ENZYMOPATHIES

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CONTENTS

Medical enzymology. Enzymopathies. Classification. The mechanism of development of primary and secondary metabolic disorders in enzymopathies.

- Primary enzymopathies. Impairment of final products formation (albinism). Accumulation of intermediates (alkaponuria). Impairment of final products formation, and the accumulation of substrates-precursors (von Girke's disease – type I glycogenosis).
- Secondary enzymopathies: toxic and alimentary enzymopathies. Etiology. The mechanism of metabolic disorders development. Clinical manifestations.
- Review of carbohydrate and lipid metabolism enzymopathies. Amino acids enzymopathies.
- Purines and pyrimidines metabolism impairments. Lesch-Nihan syndrome, gout, xanthinuria.
- Porphyrins metabolism impairments. Porphyria.

MEDICAL ENZYMOLOGY

Enzymopathology

Enzymodiagnostics

Enzymotherapy

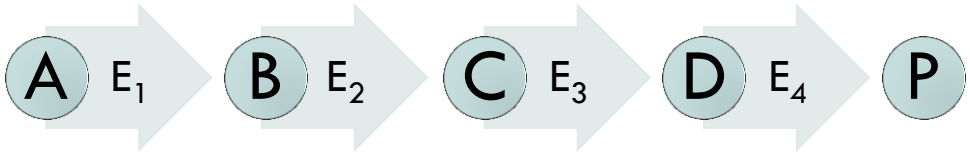
ENZYMOPATHOLOGY

Pokrovsky Enzymopathies classification:

- **Primary**: inherited (e.g. PKU)
- **Secondary**: non-inherited, acquired.
  - Alimentary: poor nutrition.
  - Toxic: poisoning with heavy metals (lead poisoning, mercury poisoning).

PRIMARY ENZYMOPATHIES

- In primary (inherited) enzymopathy enzyme defects are inherited mainly in autosomal recessive manner.
  - Heterozygotes often have no symptoms.
- First clinical signs are detected in early childhood,
  - but in some cases the disease is clinically manifested in older children or adults.
- Primary enzymopathies usually related to **metabolic diseases,**
  - as there is an impairment of certain **metabolic pathways**.



Normal metabolism

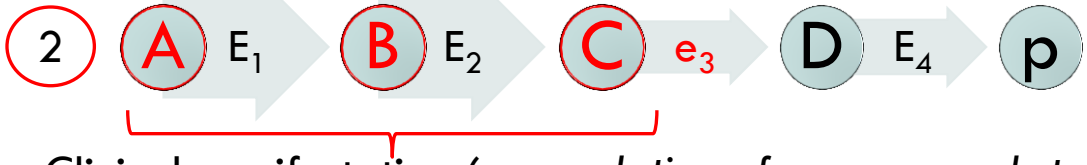
PRIMARY ENZYMOPATHIES MANIFESTATION

- The development of the disease can occur by one of the following scenarios (E_3 is mutated):

1. **Impaired final products formation**

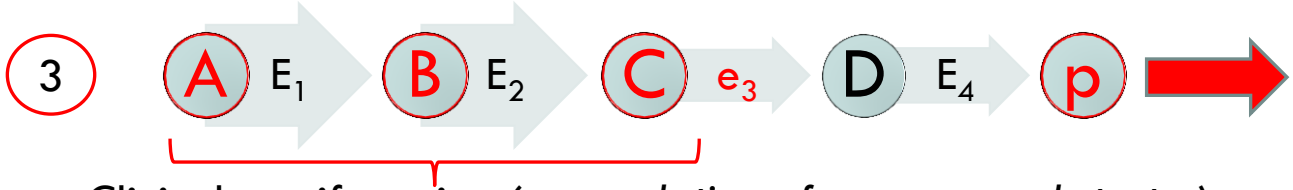
Clinical manifestation *(lack of the product, e.g., in albinism)*

2. **Accumulation of precursor substrates**



Clinical manifestation *(accumulation of precursor substrates, e.g., in alkaptonuria)*

3. **Both impaired final products formation and accumulation of precursor substrates**



Clinical manifestation *(lack of the product)*

Clinical manifestation *(accumulation of precursor substrates)*

(e.g., von Gierke's disease)

ENZYMODIAGNOSTICS

Enzymes in clinical diagnosis

- Enzyme assays provide important information (presence and severity of a disease).
- Enzyme assay allow to monitor a patient's response to therapy.
- Genetic predispositions is possible to determine using enzyme assay.

Several enzymes are used as reagent.

SOME ENZYMES USED FOR CLINICAL DIAGNOSIS OF DISEASE

Enzyme	Tissue source(s)	Diagnostic use
AST	heart, skeletal muscle, liver, brain	liver disease
ALT	liver	liver disease, e.g. hepatitis (ALT > AST)
amylase	pancreas, salivary gland	acute pancreatitis, biliary obstruction
CK	skeletal muscle, heart, brain	muscular dystrophy, myocardial infarction
GGT	liver	hepatitis, alcohol excess
LDH	heart, liver erythrocytes	lymphoma, hepatitis
lipase	pancreas	acute pancreatitis, biliary obstruction
alkaline phosphatase	osteoblast	bone disease, bone tumors
acid phosphatase	prostate	prostate cancer

AST = aspartate amino transferase; ALT = alanine amino transferase; CK = creatine phosphokinase; GGT = gamma glutamyl transferase; LDH = lactate dehydrogenase.

