Molecular Pathology

The enzyme defects
and
Their consequences

For educational use only

DEFECTS OF ENZYMATIC PROTEINS

Catalysts that increase (accelerate) the rate of chemical reactions

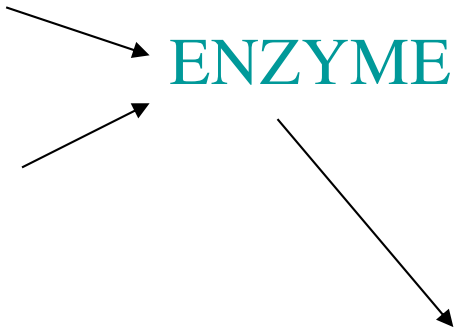
A specific substrate

Small quantities

Active Site

Qualitative Alteration

Quantiative Alteration



ENZYME DEFICIENCY

Biochemical mechanisms in inborn errors of metabolism

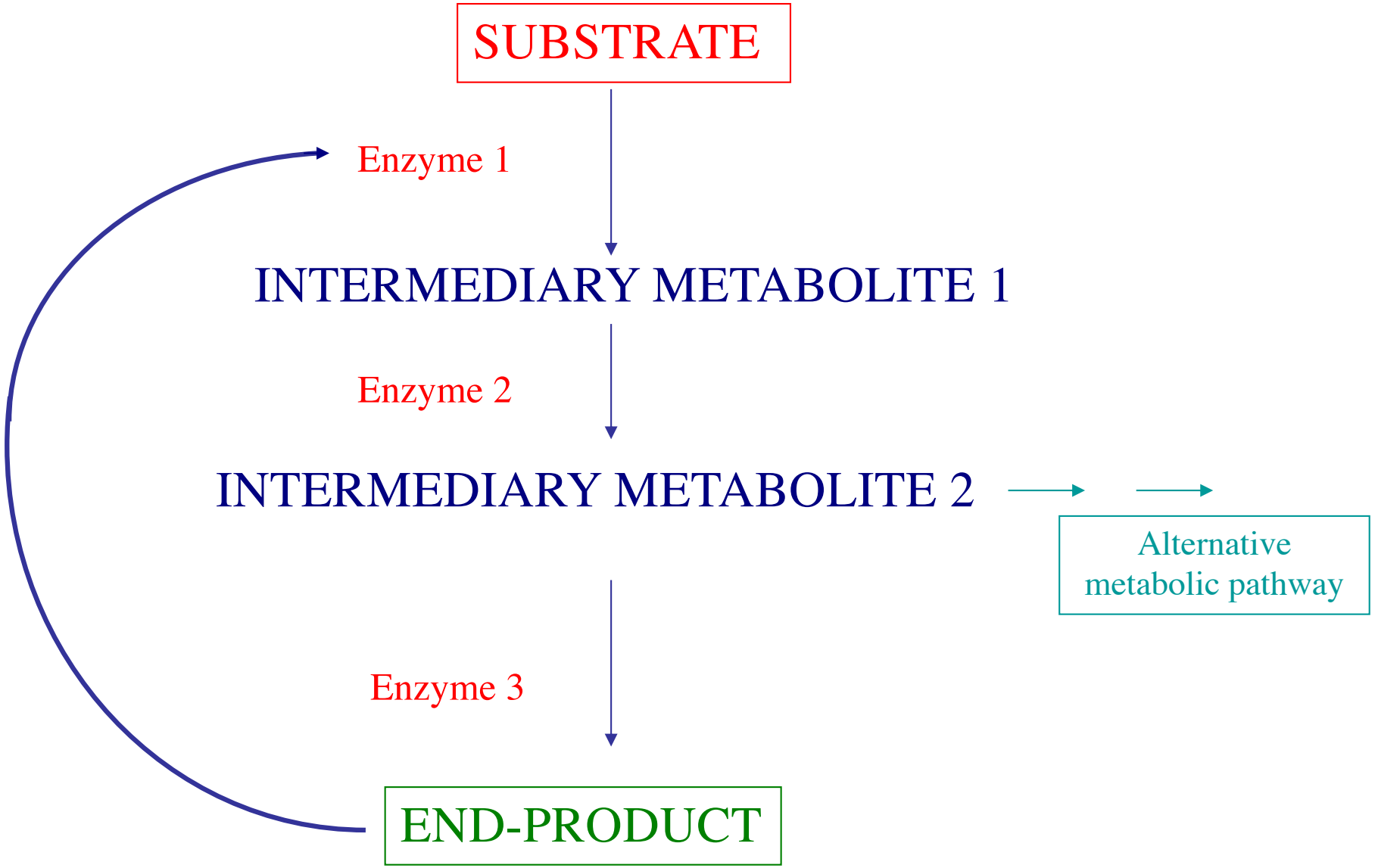
A single gene defect can have several impact on metabolic pathways that can lead to four main biochemical consequences:

1. **Failure to complete a metabolic pathway→ metabolic block:** the end-product is not formed because the enzyme that is required for the completion of a metabolic sequence is missing. (Albinism)

