Molecular Pathology

The enzyme defects
and
Their consequences

Exclusively for education

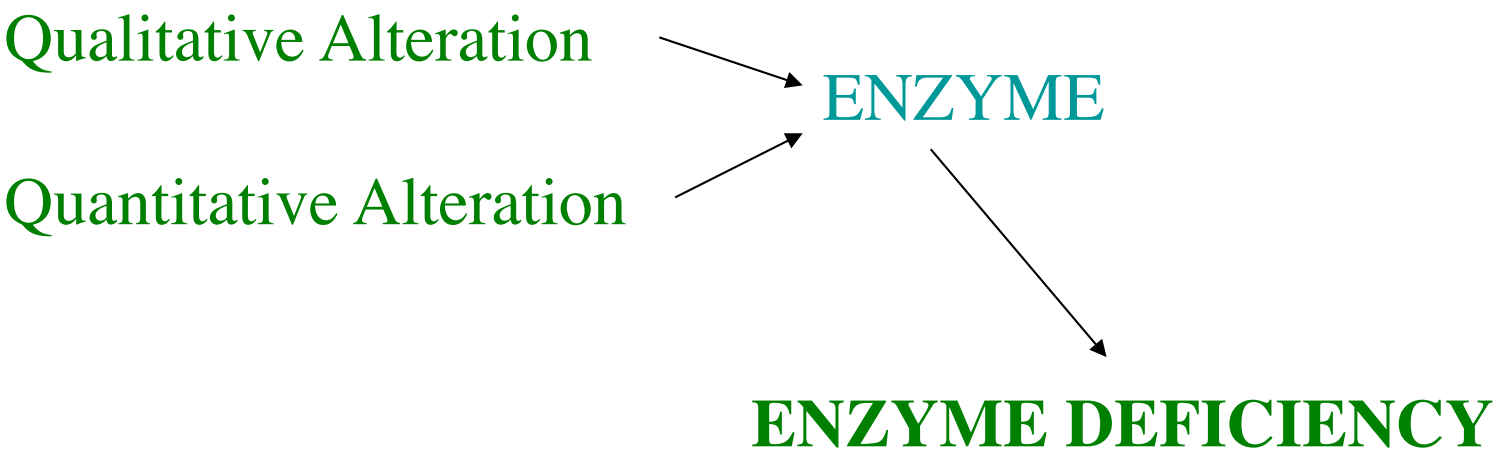
DEFECTS OF ENZYMATIC PROTEINS

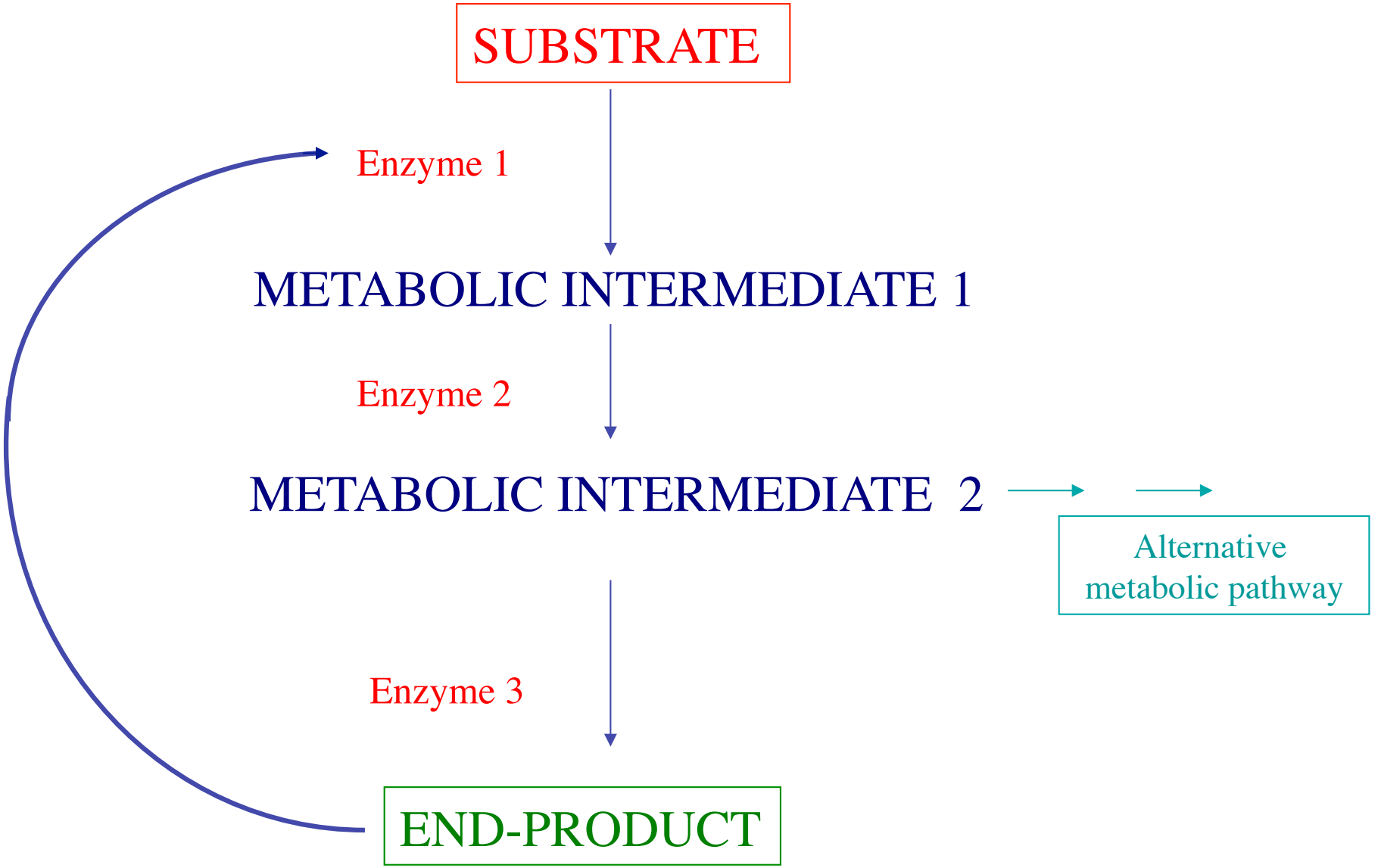
Catalysts that increase (accelerate) the rate of chemical reactions