ENZYMOTHERAPY

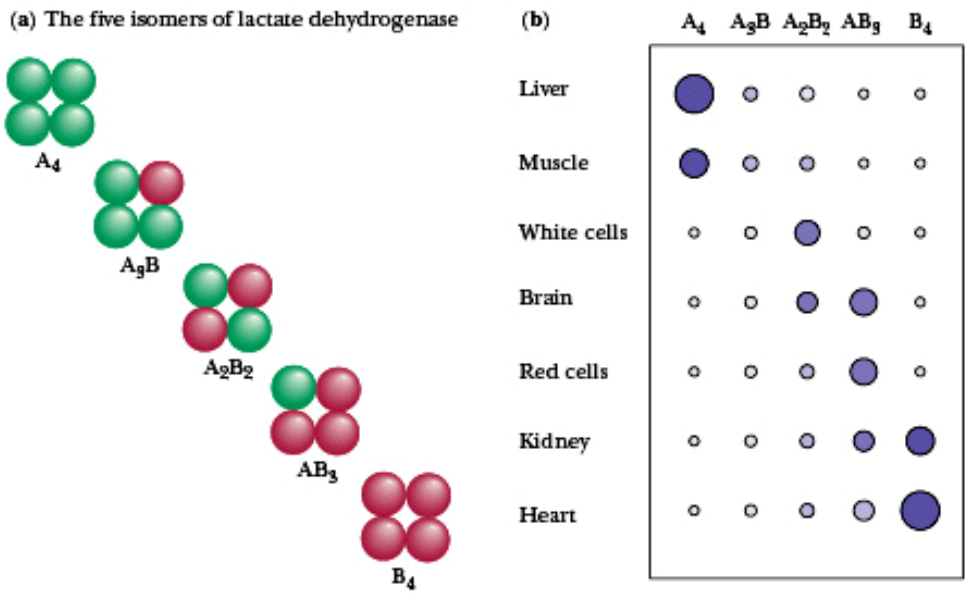
Festal, pancreatine – for substitution of lacking enzymes of GIT;

Streptokinase – in treatment of myocardial infarction;

Asparaginase – treatment of several types of cancer.

ISOZYMES

Multiple forms of enzymes, catalyzing the same reaction.

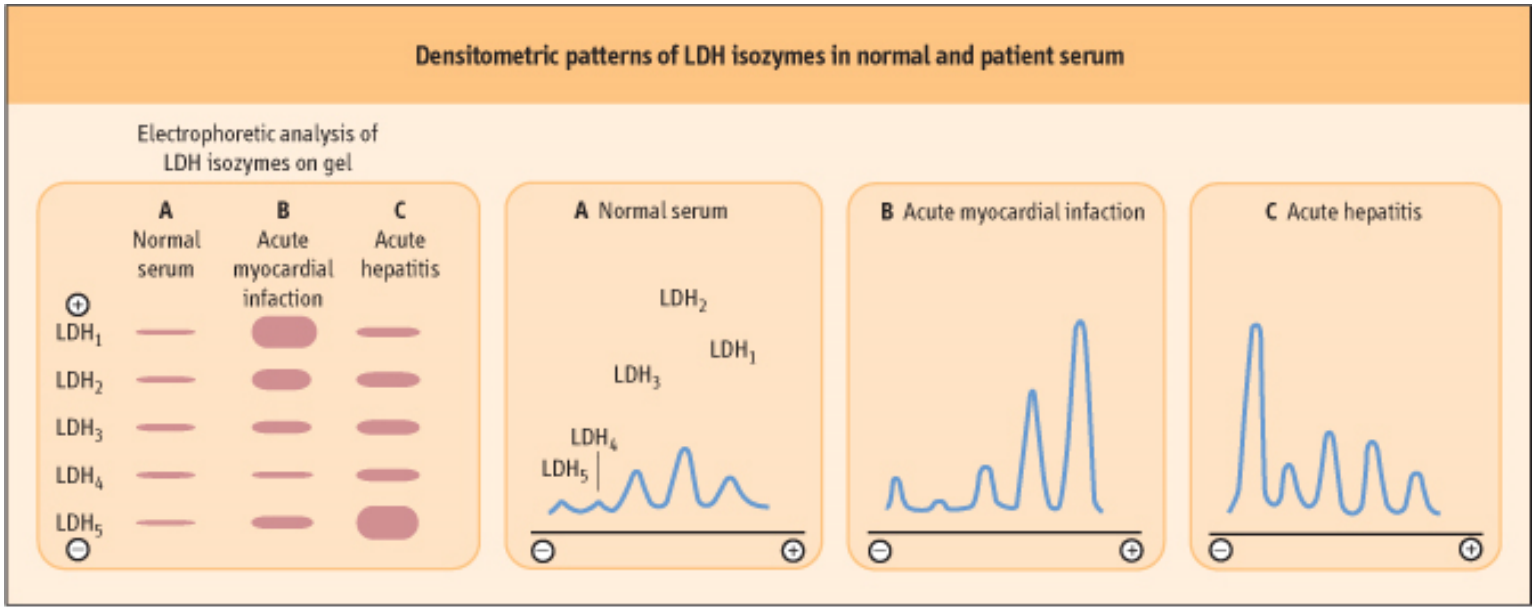


Lactate dehydrogenase:	
▪tetrameric, ▪2 subunints, ▪5 isoforms are possible:	LDH 1 (B4) LDH 2 (AB3) LDH 3 (A2B2) LDH 4 (A3B) LDH 5 (A4)

Subunit designation variants: A=M; B=H
(M – muscle, anaerobic izozyme
H – heart, aerobic izozyme)

There is also 3rd subunit is X – in testis.
Analogous to subunit A.

