



CDL CHIMICA



Corso di Biochimica (6 CFU) 48 ore

Prof.ssa **Alessandra Olianas**

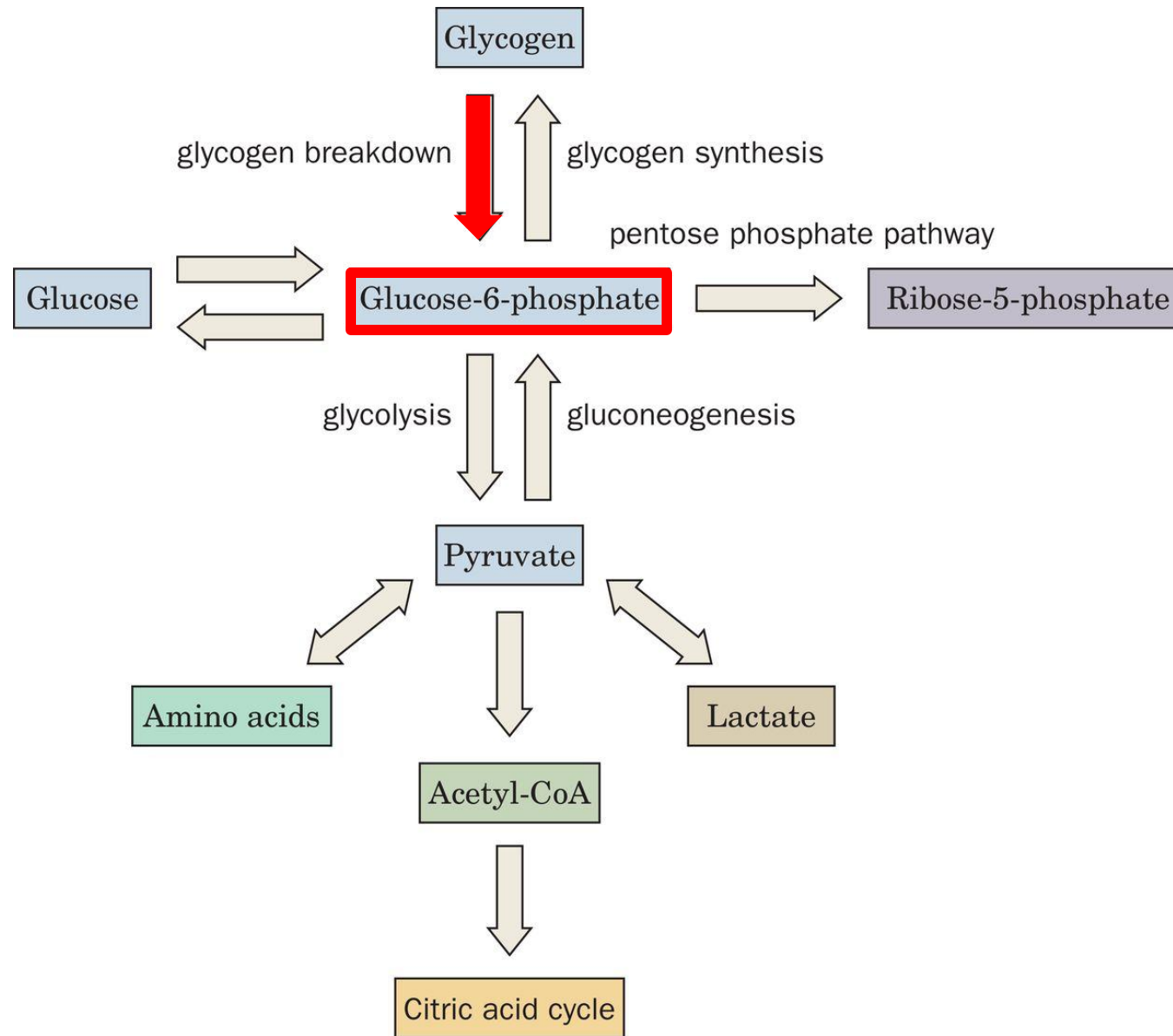


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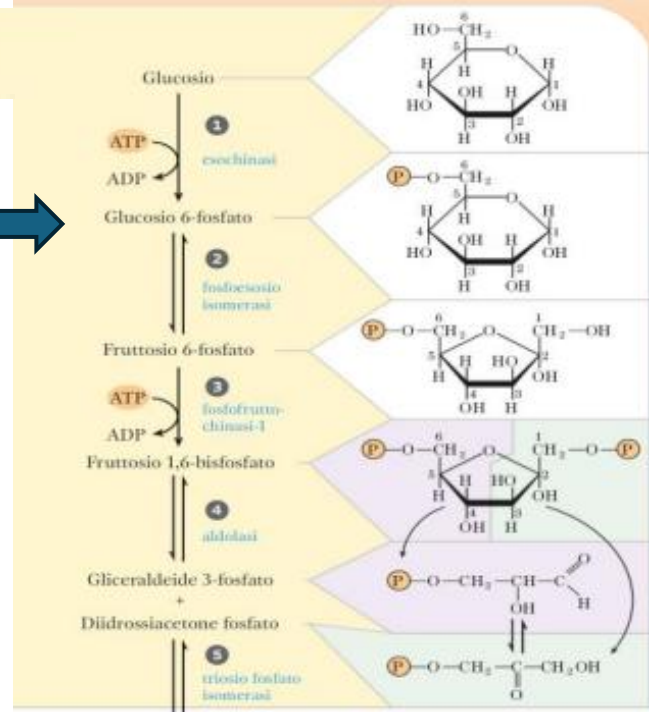
Ricevimento studenti: **si riceve per appuntamento**