A specific substrate

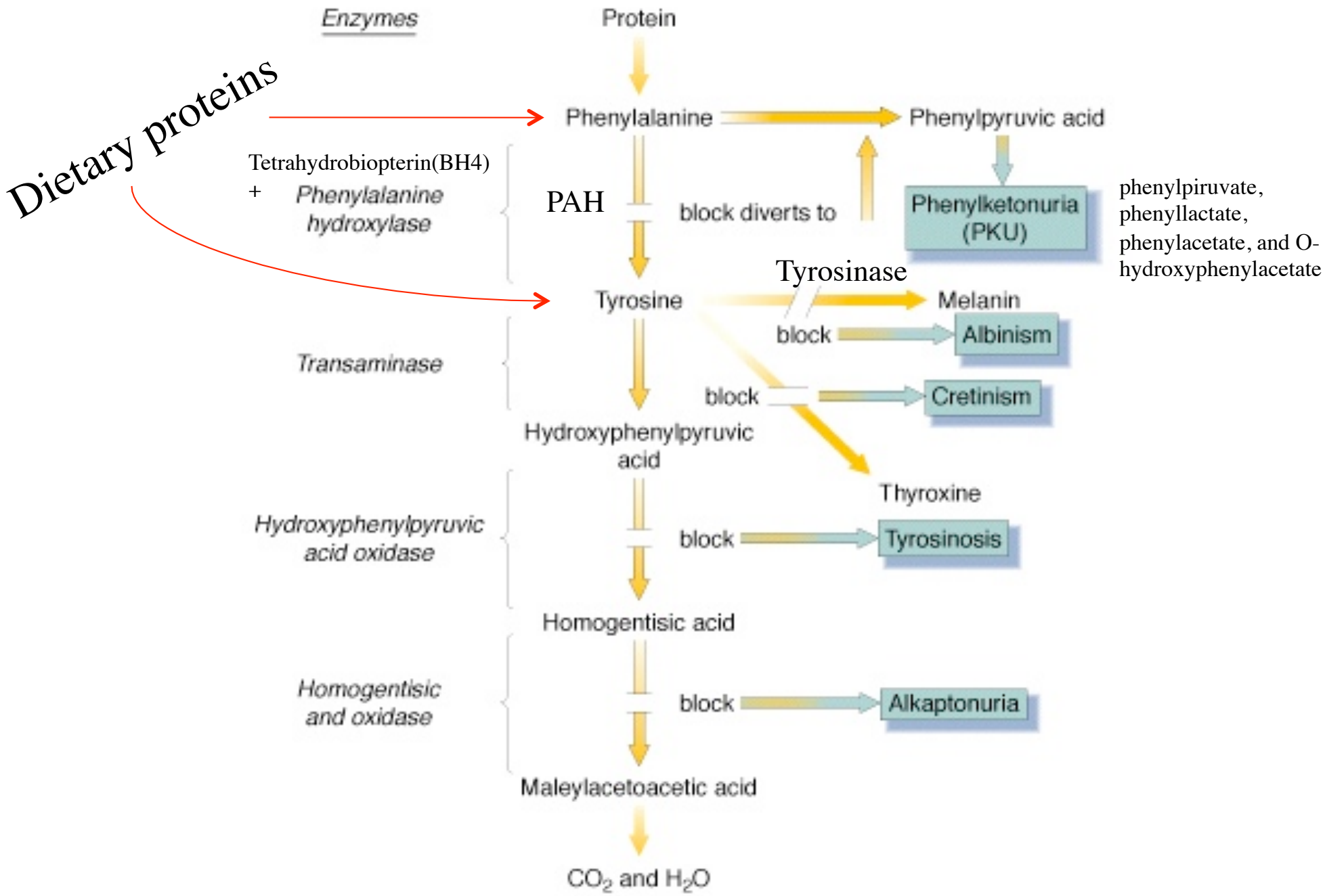
Small quantities

Active Site




SUBSTRATE
Enzyme 1
METABOLIC INTERMEDIATE 1
Enzyme 2
METABOLIC INTERMEDIATE 2
Alternative metabolic pathway
Enzyme 3
END-PRODUCT

Diseases by disturbance of Phenylalanine and Tyrosine metabolism



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 required enzyme to complete the metabolic sequence is missing.

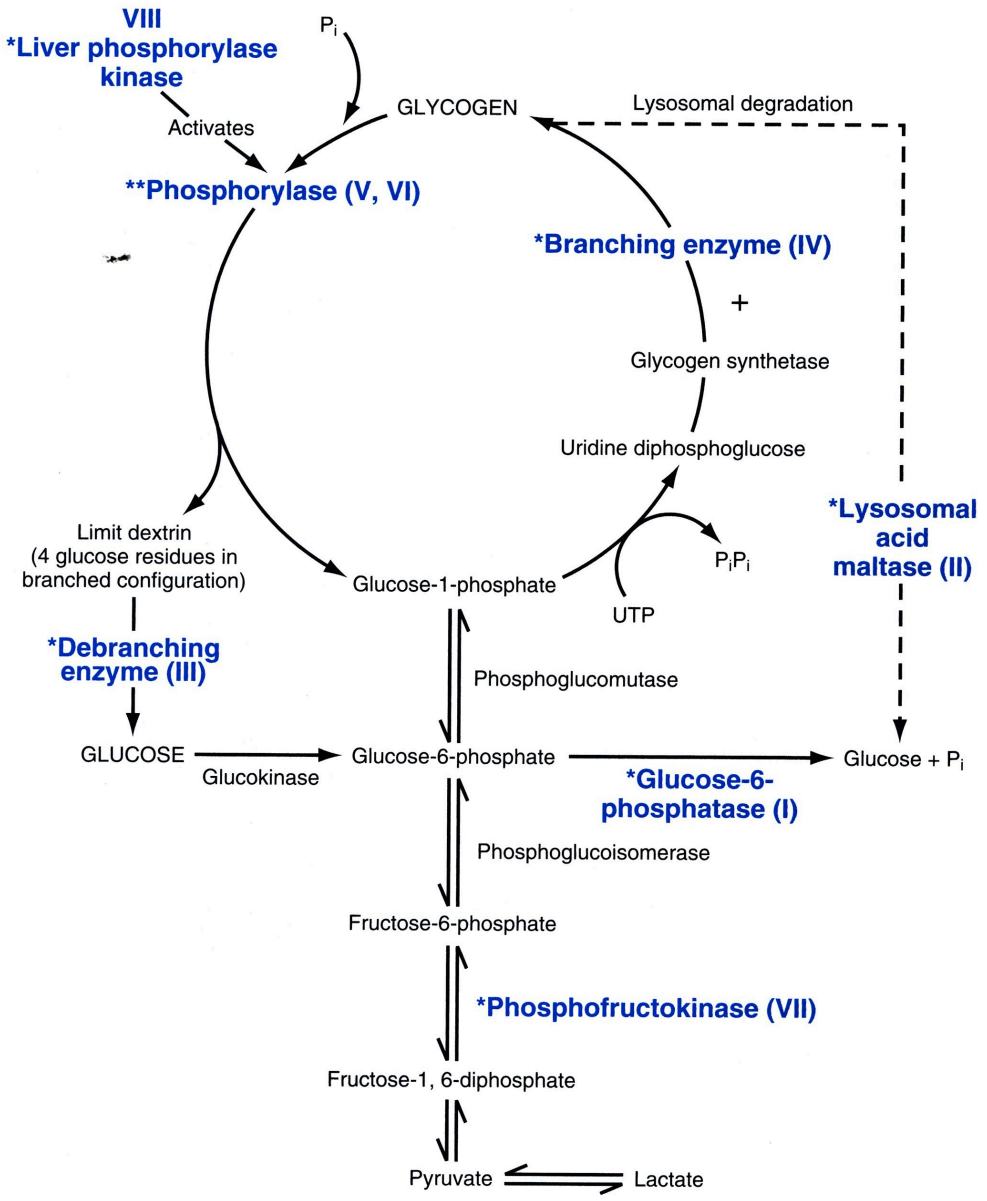
2. **Accumulation of unmetabolized substrate:** the enzyme that converts the initial substrate into the first matabolic intermediates may be missing, inthat case the initial substrate accumulates in excess.

3. **Storage of an intermediary metabolite:** a metabolic intermediates, 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)

Glycogem metabolism

Pathway of Glycogenosynthesis and Glycogenolysis in liver



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

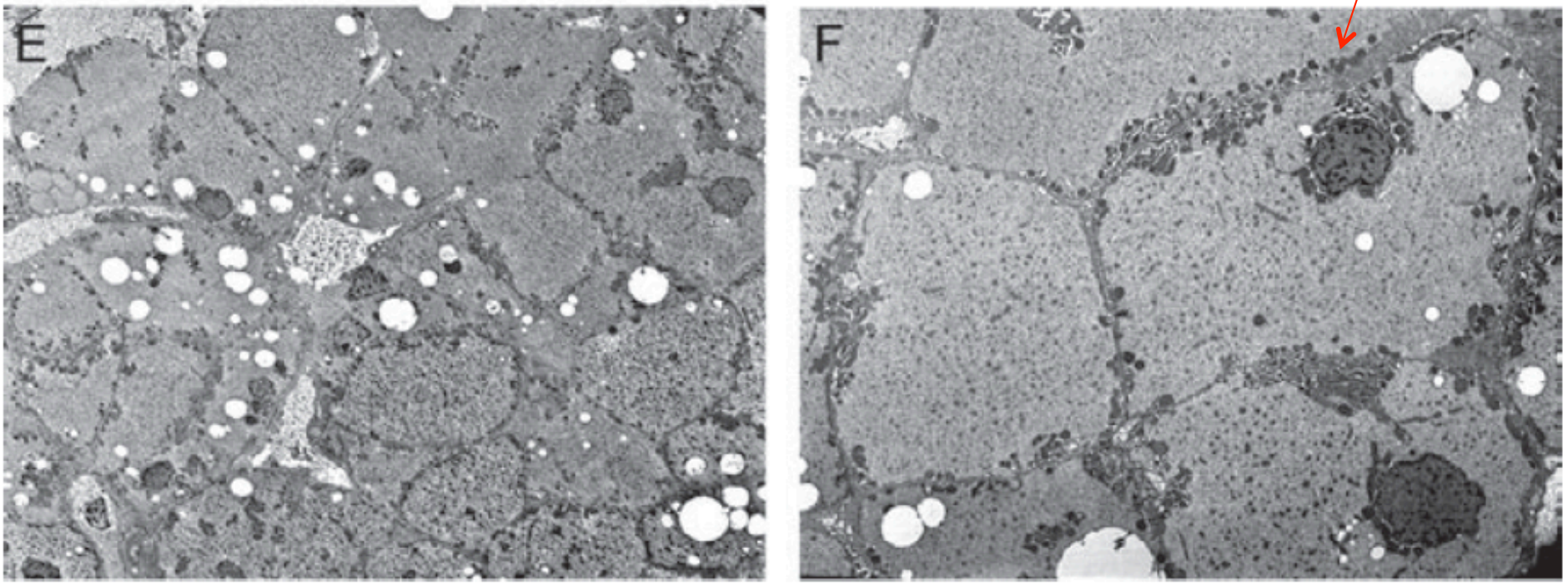
- Type IV (Anderson) – branching enzyme, ubiquitous deposition of abnormal glycogen, damages in nervous system, heart, muscles, hepatocytes.