LDH ISOZYMES IN NORMAL AND PATIENT SERUM



Densitometric patterns of the LDH isozymes in serum of patients diagnosed with myocardial infarction or acute hepatitis.

- Isozymes, differing slightly in charge, are separated by electrophoresis on cellulose acetate, visualized using a chromogenic substrate, and quantified by densitometry. Total serum LDH activity is also increased in these patients.
- Since hemolysis releases LDH from red blood cells and affects diagnosis, blood samples should be treated with care.
- The LDH measurements for the diagnosis of myocardial infarction have now been superseded by plasma troponin levels.

CONTROL OF ENZYME SYNTHESIS (1/2)

Enzymes whose concentrations remain essentially constant over time are termed **constitutive enzymes.**

The concentrations of many other enzymes depend upon the presence of **inducers,** typically substrates or structurally related compounds, that initiate their synthesis.

- *Escherichia coli* grown on glucose will only catabolize lactose after addition of a **β-galactoside,** an inducer that initiates synthesis of a β-galactosidase and a galactoside permease.
  - Inducible enzymes of humans include tryptophan pyrrolase, threonine dehydrase, tyrosine-α-ketoglutarate aminotransferase, enzymes of the urea cycle, HMG-CoA reductase, and cytochrome P450.

CONTROL OF ENZYME SYNTHESIS (2/2)

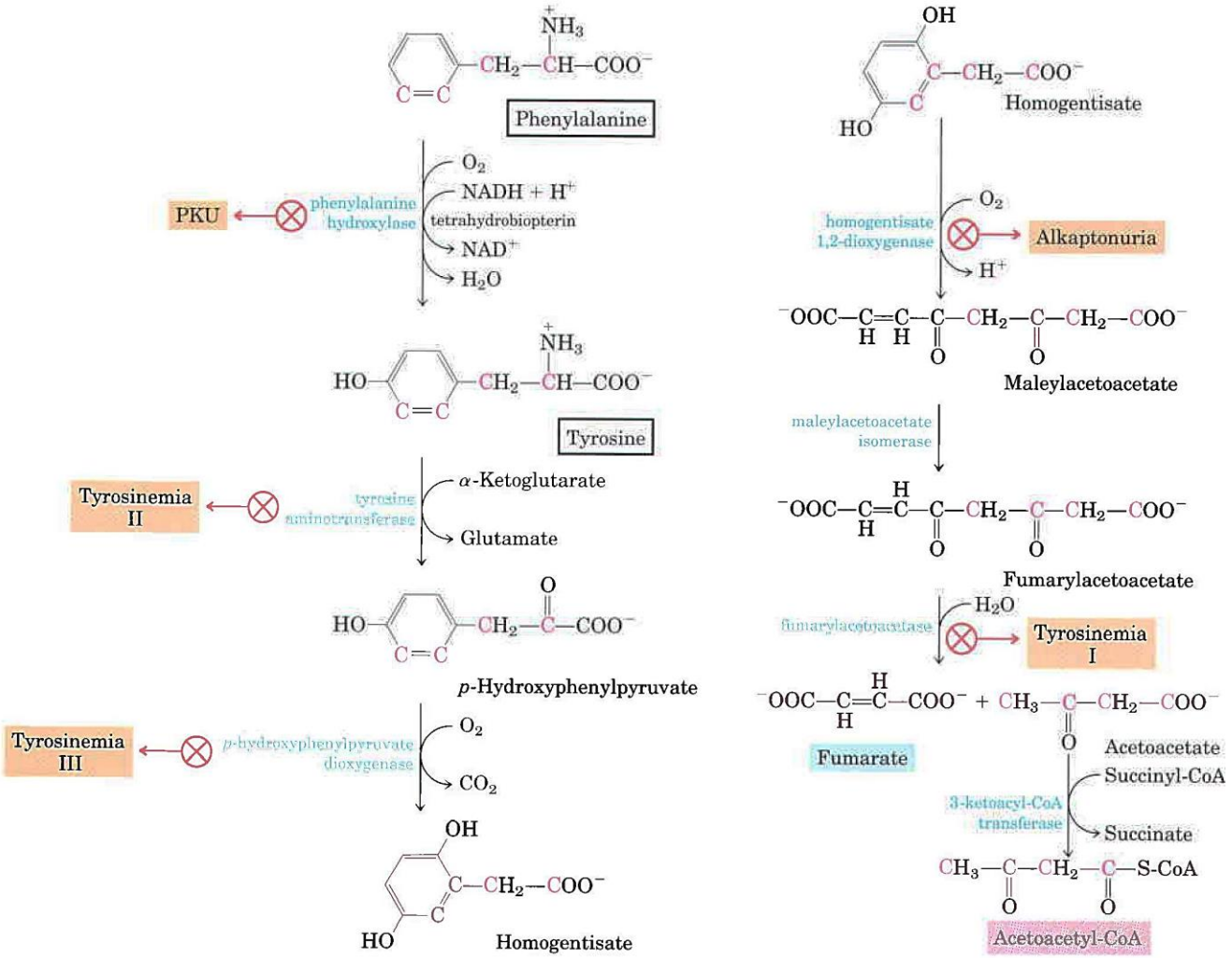
Conversely, an excess of a metabolite may curtail synthesis of its cognate enzyme via repression.