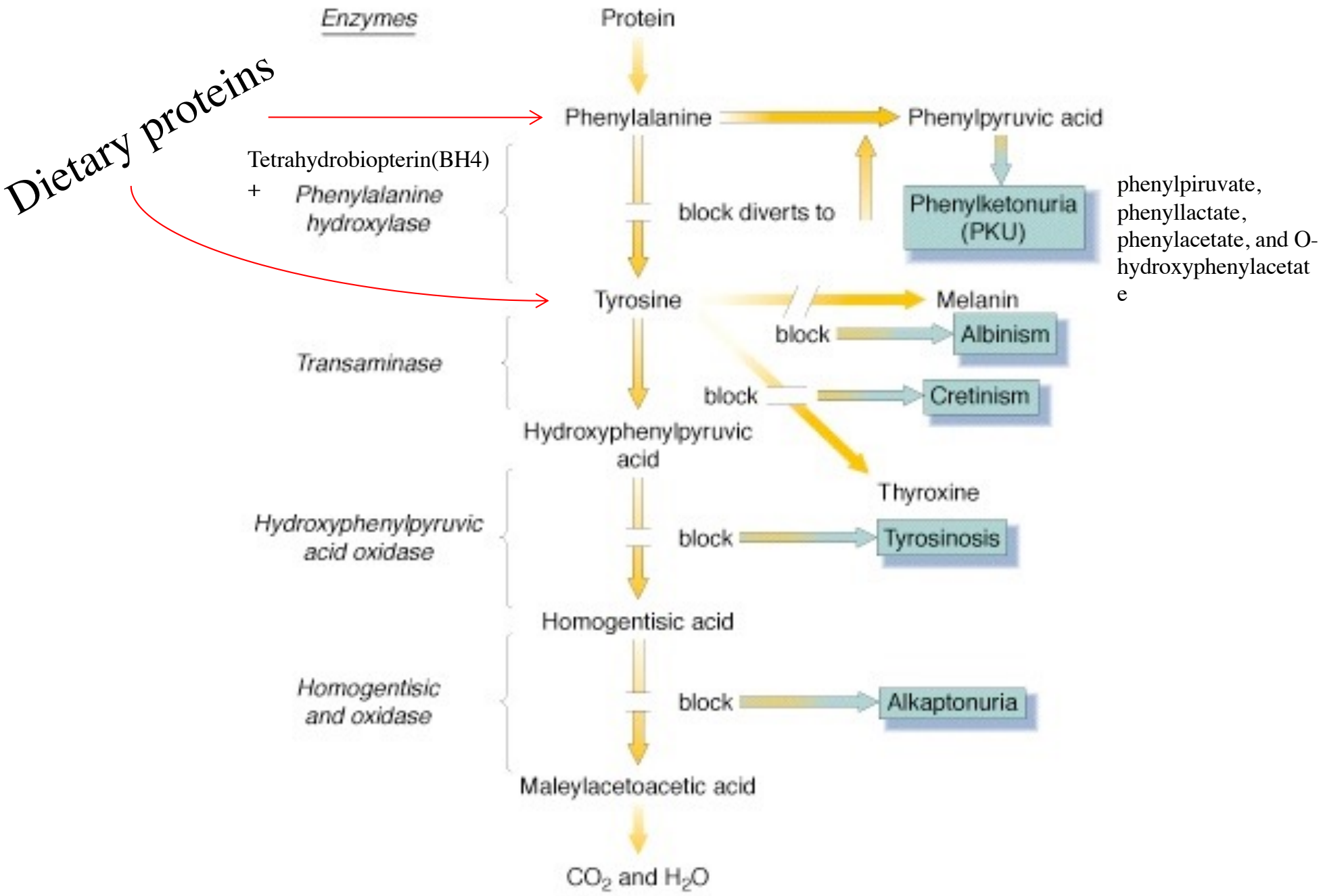
2. **Accumulation of unmetabolized substrate:** the enzyme that converts the initial substrate into the first intermediary metabolite may be missing, in which case the initial substrate accumulates in excess. (Phenylketonuria)