TABLE 2 Diagnosis of glycogen storage disease by molecular sequencing.

	Gene sequencing	Prenatal diagnosis available
Type 0		No
Liver isoform	*GYS2*	
Muscle isoform	*GYS1*	
Type I b, c, d	*SLC37A4*	Yes
Type I a	*G6PC*	Yes
Type II	*GAA*	Yes
Type III	*AGL*	Yes
Type IV	*GBE1*	Yes
Type V	*PYGM*	Yes
Type VI	*PYGL*	Yes
Type VII	*PFKM*	Yes
Type IX a	*PHKA2*	Yes
Type IX b	*PHKB*	Yes
Type IX c	*PHKG2*	Yes
Type IX d	*PHKA1*	Yes
Type X	*PGAM2*	Yes

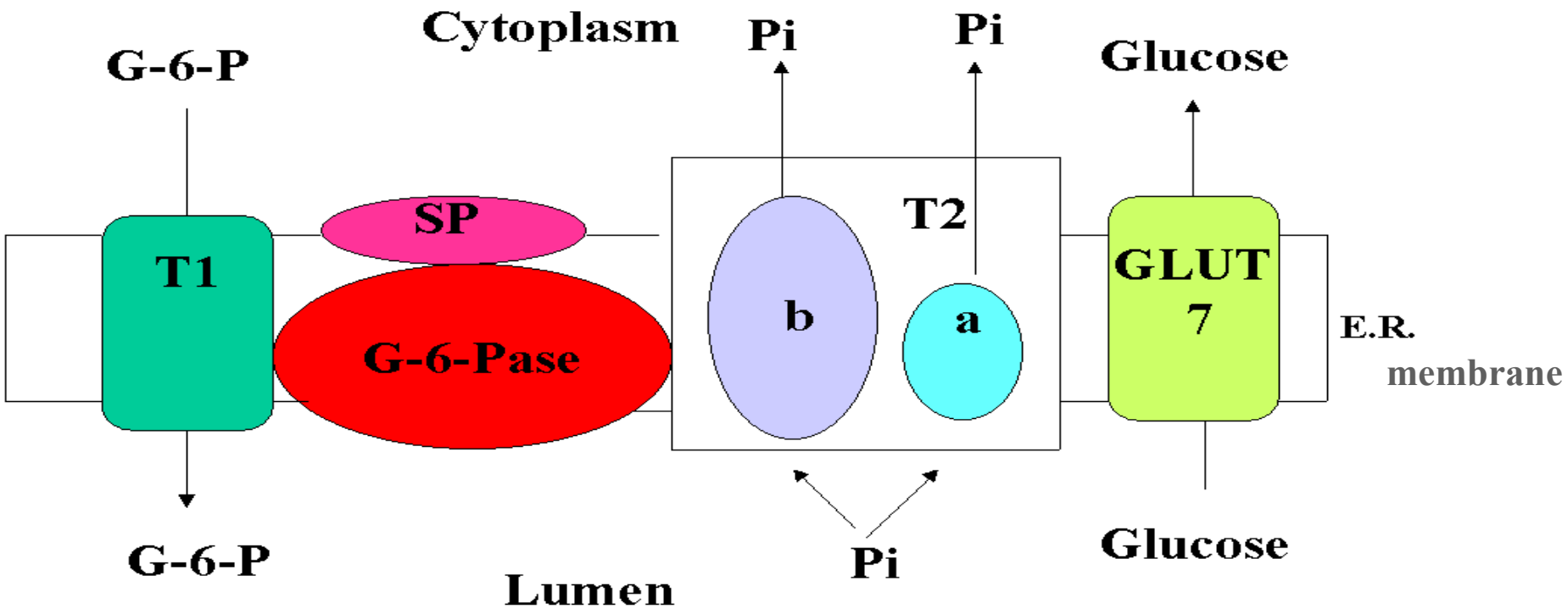
Glycogenoses Type I
vonGierke disease

Thickened plant-like membrane



Hepatocytes

Glucose-6-posphatase



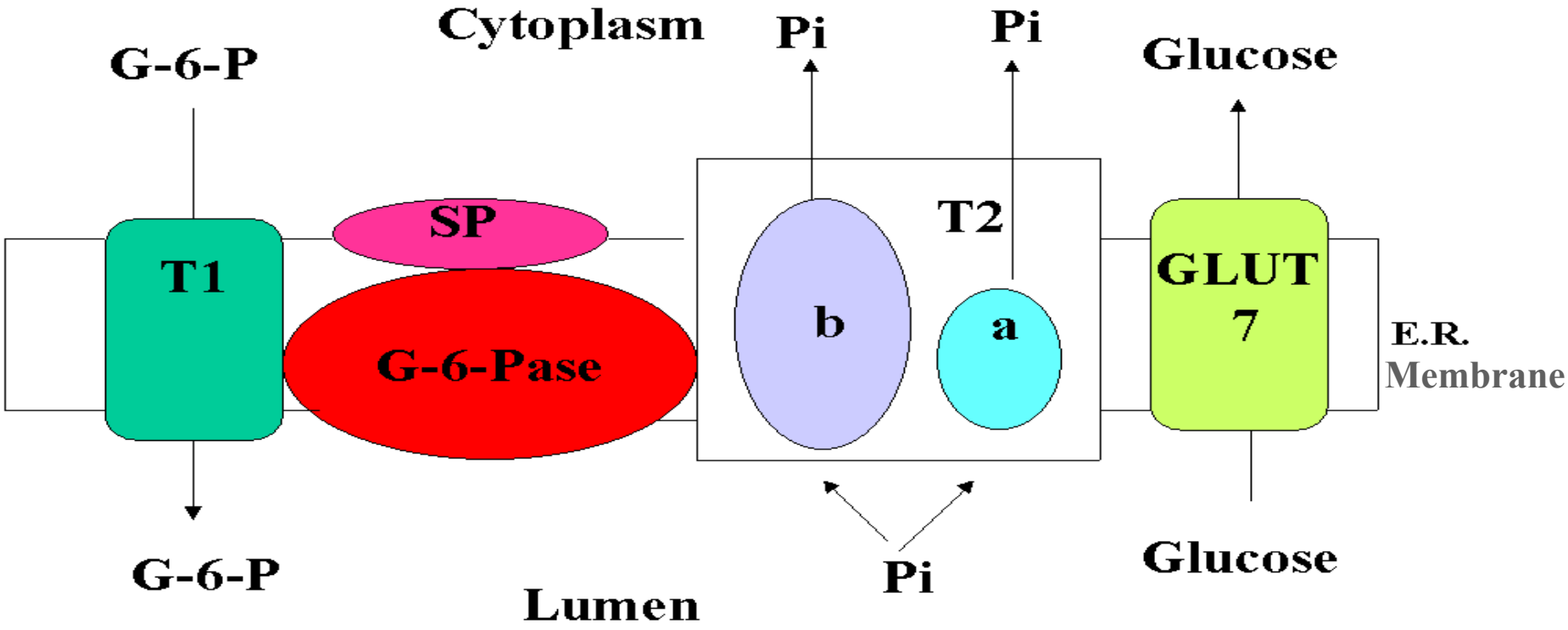
Glucose-6- phosphatase is a complex enzymatic system in liver, kidney and present in small amount in platelets.