Both induction and repression involve cis elements, specific DNA sequences located upstream of regulated genes, and trans-acting regulatory proteins.

EXAMPLES OF PRIMARY ENZYMOPATHIES

- **Hereditary diseases of amino acid metabolism:**
  - Phenylketonuria, albinism, alkaptonuria ("black diaper" disease), "blue diaper" disease, "maple syrup urinary disease“, et al.
- **Hereditary diseases of carbohydrate metabolism**
  - glycogenoses, galactosemia, fructosuria, disaccharidase deficiency (malabsorption), mucopolysaccharidoses.
- **Hereditary diseases of lipid metabolism**
  - lipidoses are characterized by elevated serum blood level of lipids, lipoproteins and cholesterol or
  - lipidoses with intracellular inclusions.
- **Hereditary diseases of purine and pyrimidine metabolism**
  - They include some form of gout, Lesch-Nyhan syndrome.

CATABOLISM OF PHE AND TYR



ALBINISM

- Albinism defines a genetically and clinically heterogenous group of diseases characterized by reduction in melanin in the skin, hair and eye (oculocutaneous albinism, OCA, mostly autosomal recessive), or primarily in the eye (ocular albinism, OA, X-linked recessive).
- Among the different forms of albinism OCA2 has the highest **prevalence** of
  - 1:37,000 in Caucasians,
  - 1:15,000 in African-Americans, and
  - 1:3,900 in Southern Africans with Bantu-speaking origin.

- Mutated genes in albinism:
  1. Tyrosinase gene (Tyr), MIM 203100;
  2. P gene, MIM 203200;
  3. Tyrosinase-related protein-1 gene (TYRP1), 203290;
  4. Membrane-associated transporter protein (MATP), MIM 606574;
  5. HPS1 gene, MIM 604982;
  6. Beta-3 A-adaptin gene (ADTB3A), MIM 603401;
  7. HPS3 gene, MIM 606118;
  8. HPS4 gene, MIM 606682;
  9. HPS5 gene, 607521;
  10. HPS6 gene; MIM 607522;
  11. CHS1 gene; MIM 214500;
  12. OAI gene MIM 300500 (1).