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Fase preparatoria

5 reazioni

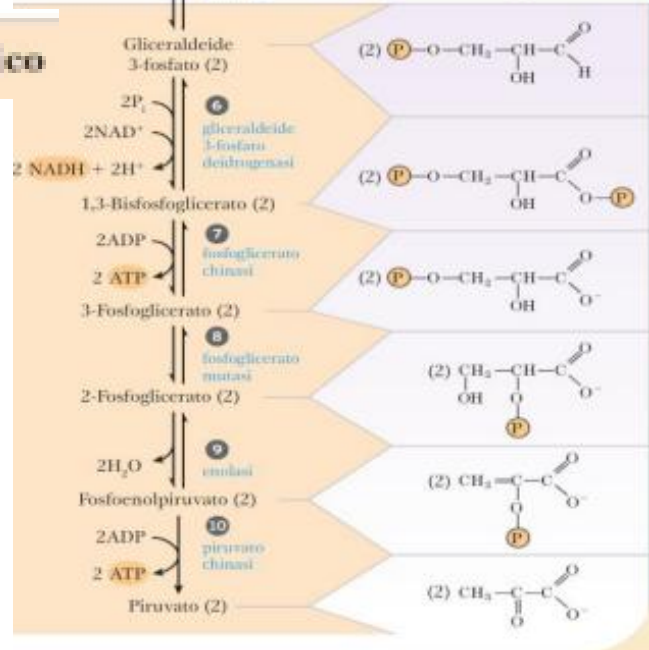


Non ha bisogno di ossigeno

Produce ATP e NADH

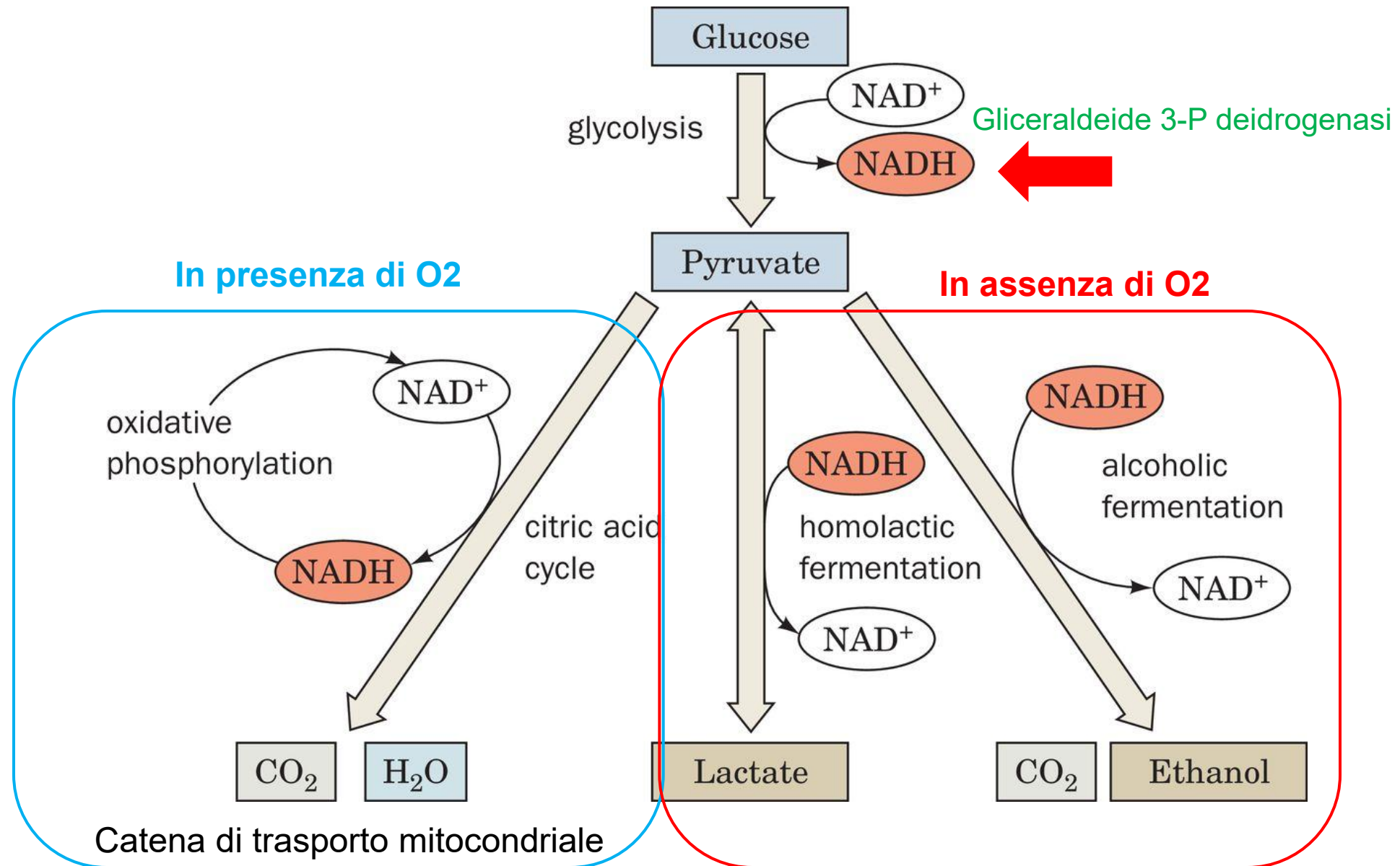
Fase di recupero energetico

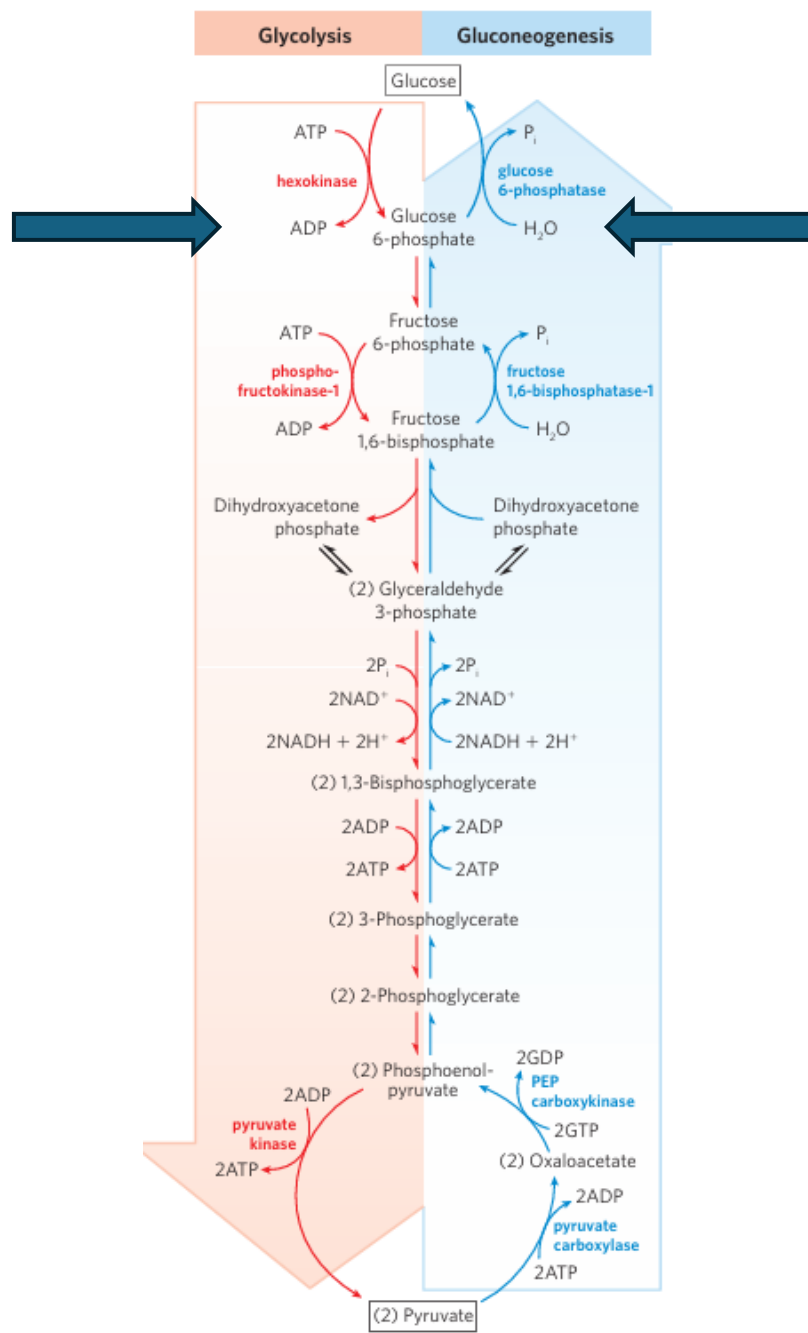
5 reazioni



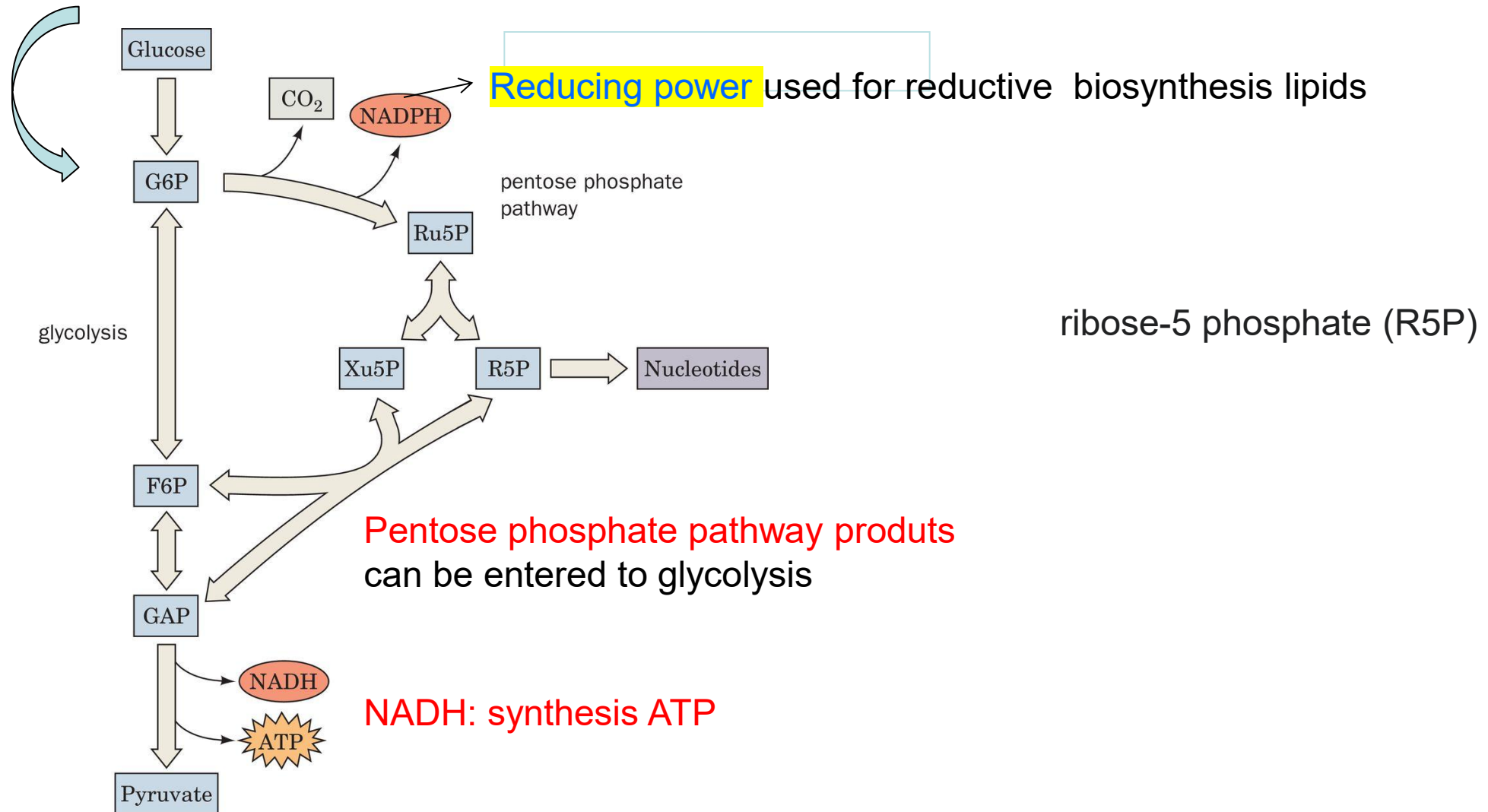