Located in Reticulum endoplasmic membrane (active site in lumen)

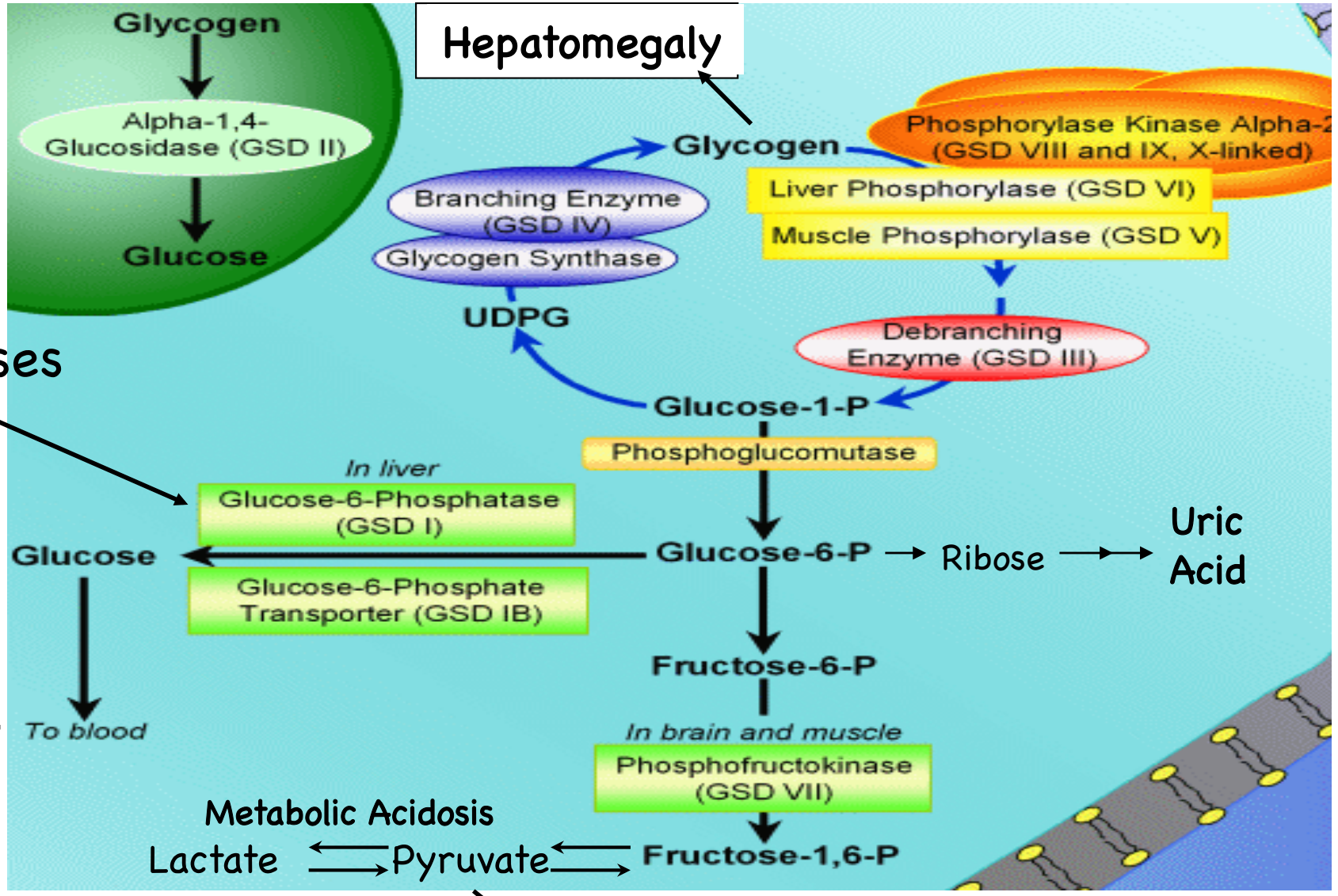
Normal enzymatic activity: catalytic activity of G-6-Pasi and regulatory protein (SP)

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 tipo I

Insulin ⊣

HYPO-GLICEMIA

Mobilization of Fatty Acids and Block of protein synthesis → Steatosis

Acetil-Co-A → Cholesterol synthesis

Glycogenoses type 1a

Important hypoglicemic crisis in the first year

Later low of glycemia are tolerated

Brain start using Keton bodies

HYPOGLICEMIA:

-Block insulin secretion

-Stimulates glucagone result in Hepatocytes proliferation

-Acelerates lipolysis in adipose tissue

-Stimulates β−oxydation of Fatty Acids and neoglucogenesis in liver

(Hyperlipidemia, hepatic steatosis, Ketosis)

-Accumulation of glucose –6-phosphate increases glicolisis

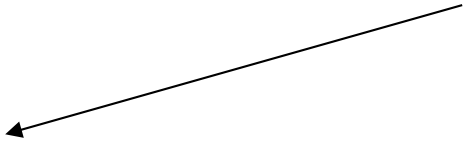
Hyperpiruvicemia, Hyperlactacidemia

Glycogenoses : myopathic forms

Glycogenoses causing constant weakness

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

example: Acid Maltase deficiency (17q23)

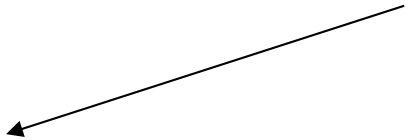


Pompe Disease (Type II)

Glycogenoses that cause a reduced exercise tolerance, cramps and myoglobinuria

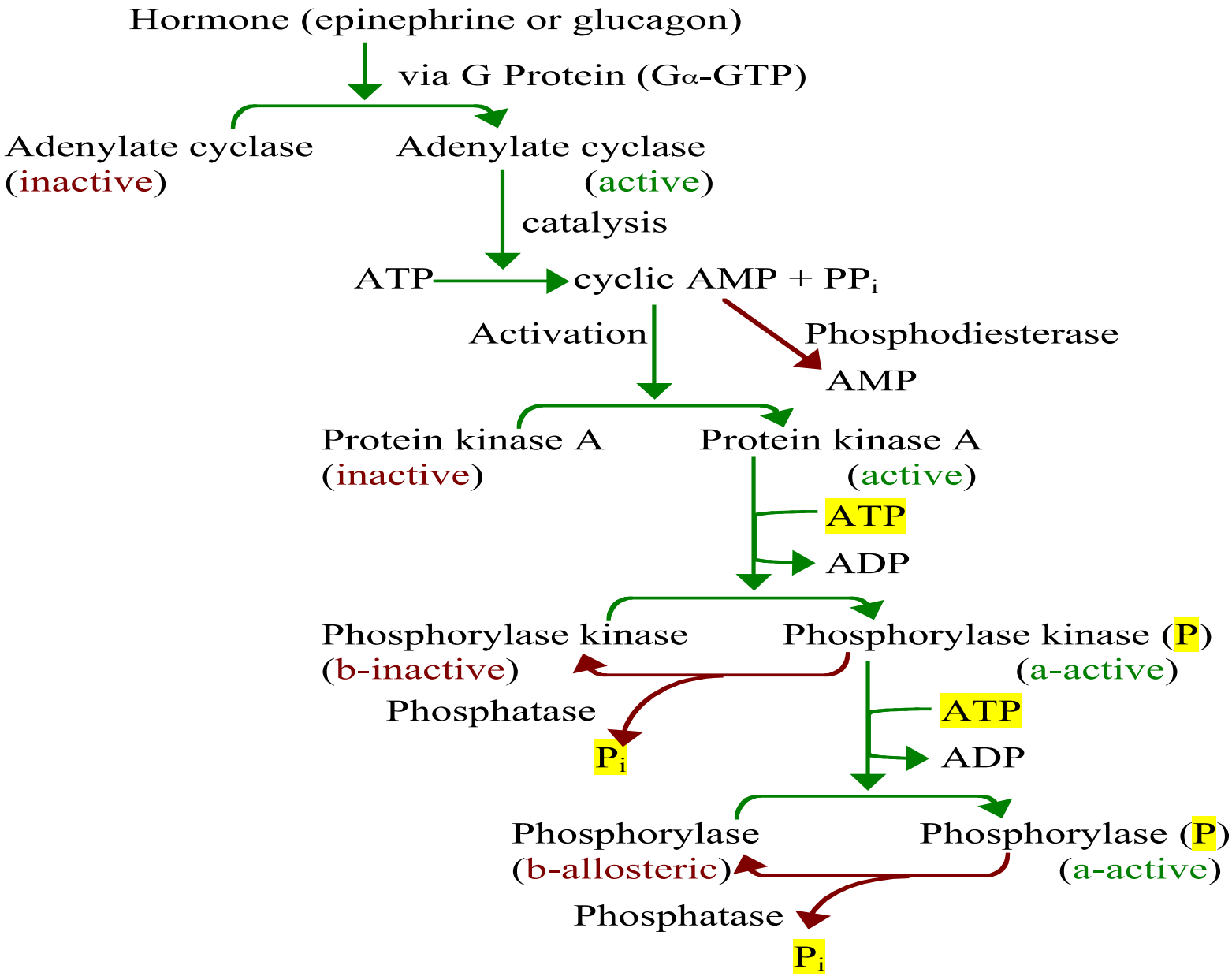
- Generally after intense exercise
- Dependent on specific enzyme defects

example: Phosphorylase deficiency (11q13)