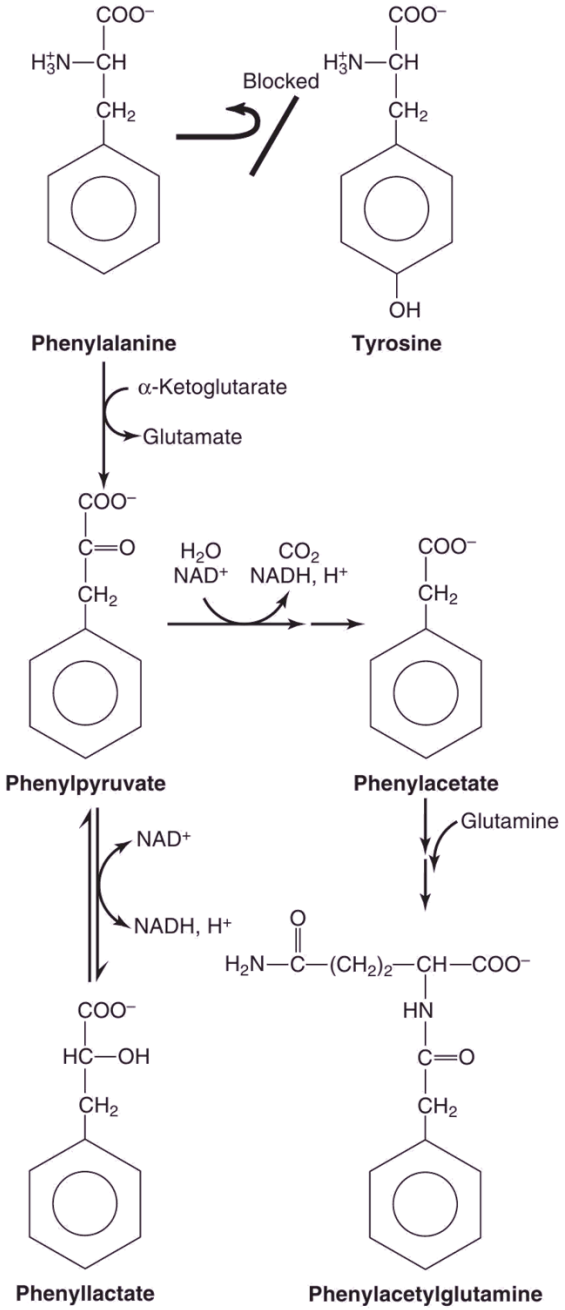
A SELECTION OF GENES AND LOCI OF ALBINISM

Type of albinism	MIM#	Human chromosome	Human locus	Encoded protein	Murine locus	Functional role in pigmentation
OCA1	203,100	11q14–21	*TYR*	Tyrosinase	*albino (c)*	Melanogenic enzyme
OCA2	203,200	15q11–13	*OCA2*	Melanomsomal membrane protein[1]	*pink-eyed dilution (p)*	Stabilization of melanosomal pH
OCA3	203,290	9q23	*TYRP1*	Tyrosinase-related protein (TPP-1)	*brown (b)*	Melanogenic enzyme/stabilizing factor
HPS	604,982	10q24	*HPS1*	Membrane protein	*pale ear (ep)*	Lysosome/melanosome structure/ function
CHS	214,500	1q43	*CHS1*	Membrane protein	*beige (bg)*	Lysosome/melanosome structure/ function
OA1	300,500	Xp22.3–22.3	*OA1*	Melanosomal membrane protein	*OA1 (oa)*	Intracellular signaling/melanosomal biogenesis

Therapy: photoprotection is essential to minimize the risk of cutaneous cancers (esp. in OCA1 and OCA2 patients). Topical broad-spectrum sunscreens, physical sun protection and sunglasses are necessary and regular clinical examination on a yearly basis are advised.

PHENYLKETONURIA

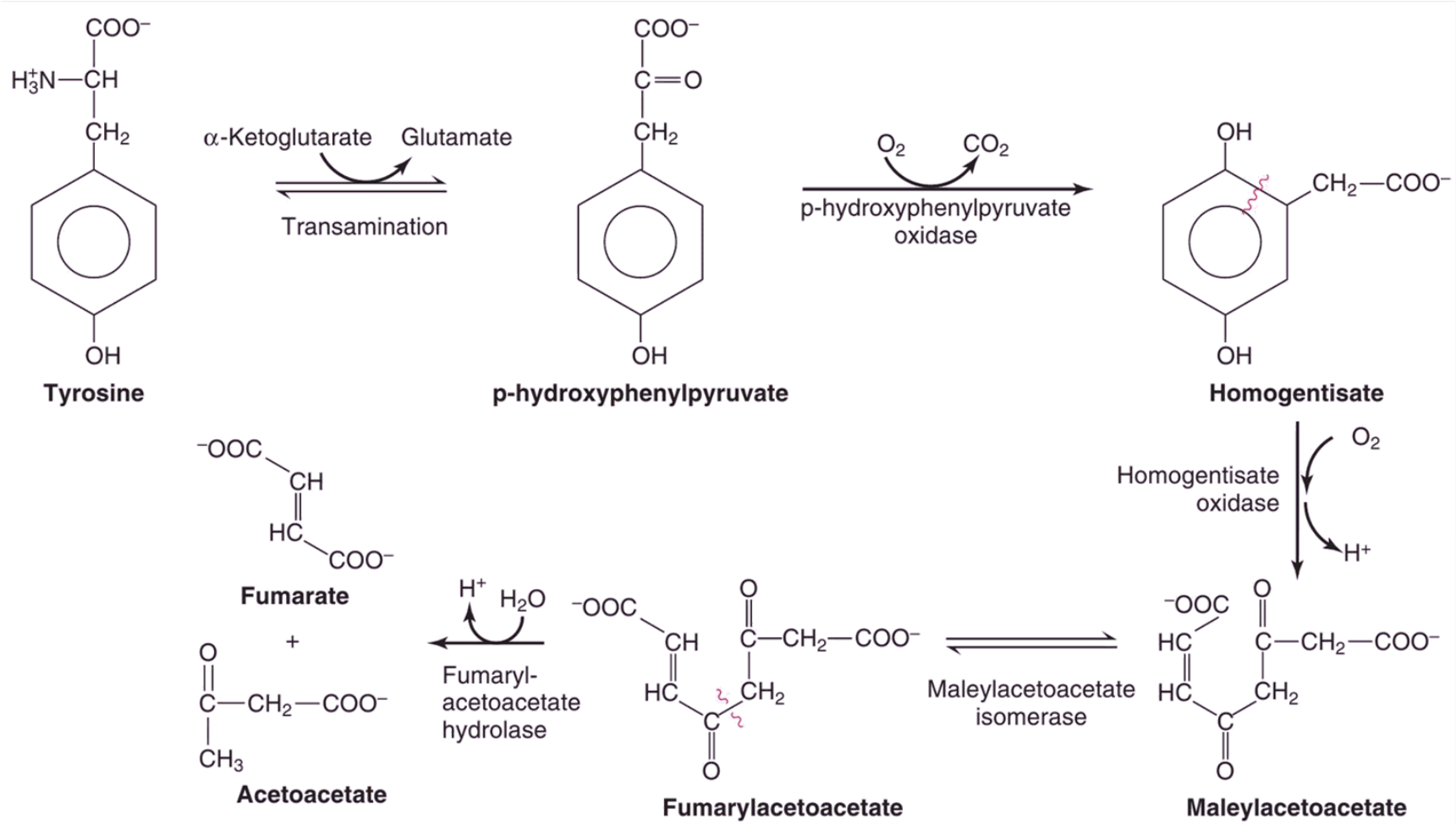
Definition and Characteristics. Autosomal recessive defect in untreated patients usually results in profound mental retardation and neurodegenerative changes.