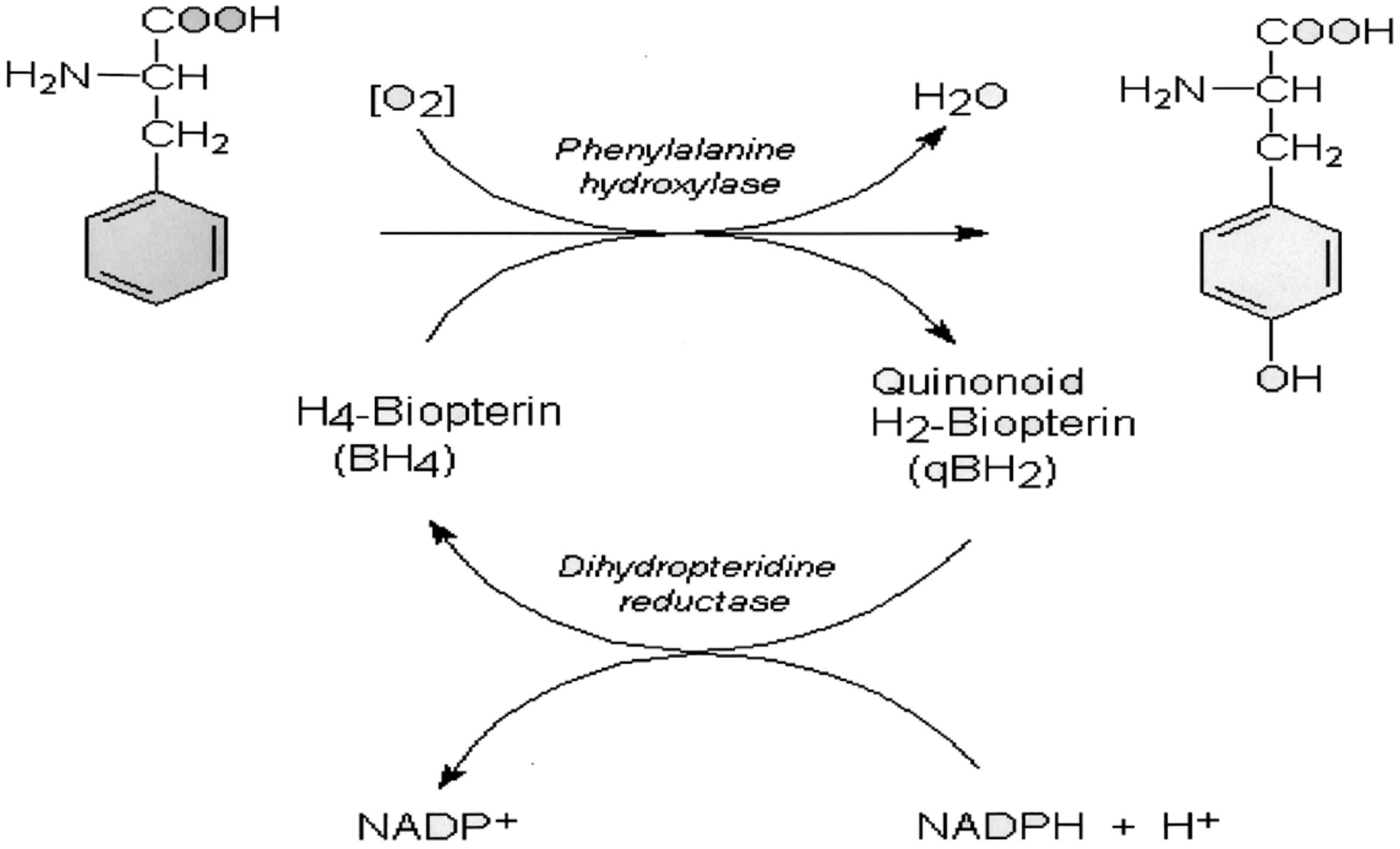
3. **Storage of an intermediary metabolite:** an intermediate metabolite, which is normally quickly processed into the final product and so is usually present only in minute amounts, accumulates in large quantities if the enzyme for its metabolism is lacking. (vonGierke)

4. **Failure to inactivate a tissue-damaging substrate.** (α1-antitrypsin)


SUBSTRATE
Enzyme 1
INTERMEDIARY METABOLITE 1
Enzyme 2
INTERMEDIARY METABOLITE 2
Alternative metabolic pathway
Enzyme 3
END-PRODUCT

Diseases by disturbance of Phenylalanine and Tyrosine metabolism





BH4 is a cofactor required fo hydroxylation of Phe by PAH. Defect results from a failure to regenerate BH4.

Malignant Hyperphenilalaninemia

Deficinecy of tetrahydrobiopterin (BH4), cofactor required for hydroxylation of Phenylalanine by PAH.

A failure to regenerate BH4 due to hereditary lack of dydropteridin reductase (DHPR) reduces BH2 to BH4.

DHPR gene is on the short arm of chromosome 4.

Impaired synthesis of BH4 has been described

Phenotipically indistinguishable from classic PKU.

BH4 deficiency interfers with neurotrasmetters synthesis

No treatment.

PHENYLKETONURIA

- Newborn Screening programs
- Increased Phe concentrations may be transient due to non PKU disease.
- Heelprick onto filter paper (spectrometry)

- For prenatal diagnosis molecular genetic techniques:
- Sothern blotting, restriction enzyme digestion,
- Detection of mutations for differential diagnosis by sequencing.
- Emerging