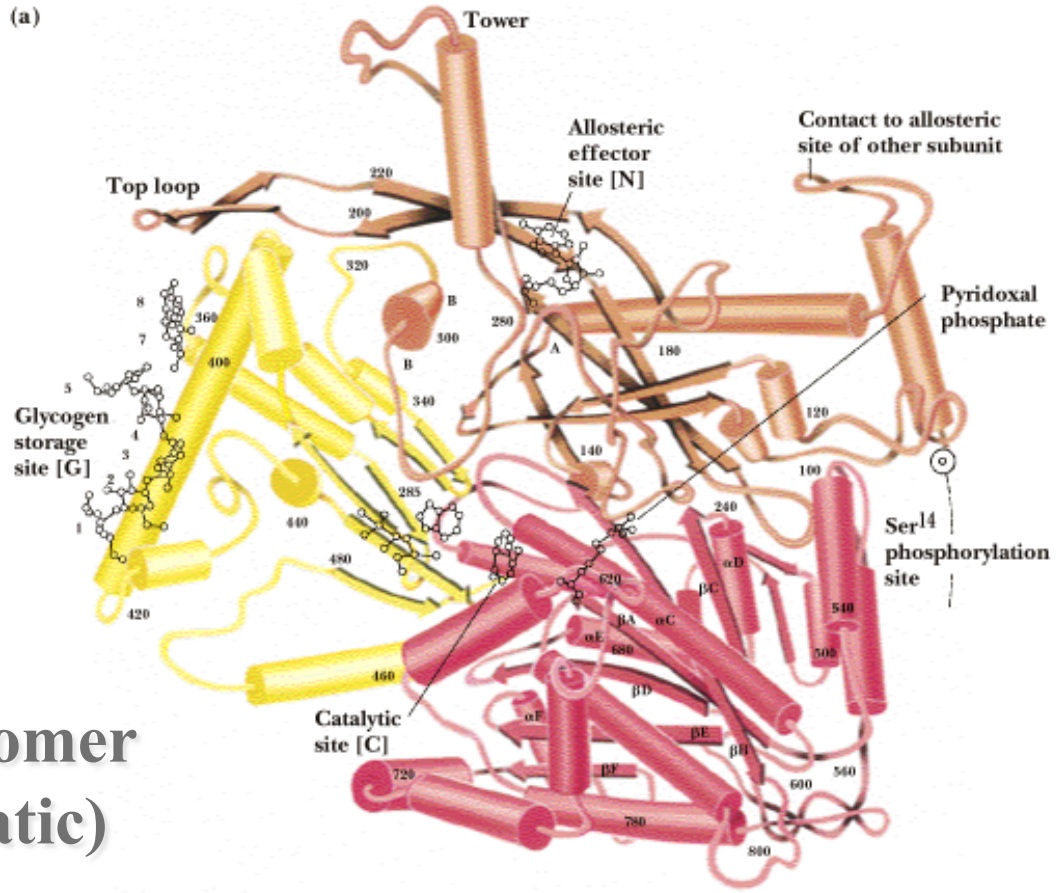
McArdle Disease (Type V)

Regulation of glycogen phosphorylase



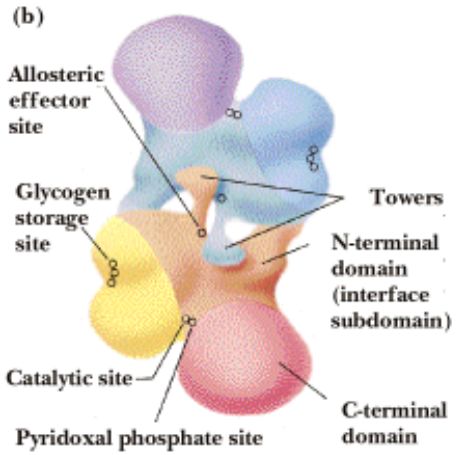
Active forms of glycogen phosphorylase

Garrett & Grisham: Biochemistry, 2/e
Figure 15.15



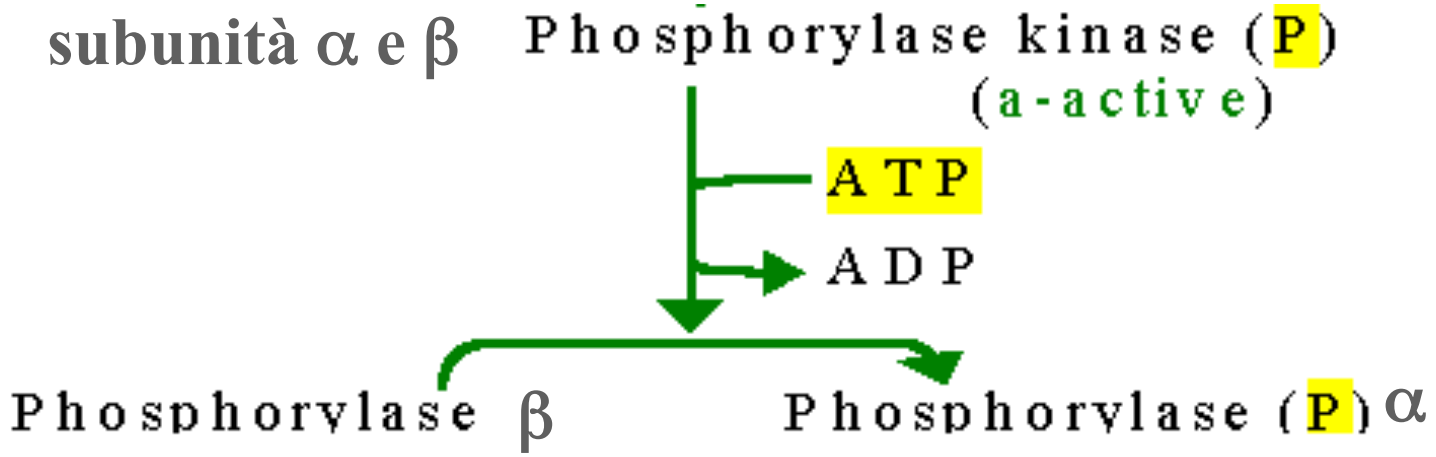
Monomer (hepatic)

Dimer (muscolar)



Saunders College Publishing

Alterations of glycogen phosphorilase

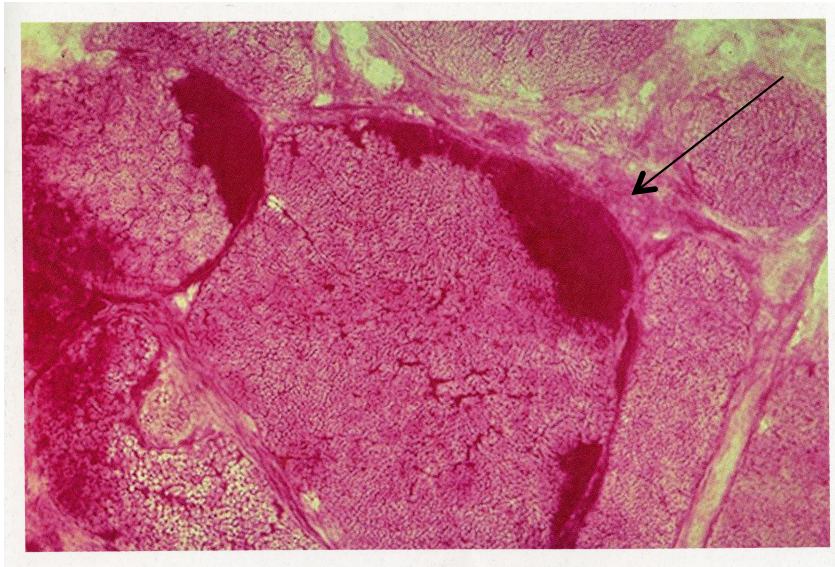


- Deficiency of muslce glycogen phosphorilase: GSD V
- Deficiency hepatic glycogen phosphorilase: GSD VI
- Deficiency phosphorilase kinase: GSD IX

Glycogenoses type V
McArdle Disease

- Deficiency of **Muscle Phosphorylase**
- Accumulation of normal Glycogen in muscles

Clinical features:

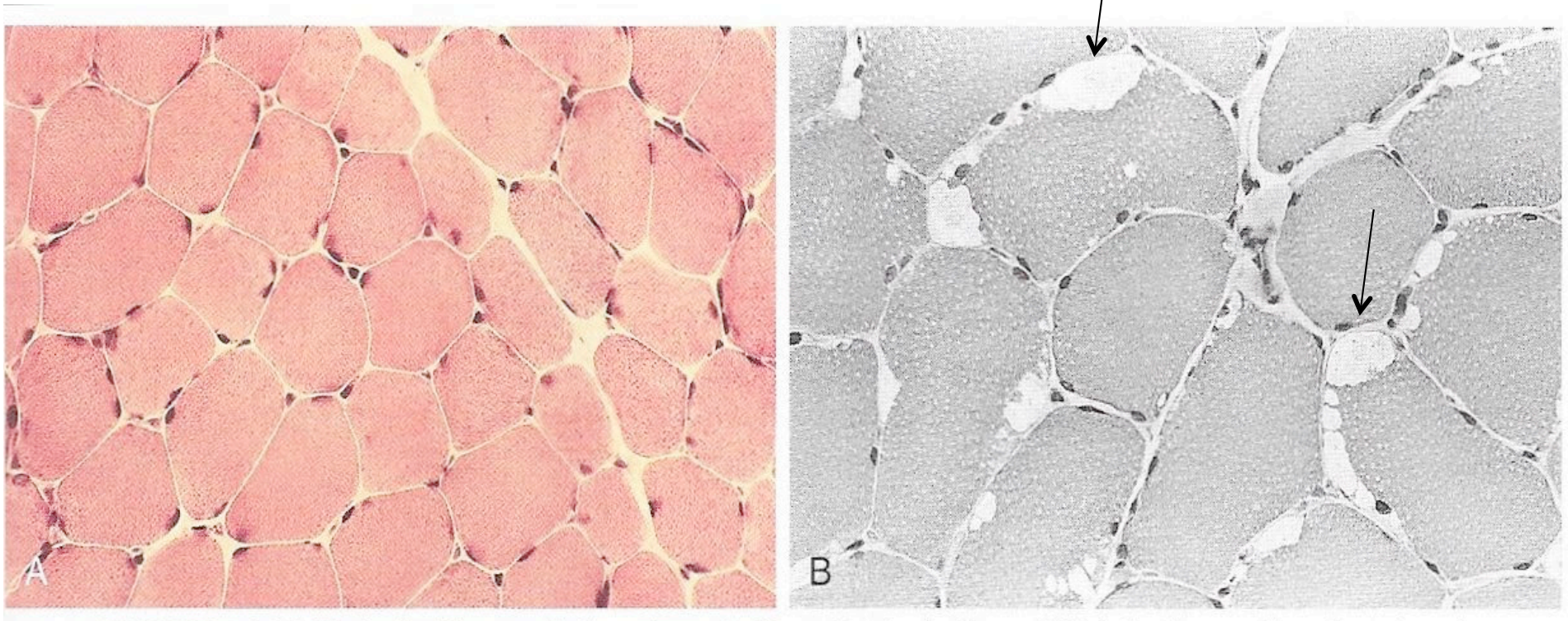


- Myalgia
- Cramps
- Muscle hardening after intense exercise
- Myoglobinuria causing Renal failure
- No increase in lactacidemia after muscle exercises (altered glycogenolisis)
- Normal Glicemia

McArdle Disease

muscle fibers

Large peripheral vacuoles



Normal

McArdle

Glycogenoses 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

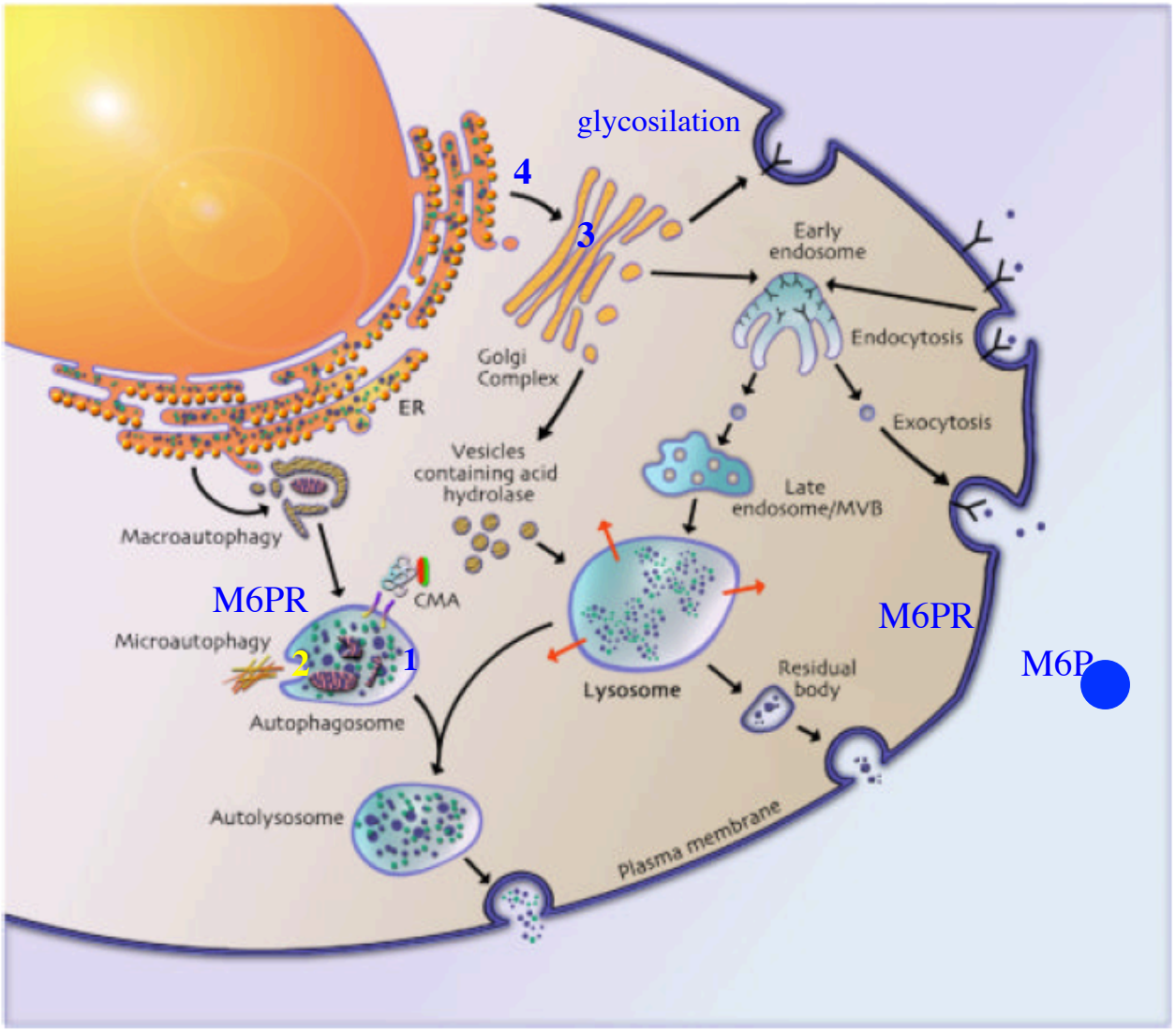
Exone 17 D TTC Deletione of AA: altered protein folding

Lysosomal storage disorders

Disease	Affected protein	Storage material	Mechanism	Affected organs	Pathology
Niemann-Pick disease type C	NPC1, NPC2	Cholesterol, Sphingolipids	Lysosomal cholesterol and lipid export, Foam cells in visceral organs and neuronal storage	Liver, CNS	Ataxia, Dysarthria, Dysphagia, Dystonia, Dementia, Seizures, Hepatosplenomegaly, Thrombocytopenia
Fabry disease	α-Galactosidase A	Globotriaosylceramide, Galabiosylceramide, Globotriaosylsphingosine, blood-group-B glycolipids	Lipid storage in endothelial and smooth muscle cells of blood vessels	Kidney, Heart	Acroparesthesias, Angiokeratoma, Renal failure, Cardiomyopathy, Stroke, Gastrointestinal symptoms
Gaucher disease	β-Glucosidase/ Glucocerebrosidase	Glucosylceramide, GM1, GM2, GM3, GD3, Glucosylsphingosine	Lipid storing macrophages	Spleen, Liver, Bone marrow, CNS (not type 1)	Hepatosplenomegaly, Thrombocytopenia, Anemia, Skeletal deformations, Bone fractures
Pompe disease	α-Glucosidase	Glycogen	(Autophagic) accumulation in type II muscle fibers	Skeletal muscle, Cardiac muscle	Cardiomegaly, Hypotonia, Cardiorespiratory failure, Hepatomegaly, Muscle weakness

Summarised from Ballabio et al. [4], Parkinson-Lawrence et al. [5], Platt et al. [6].