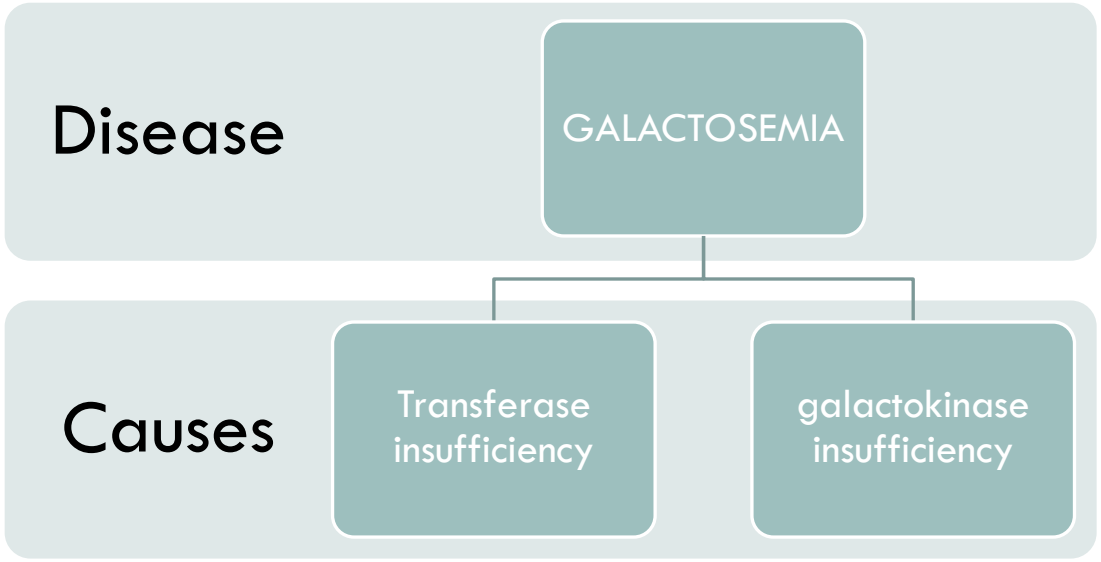
ALKAPTONURIA (BLACK URINE DISEASE)

- Alkaptonuria (AKU) is caused by homozygous or compound heterozygous mutation in the **homogentisate 1,2-dioxygenase** gene (HGD; 607474) on chromosome 3q13.
  - autosomal recessive metabolic disorder characterized by accumulation of homogentisic acid, leading to darkened urine, pigmentation of connective tissue (**ochronosis**), joint and spine arthritis, and destruction of the cardiac valves.
- **The manifestations** are urine that turns dark on standing and alkalinization, black ochronotic pigmentation of cartilage and collagenous tissues, and arthritis, especially characteristic in the spine.

ALKAPTONURIA: IMPAIRED TYR CATABOLISM



GALACTOSEMIA

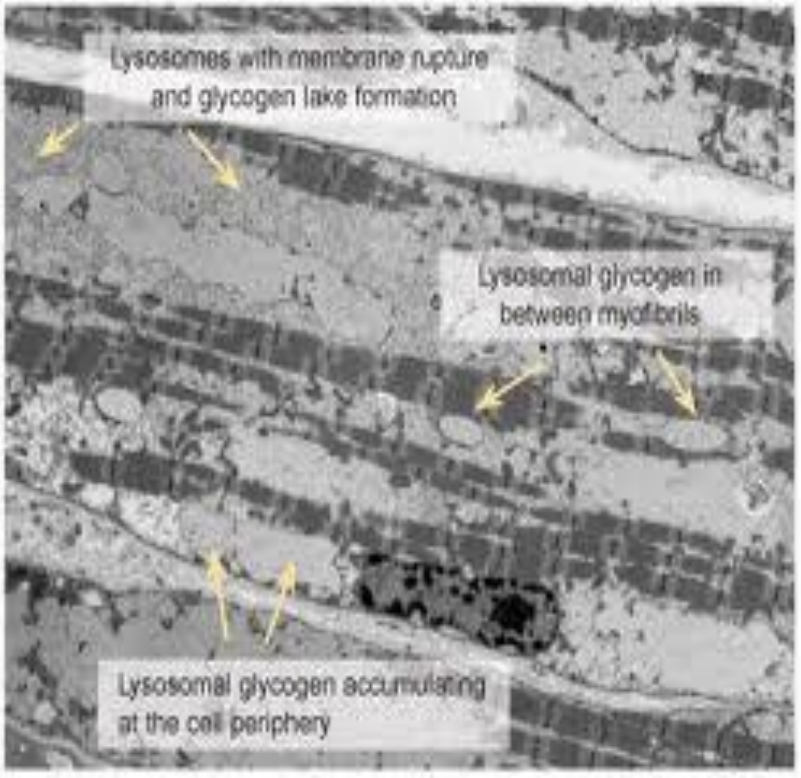


- This is hereditary illness. In it's base lies an blockade of galactose metabolism.
- In organism intermediate metabolits accumulate.
- There are two the main forms of galactosemia on base of transferase insufficiency and on base of galactokinase insufficiency.