GLYCOGENOSES

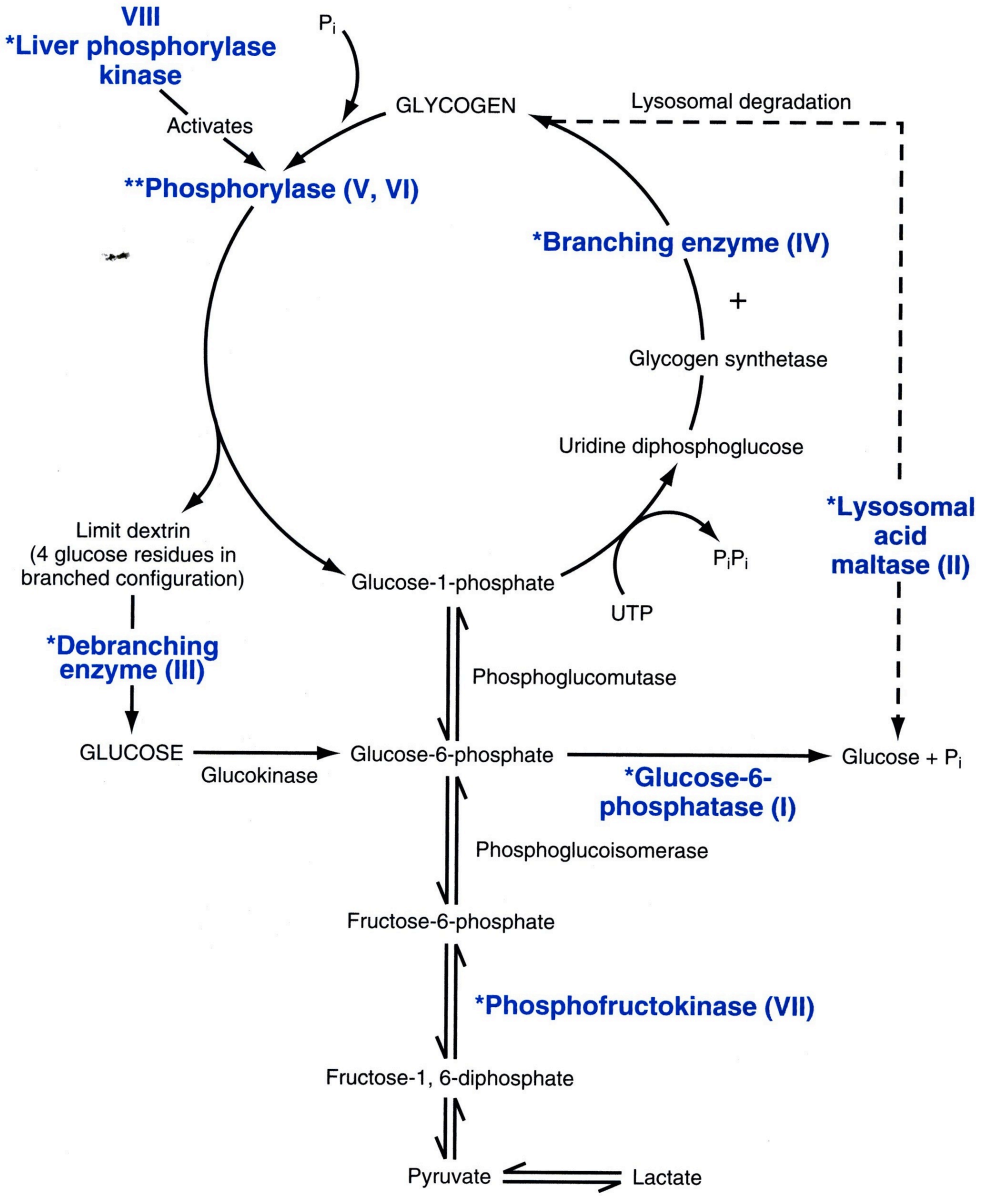
Glycogen storage diseases

Glycogenoses

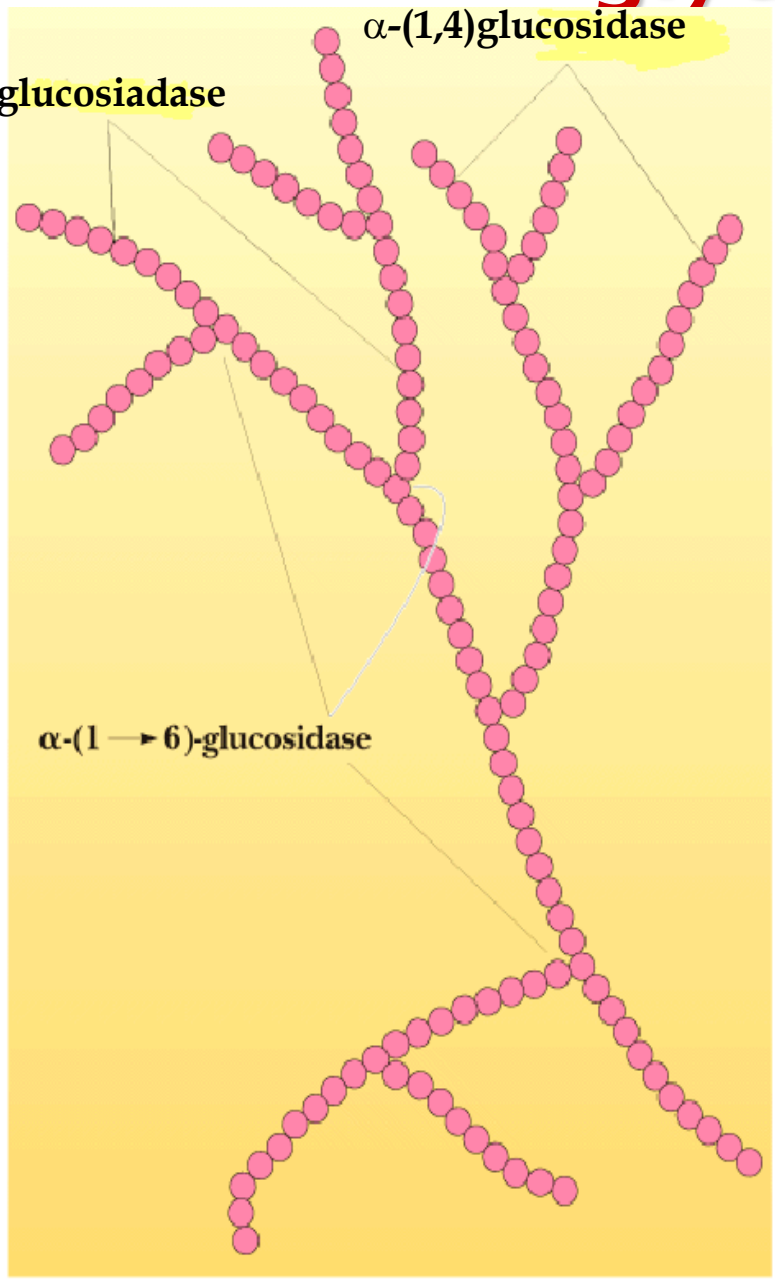
- Glycogen storage diseases:
- Characterized by pathologic accumulation of glycogen
- Strutturally normal or abnormal,
- Due to genetic enzymatic deficiency in one of the enzymes involved in glycogen metabolism
- Hereditary diseases { ausomal recessive; X-linked
- Target organs: liver, muscle tissue, kidney, myocardium

