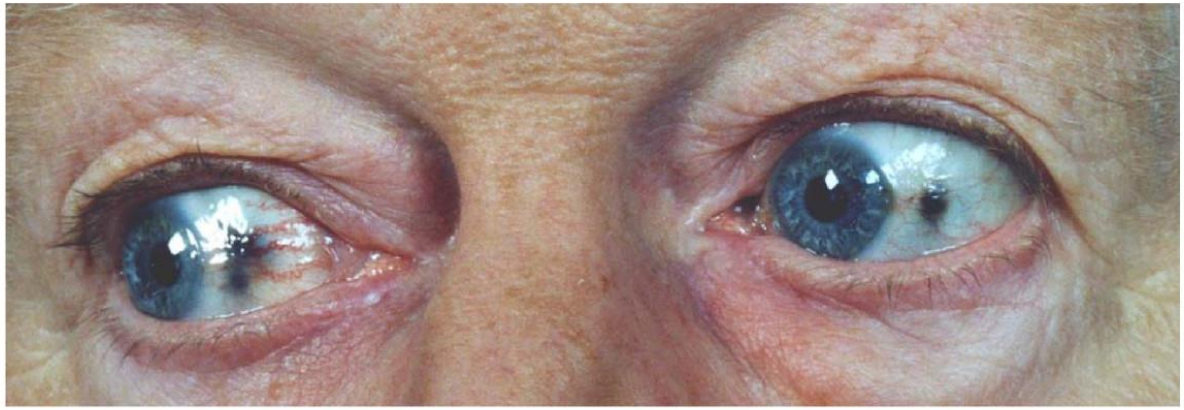
Treatment

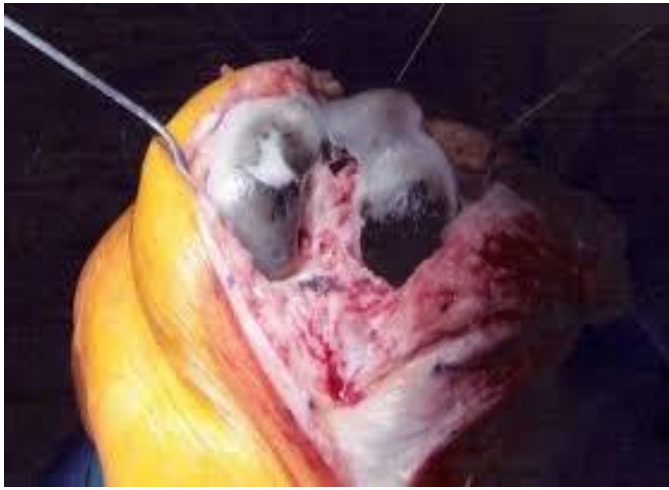
- A strictly controlled **phenylalanine** free diet up to the age of about **14 years old**
- **phenylalanine** is itself an essential amino acid small doses must be supplied
- After this age the growth and development of the brain is not affected by high levels of **phenylalanine** in the body

Albinism - ***tyrosinase*** is deficient and **melanine** (black pigment of skin, hear and eyes) not produce, and affected individuals (called **albinos**) are extremely sensitive to sunlight.

Alcaptonuria - coused by a deficiency of ***homogentisate oxidase***. Large quantities of **homogentisate** (product of **Tyr** metabolism) excreted in urine and autooxidizes, forming dark colored pigment (**black urine**). In additional, pigment accumulates in various tissue and cause a **degenerative arthritis**.

Fig. 3: Comparison of Colour of Freshly Voided Urine and Urine after 24 Hours





Secondary enzymopathy

PYRIDOXINE vitamin B_6 - 1.3 mg/d for adults.

In the liver, **pyridoxine** are phosphorylated by ***pyridoxal kinase*** to form **pyridoxine-P** and **PLP**, the active cofactor form. **PLP** is utilized by **112** (3%) of the **3870** enzymes catalogued in the ENZYME database.

Ornithine aminotransferase (OAT) is a **PLP**-dependent mitochondrial matrix protein that catalyzes the breakdown of **ornithine** to **pyrroline-5-carboxylic acid**, which is then converted into **proline**.

Defects in OAT lead to ***gyrate atrophy*** of the choroid and retina, an autosomal recessive disease that affects persons of all ages **OMIM*** 258870

* - **O**nline **M**endelian **I**nheritance in **M**an

PYRIDOXINE vitamin B_6 - 1.3 mg/d for adults.

Alanine–glyoxylate aminotransferase is a liver-specific enzyme that uses a **PLP** cofactor to transfer the amino group from **Ala** to **glyoxylate**, forming **Gly** and **pyruvate**.

A primary **hyperoxaluria** (see OMIM 259900) caused by a functional deficiency of the ***alanine–glyoxylate aminotransferase*** results in an accumulation of **glyoxylate** that is converted to **oxalate**, resulting in renal deposits of **calcium oxalate** - **urolithiasis disease** - and **renal failure**.

Large doses of **pyridoxine** reduced urinary

oxalate excretion

in **~30**% patients

with **primary hyperoxaluria**.

Thiamine (vitamine B_1)

- 1.2 and 1.1 mg/d for men and women.

Thiamine is phosphorylated to form **TPP**,

the cofactor used by 4 enzymes.

Branched-chain α-ketoacid dehydrogenase:

maple syrup urine disease

- ketoacidosis,
- mental retardation,
- ataxia.
- blindness as a result of the accumulation of α-keto acids.

Pyruvate decarboxylase:

Leigh disease

- lactic acidosis,
- ataxia,
- mental retardation,
- cardiomyopathy,
- psychomotor retardation,
- central nervous system damage,
- muscle fiber atrophy, and
- developmental delay.

Thiamine transporter, thiamine pyrophosphokinase, and α-ketoglutarate dehydrogenase:

- thiamine-responsive megaloblastic anemia,
- mental retardation,
- cardiomyopathy,
- psychomotor retardation,
- central nervous system damage.
- Sometimes blindness as a result of the accumulation of α-keto acids.

Riboflavin – vitamin B_2

Defects in mitochondrial **complex I** cause **myopathies**

OMIM 252010.

Decreased affinity for the **FMN cofactor** (**B_2**) may explain the cases of high level riboflavin responsiveness.

Large doses of vitamins

in 30% of cases

is normalized condition.