GLYCOGENOSES

- Simple carbohydrates deposit in organism as polysaccharides. In muscles and liver accumulates glycogen. It consist of 4 % of liver weight and 2 % of muscles weight. Muscles glycogen is used as of ready fuel source for immediate guaranteeing by energy. Liver without interruption provides cerebrum and erythrocytes with glucose .

- Synthesis and splitting of glycogen are exactly adjusted and coordinated processes. Attached to immediate need in glucose α–cells of pancreas secret glucagone. It activates adenylatcyclase of hepatic cells. Adenilatcyclase stimulates derivation of cAMP. Under action of cAMP takes place activation of proteinkinase and this enzyme raises activity glycogenphosphorilase and oppresses activity of glucogensynthase.



GLYGENOSES: TYPE I

- ***Glycogenosis type I – von Girke's disease.*** Von Girke's disease cause deficit of glucose-6-phosphatase. This enzyme provides 90 % of glucose which disengages in liver from glycogen.
- It play central role in normal glucose homeostasis. Glucose which disengages attached to disintegration of glycogen or is derivated in process of gluconeogenesis obligatory goes over stage of glucose-6-phosphate.
- Enzyme glucose-6-phosphatase tears away a phosphate group from glucose. There free glucose is formed it goes out in blood.
- Attached to Girke's disease stage of tearing phosphate group is blocked. There are no free glucose hypoglycemia occur.
- Hypoglycemia arises. Attached to Girke's disease glycogen is deponed in liver and kidneys.

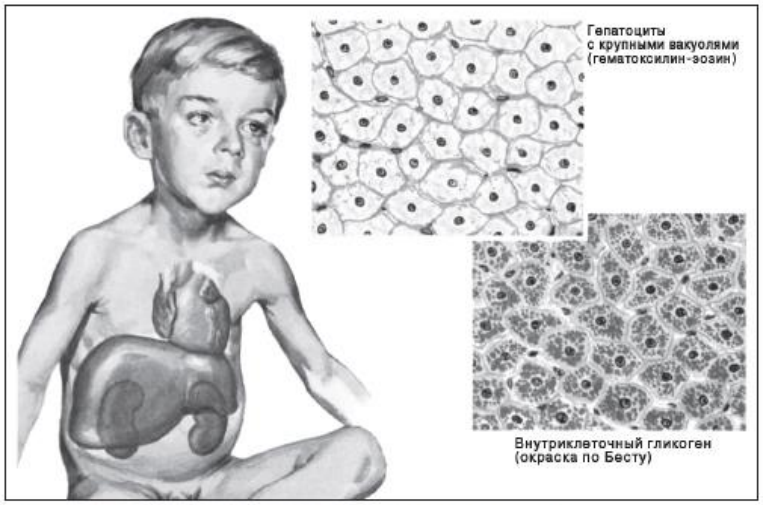
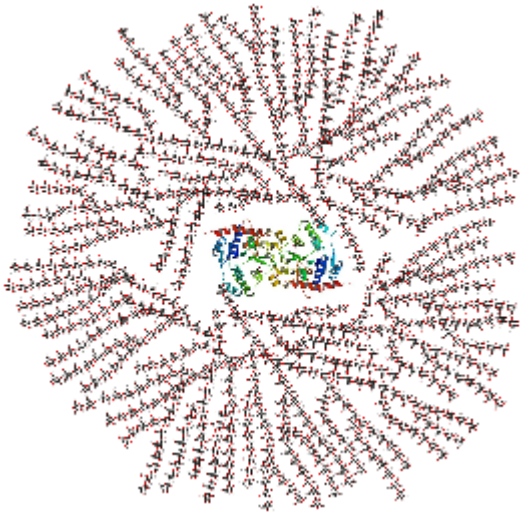


Рисунок 2. Симптомы болезни Гирке (по F. Netter, 2001)

Girke's disease

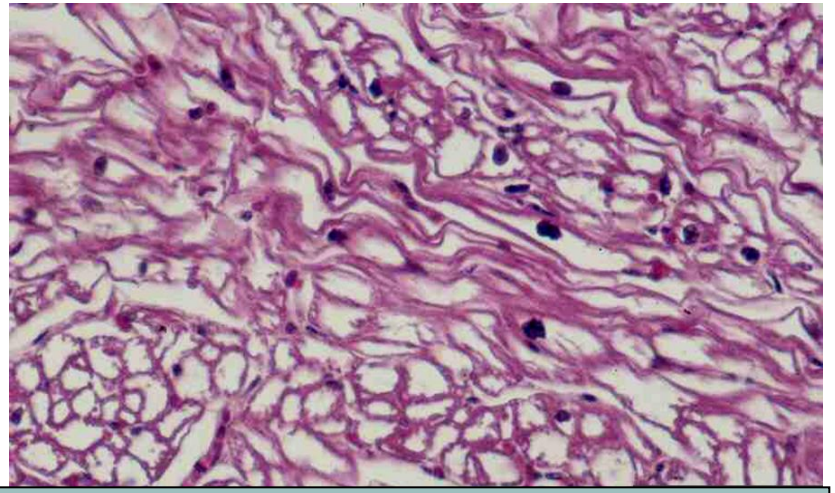
GLYCOGENOSES: TYPES II, III, IV



Type II glycogenosis – Pompe's disease. Illness is related to deficit of lysosomal enzyme – sour maltase, or α-1,4-glucosidase. This enzyme slits glycogene to glucose in digestive vacuoles. Attached to it's deficit glycogen accumulates at first in lysosomes and then in cytosole of hepatocytes and myocytes.