Glycogem metabolism

Pathway of Glycogenosynthesis and Glycogenolysis in liver



Glycogen degradation and mobilization of glycogen



Phosphorylase: catalyzes cleavage of α(1,4) bond, releasing glucose-1-phosphate

Debranching enzyme (amylo 1,6 glicosidase): hydrolizes the α(1,6) bond, releasing free glucose

Acid Maltase (α1,4-glucosidase): Enzyme located in lisosomes, ubiquitous, hydrolizes external linear chains (maltose and other linear oligosaccarides).

Glycogenoses

Autosomal recessive

Two main causes of damage

- •Cell damage by accumulation of glycogen
- •Energy deficiency for nearly absent glycolisis

Hepatic forms - Types I,III,VI,VIII

-Hepatomegaly by accumulation of glycogen in liver (and other organs)

-Hypoglicemia by low glucose

Muscle Forms – Types (II), V, VII

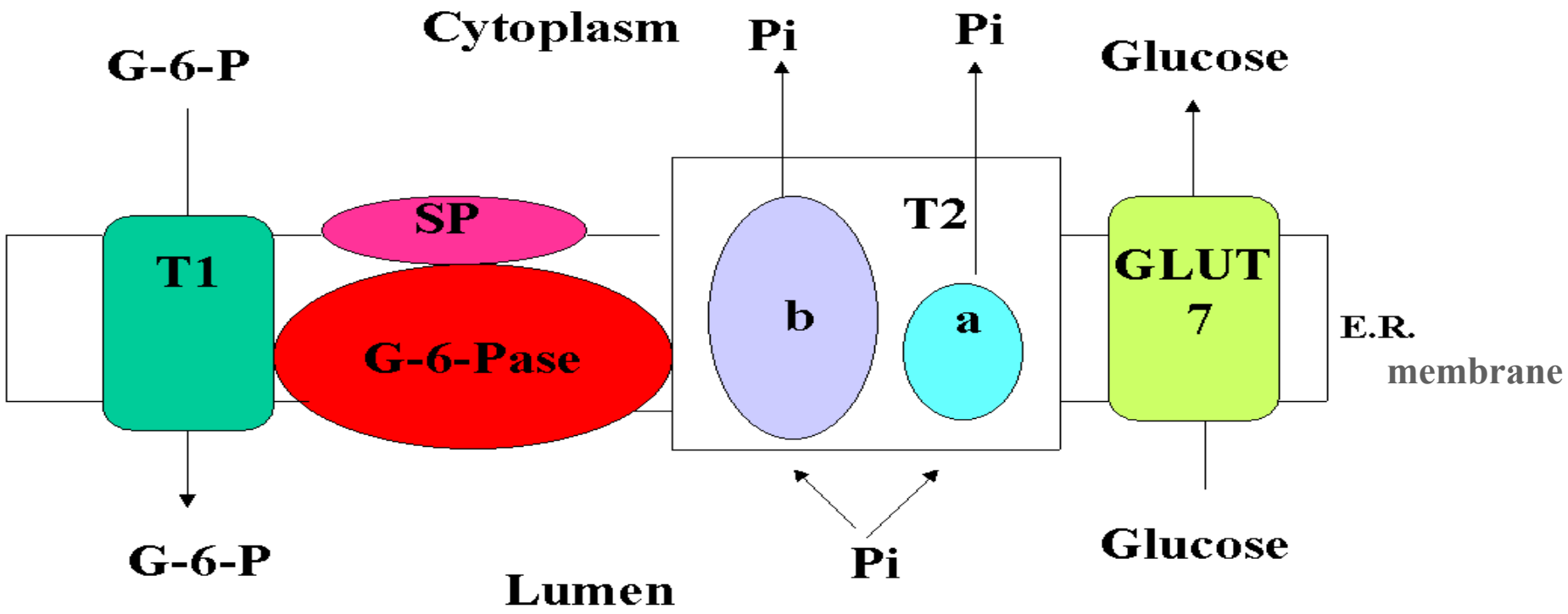
- low glicolysis in muscles➔lack of energy➔ Muscle weakness, cramps

Others:

- Type II Pompe – Lysomial acid maltase, accumulation of glycogen in lysosomes, prevalent heart damage

- Type IV (Anderson) – branching enzyme, ubiquitous deposition of abnormal glycogen, damages in nervous system, heart, muscles, hepatocytes.