Protein trafficking as a basis of lysosmal storage disorders



Mutations in:

1. lysosomal protein (inactive)
2. integral lysosomal proteins, NPC1for export of lysosomal products
3. Defective folding
4. Inability to exit the ER
5. Lysosomal uptake (Mannose-6-phosphate receptor)

Glycogenoses 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)

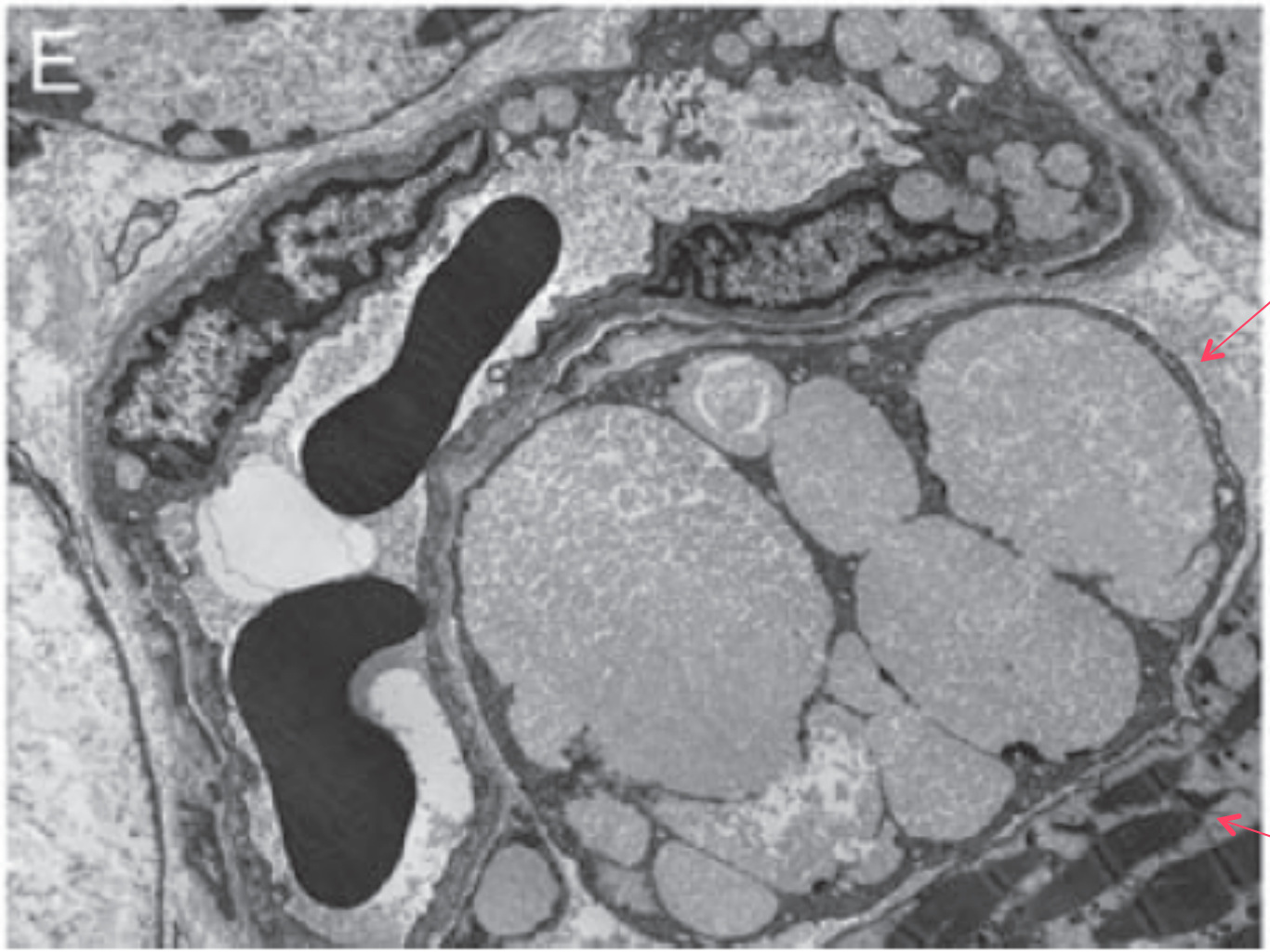
Glycogenoses Type II
Pompe disease

Storage of glycogen in autophagic vacuoles, where other molecules are digested

In autophagic vacuoles glycogen is not metabolized by cytoplasmic glicogenolytic enzymes

Lysosomial degradation of lysosomial glycogen is necessary to mobilize glycogen in neonatal liver

Glycogenoses Type II
Pompe disease



Glycogenoses 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

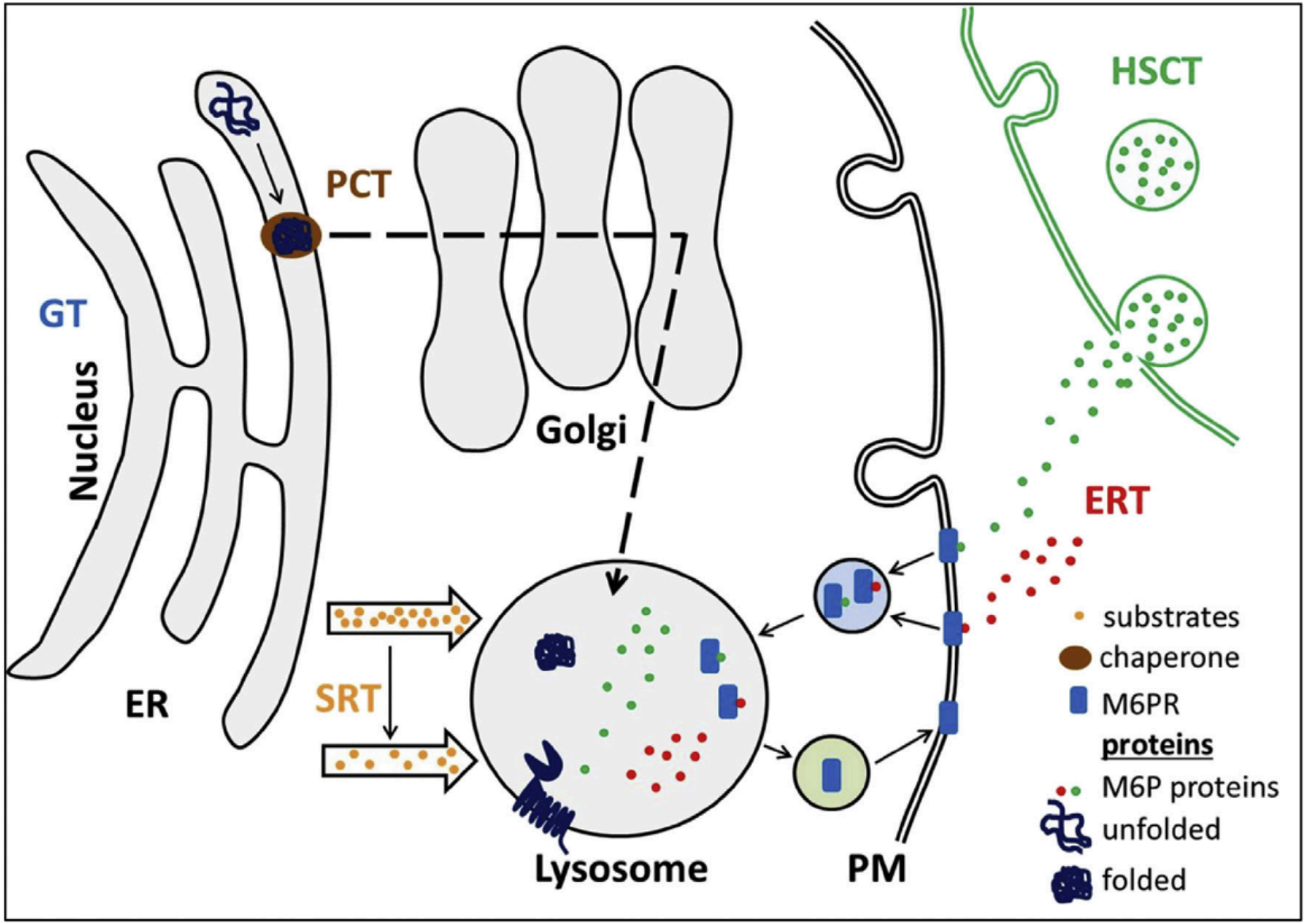
Pompe disease

Clinical-genetic correlations:

- Level of residual enzyme activity correlates with:

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

General strategies for the treatment of lysosomal storage disorders



Enzyme replacement therapy (ERT)

Molecular Genetics and Metabolism 122 (2017) 80–85

Contents lists available at ScienceDirect

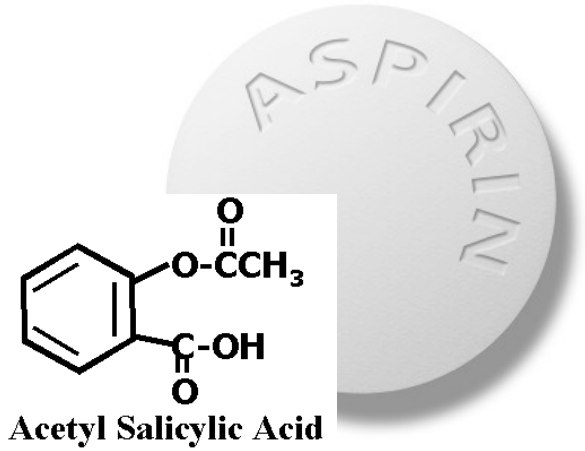
Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Effect of enzyme replacement therapy with alglucosidase alfa (Myozyme®) in 12 patients with advanced late-onset Pompe disease

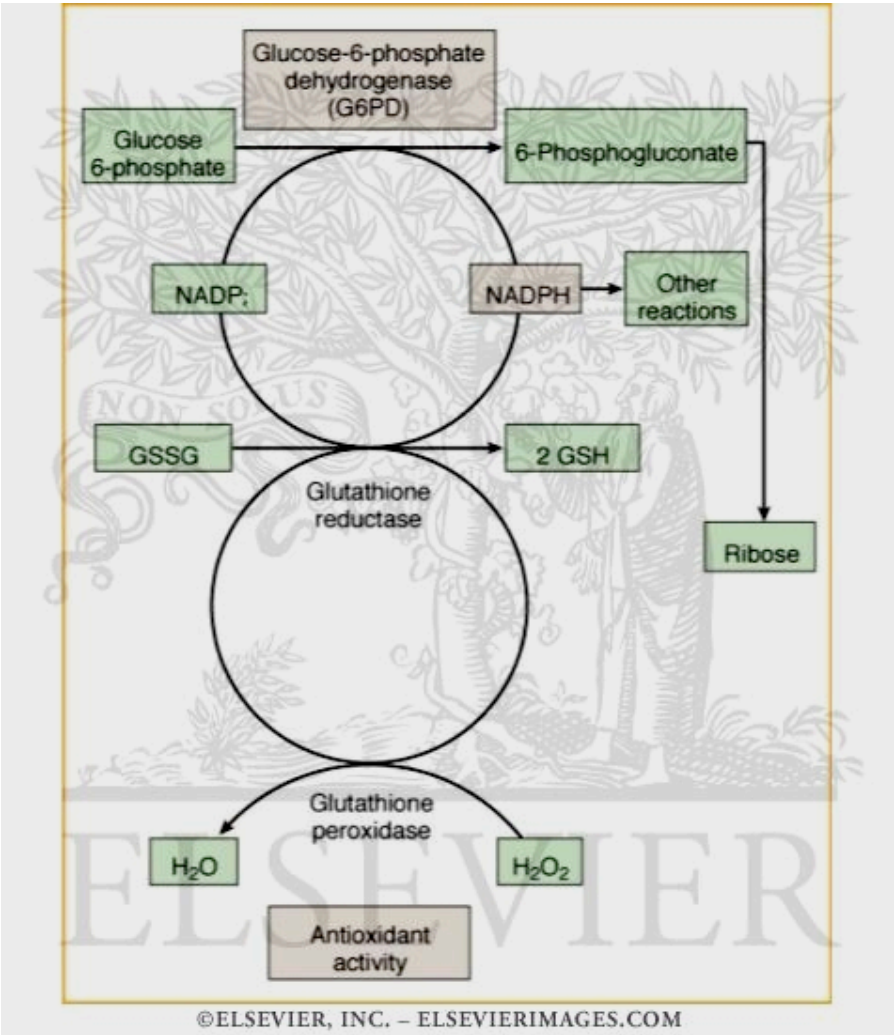
- ✓Recombinant human GAA
- ✓Long term effect
- ✓Increased time of autonomous ventilation
- ✓Enlarged distance in assisted walk

Genetically determined adverse reactions to drugs



GLUCOSE-6-PHOSPATE DEHYDROGENASE (G-6-PD)

G-6-PD deficency reduces the ability of red blood cells to protect themselves against oxydative injuries and lead to hemolysis



-More than 150 molecular variant of this enzyme

-Frequent in mediterranean

-Gene located on chr. X (Xq28)

-Male➔ all erytrocytes are affected

-Heterozygous female → defective erytrocytes

-Heterozygous female → normal erytrocytes

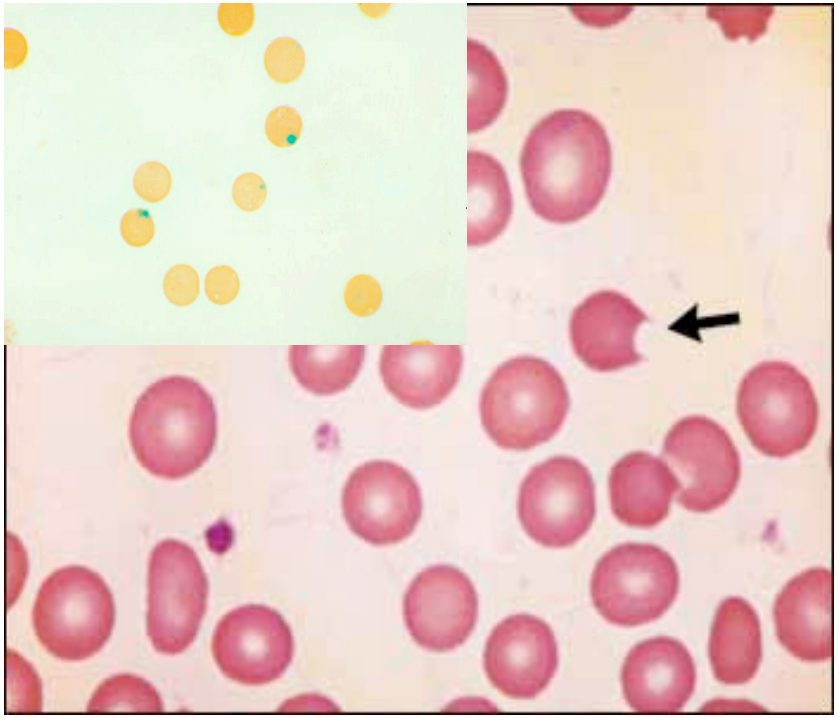
Two variants cause clinically significant Hemolytic Anemias:

Misfolding of the protein more susceptible to proteolytic degradation

$G6PD^-$	G6PD mediterranean
10% American black	Middle East
Half-life moderately reduced	markedly abnormal

inadequate protection from oxidants

Infections and drugs and certain foods can trigger hemolysis
The oxidant denatures Hb➔Heinz precipitates



Macrophages pluck out the Heinz bodies ➔ membrane damage

Clinical manifestations:

After exposure to the oxidant ➔Acute intravascular hemolysis
(2-3 days later)

⬇

Marked Anemia
Hemoglobinemia
Hemoglobinuria
Hematocrit
Self-limited

Multidrug Resistance Proteins

A family of ATP-dependent efflux pump

12 members of of the MRP/CTFR subfamily belonging to the 48 human ATP-binding cassette (ABC) transporters

Expression of Multidrug Resistance–Associated Proteins Predicts Prognosis in Childhood and Adult Acute Lymphoblastic Leukemia

Sabine L.A. Plasschaert,[1] Eveline S.J.M. de Bont,[1] Marike Boezen,[2] Dorina M. vander Kolk,[3] Simon M.J.G. Daenen,[3] Klaas Nico Faber,[4] Willem A. Kamps,[1] Elisabeth G.E. de Vries,[5] and Edo Vellenga[3]

Conclusions: The present study shows that a subset of ALL patients with high MRP expression has an unfavorable prognosis independently of age.