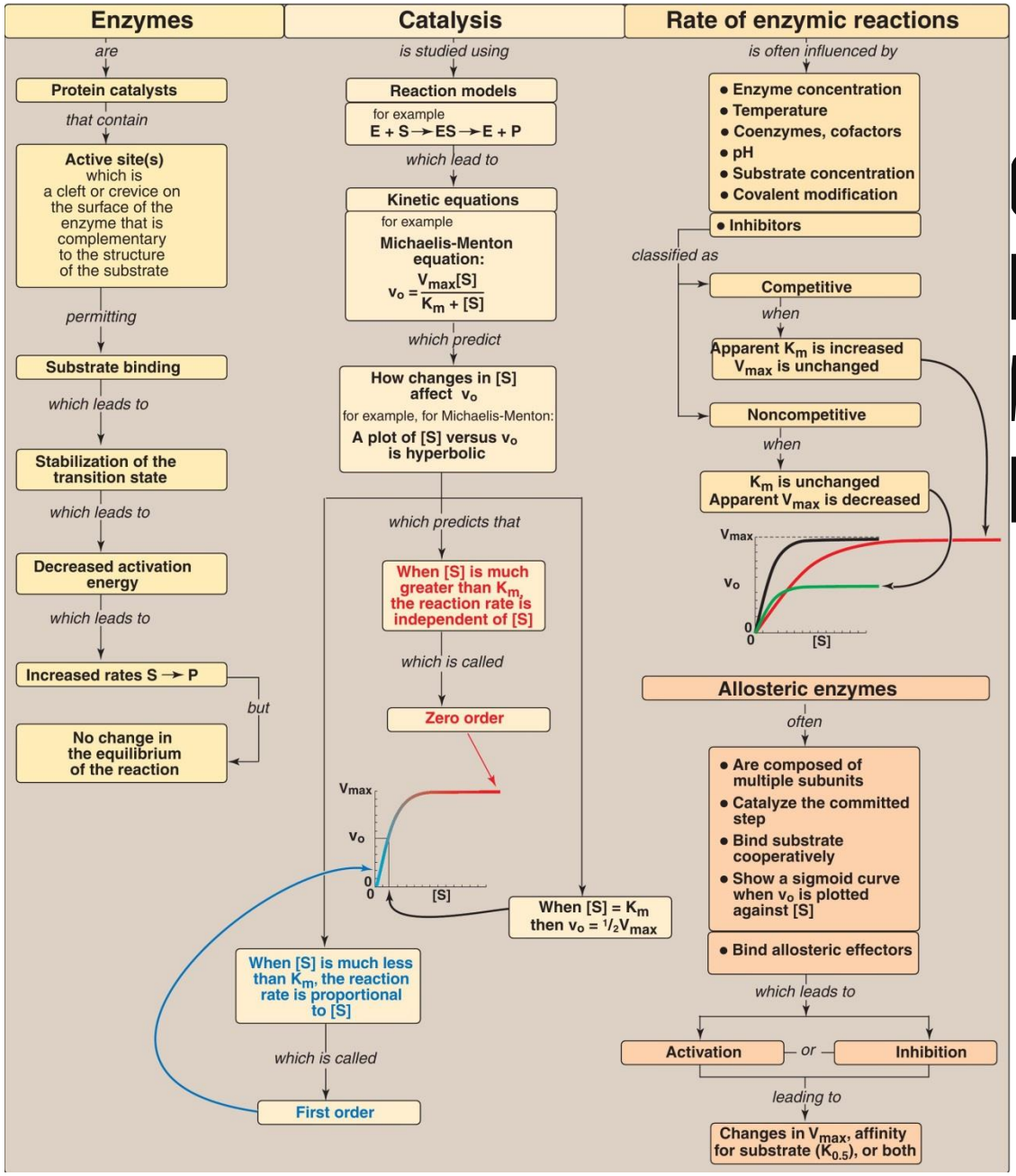
Type III glycogenosis – Cori's disease, Forbs' disease. This illness is named limitdecstrinosis. In it's base lies a deficit of amylo-1,6-glucosidase. Degradation of glycogen pauses in sites of branching. Glycogen accumulates in liver and muscles. Cure is diet with big proteins maintenance.

Type IV glycogenosis – Anderson's disease. It is called by deficit of amilo-1,4,1,6-transglucosidase (branching enzyme). As result of this There is derivated anomalous glycogen with very long branches and rare points of branching. It is not exposed to degradation and accumulates in liver, heart, kidneys, spleen, lymphatic nods, skeletal muscles.



Glycogen storage disease II (Micro)

The myofibers are engorged with glycogen. On cross sections the myofibrils are pushed to the periphery. Despite the thick walls, this is not hypertrophy. These patients present with congestive failure.



CONCLUSION: KEY CONCEPT MAP FOR THE ENZYMES

Lippincott's Illustrated Reviews: Biochemistry, 2011