Glucose-6-posphatase



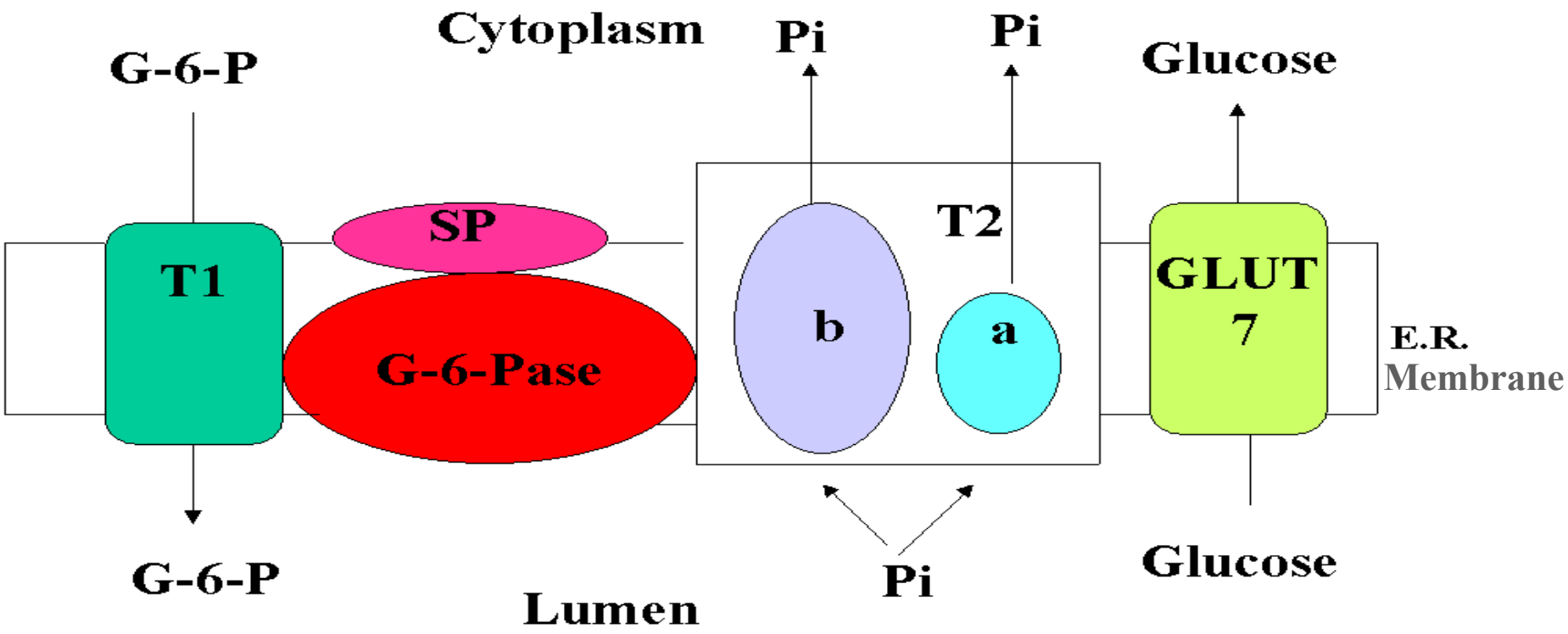
Glucose-6- phosphatase is a complex enzymatic system in liver, kidney and present in small amount in platelets.

Located in Reticulum endoplasmic membrane (active site in lumen)

Normal enzymatic activity: catalytic activity of G-6-Pasi and regulatory protein (SP)

3 proteins to trasnport G-6P, Pi and glucose

Glucose-6-posphatase



- **Deficienza della G-6-Pase** — **Sindrome di tipo 1a**
- **Deficienza di SP** — **Sindrome di tipo 1a SP**
- **Deficienza di T1** — **Sindrome di tipo 1b**
- **Deficienza di T2** — **Sindrome di tipo 1c**
- **Deficienza di GLUT7** — **Sindrome di tipo 1d**

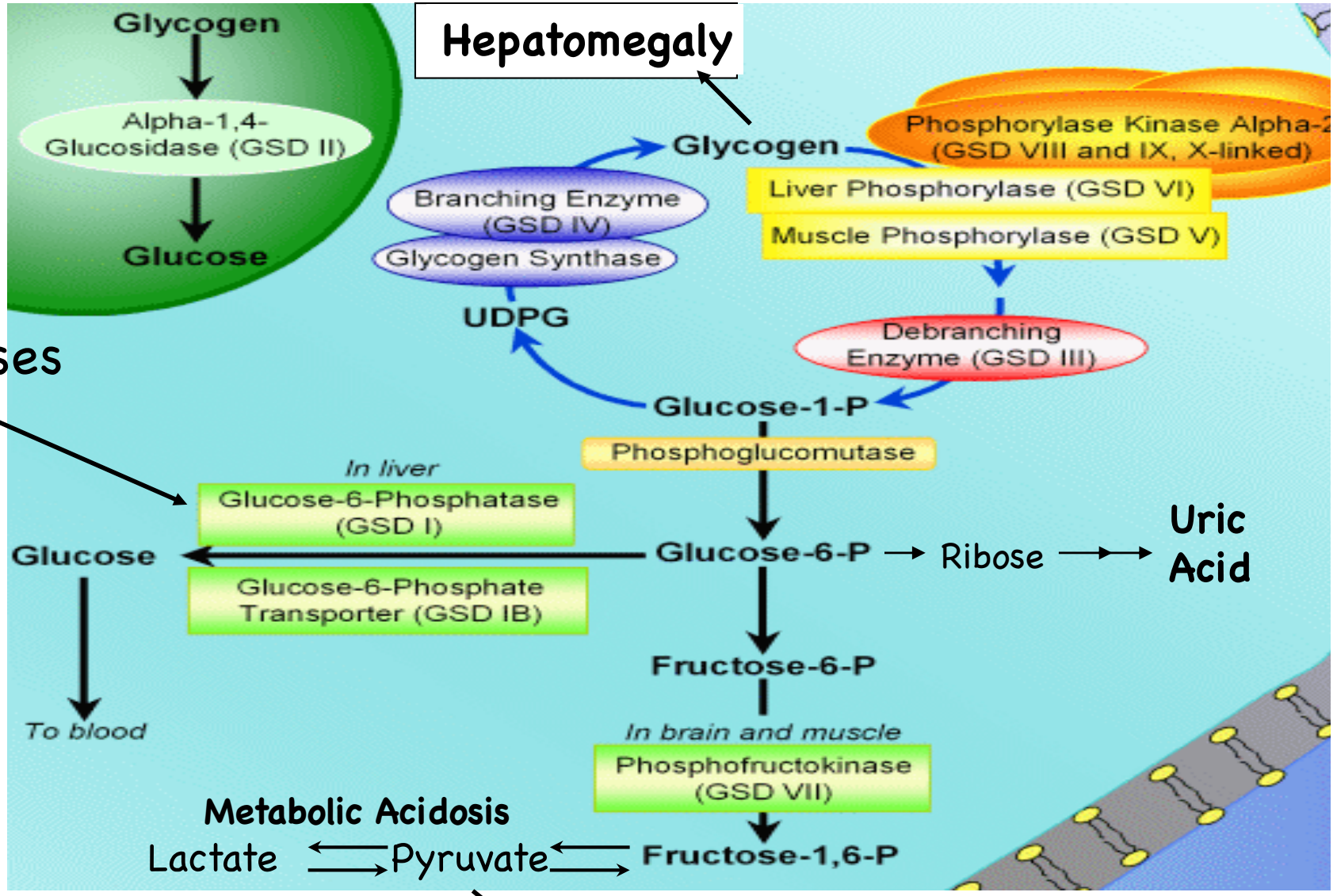
Glycogenoses type 1°
von Gierke disease

- **Deficit of glucose-6-phosphatase**

- **Accumulation of *normal glycogen* in liver and kidney**
  clinical manifestations start at age1:
- **HYPOGLICEMIA especially**
- **HYPERLIPIDEMIA lipolysis in adipose tissue**
- **HEPATIC STEATOSI and KETOSIS β–oxydation of fatty acids**
- **HYPER-PIRUVICEMIA increase of piruvis acid** } **metabolic**
- **HYPER-LACTACIDEMIA increase of lactic acid** } **acidosis**

- **HEPATOMEGALY due to accumulation of glycogen and fatty acids**
- **GROWTH FAILURE**
- **OSTEOPOROSIS (metabolic acidosis)**
- **HIGH RISK OF HEMORRHAGE abnormal platelet function**
- **ADIPOSITY localized to face and bottom**
- **CUTANEOUS XANTHOMAS**

- **SEVERE PROGNOSIS**



Glycogenoses type I

Insulin ⊣

HYPO-GLICEMIA

Mobilization of Fatty Acids and Block of protein synthesis → Steatosis

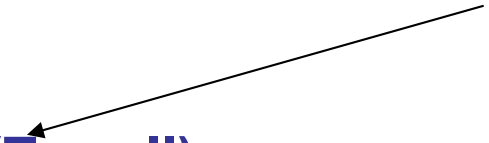
Acetil-Co-A → Cholesterol synthesis

Glycogenosis : muscle forms

Glycogenosis causing constant weakness

- Weakness related to the amount of glycogen stored in muscle cells
- Dependent on specific enzyme defects

example: Acid Maltase deficiency (17q23)



Pompe Disease (Type II)

Glycogenosis that cause a reduced exercise tolerance, cramps and myoglobinuria

- Generally after intense exercise
- Dependent on specific enzyme defects

example: Phosphorilase deficiency (11q13)

McArdle Disease (Type V)

Glycogenosis type II
Pompe Disease

- **Deficinency of lysosomial *acid Maltase***
- **Accumulation of normal glycogen in all organs, in vacuoles.**
- ***Infantile Phenotype*: in the first trimester**

Lead quickly to die (failure c.c., pulmonitis, etc)

Imporatnt muscle hypotonia, Cardiomegaly, normal Glicemia

- ***Juvenile phenotype* : onset in the first decade of life**

Muscle hypotonia, pulmonary infections, respiratory failure

Glycogen mainly increased in skeletal muscles

- ***Adult Phenotype*: reduced morbidity**

Onset in the second decade of life

respiratory failure (diaphragm muscles)

Glycogenosis type II

Infantile phenotype

- **Missense** Exon 5: Met 318 Thr
  Exon 11: Glu 521 Lys catalytic site catalytic activity
  Exon 14: Cys 647 Trp also in adult phenotype
  Exon 5: Leu 299 Arg
- **Delezioni** Exon 10 Δ13 nt (1456-1468) Stop codon truncated protein
  Exon 18 Δ18 lacking catalytic domain

Adult Phenotype

Missense

Exon 14: Asp 645 Glu **residual catalytic activity**
Exon 14: Gly 643 Arg **10-12%**
Exon 15: Arg 725 Trp

- **Non sense:** Exon 18 Arg 854 Stop codon truncated protein
- **Delezioni** Exon 10 Δ10 imporatnt mutation
  Exon 18 Δ18 loss of proteolitic cleavage site
  Exon 2 Δ2 for enzyme maturation

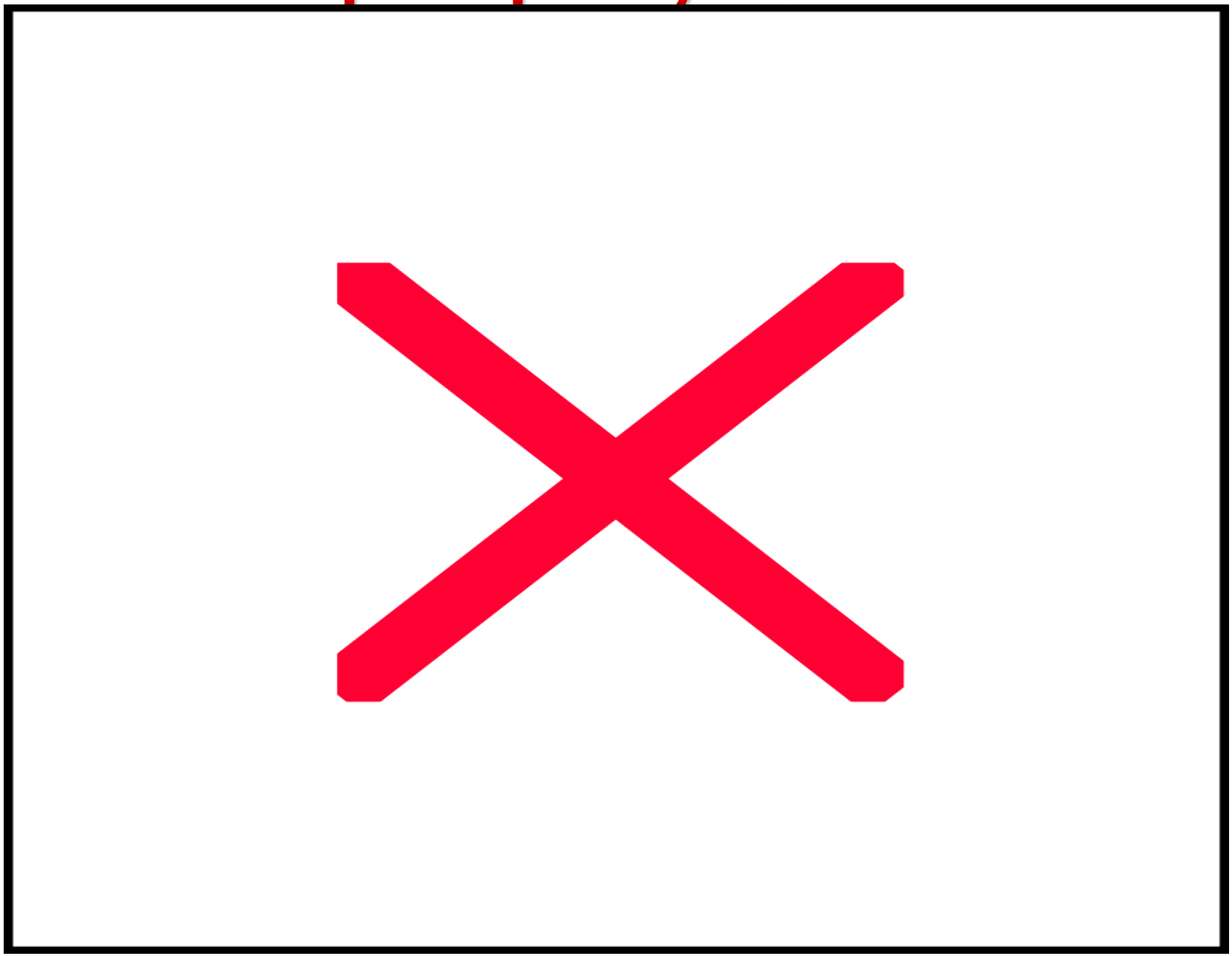
Pompe disease

Clinical-genetic correlations:

- Level of residual enzyme activity correlates with:

Severity of disease - Age of disease onset - Location of mutations

Regulation of glycogen phosphorylase



Glycogenosis type V
Mc Ardle Disease

- Deficiency of **Muscle Phosphorilase**
- Accumulation of normal Glycogen in muslces

Clinical features:

- Myalgia
- Cramps
- Muscle hardening after intense exercise
- Myoglobinuria causing renal failure
- No increase in lactacidemia after muscle exercises(altered glycogenolisis)
- Normal Glicemia

Glycogenosis type V

Molecular basis

Gene of muscle phosphorylase

Chr. 11 14 Kb 20 exons

5' region multiple promoters

Region –592 CTCCAAAAGG necessary fo effcient transcription

Non sense: Exon 1 CGA TGA Stop codon (frequent)

Missense: Exon 1 frameshift: rapidly degraded peptide

Esone 5	G 204 S	GGC	AGC	altered protein
Esone 8	L291P	CGT	CCG	less active
Esone 14	K452 T	AAG	ACG	no stabilized

Deletions: Exon 14 1844 deletion di 67 basi

Exone 17 D TTC Deletione of AA: altered protein folding