Destino metabolico del piruvato





Relationship Between Glycolysis & Pentose Phosphate Pathway





La via dei pentoso fosfati

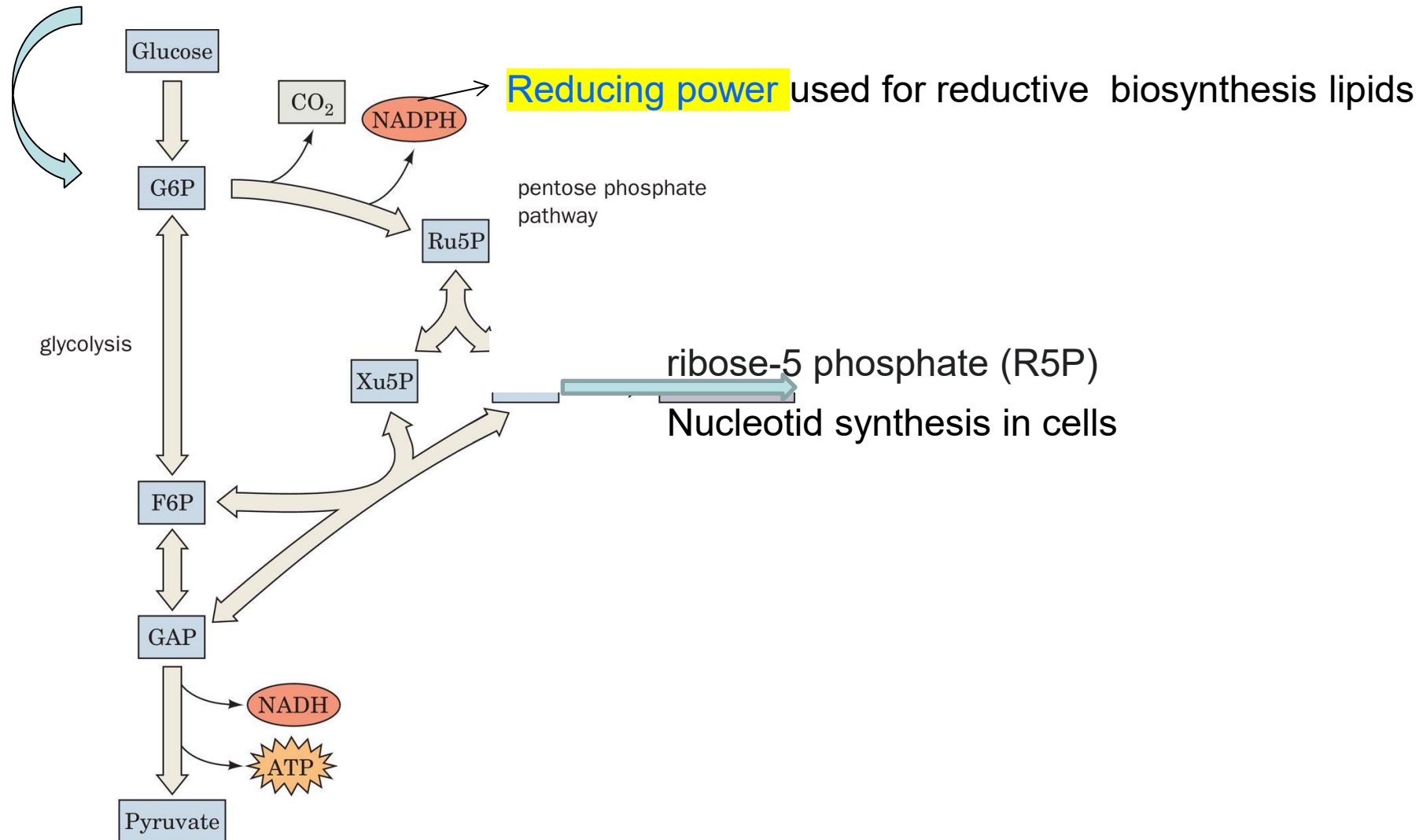
Shunt degli esosi monofosfati

Via metabolica parallela alla glicolisi

Relationship Between Glycolysis & Pentose Phosphate Pathway



Principal function:



La via dei pentoso fosfati

CITOSOL



Produzione di **NADPH** per le biosintesi riduttive e **ribosio 5-fosfato** per la biosintesi dei nucleotidi.

ribosio 5-fosfato

Sintesi di nucleotidi nelle cellule che hanno una elevata velocità di divisione
(**midollo osseo, pelle mucosa intestinale tumori**)

NADPH

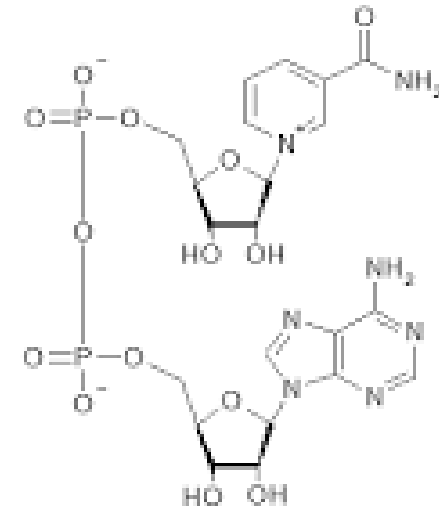
- Per la biosintesi di acidi grassi, colesterolo e ormoni steroidei
- Protegge le cellule dai danni ossidativi (eritrociti, cornea)

NADP⁺ è un accettore di elettroni

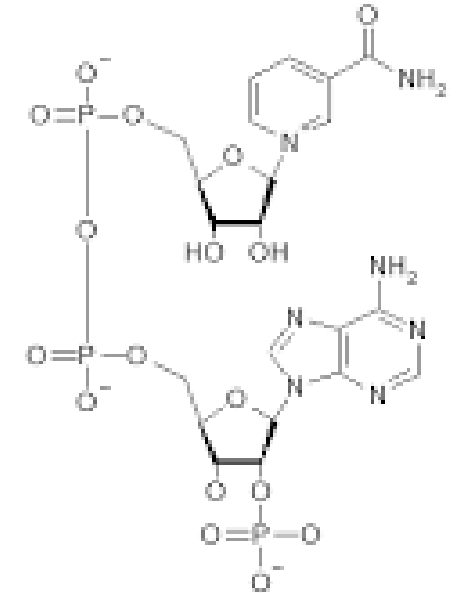
NADPH deriva dalla vitamina B3 (niacina)

NADP⁺ è un accettore di elettroni

Cellule in rapida divisione } **midollo osseo**
 } **pelle**
 } **mucosa intestinale**
 } **tumori,**



NAD

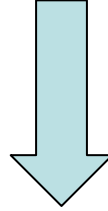


NADP

Nicotinamide
adenin dinucleotide
fosfato

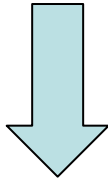


Tessuti che necessitano di una intensa sintesi di acidi grassi



Fegato, tessuto adiposo, ghiandola mammaria

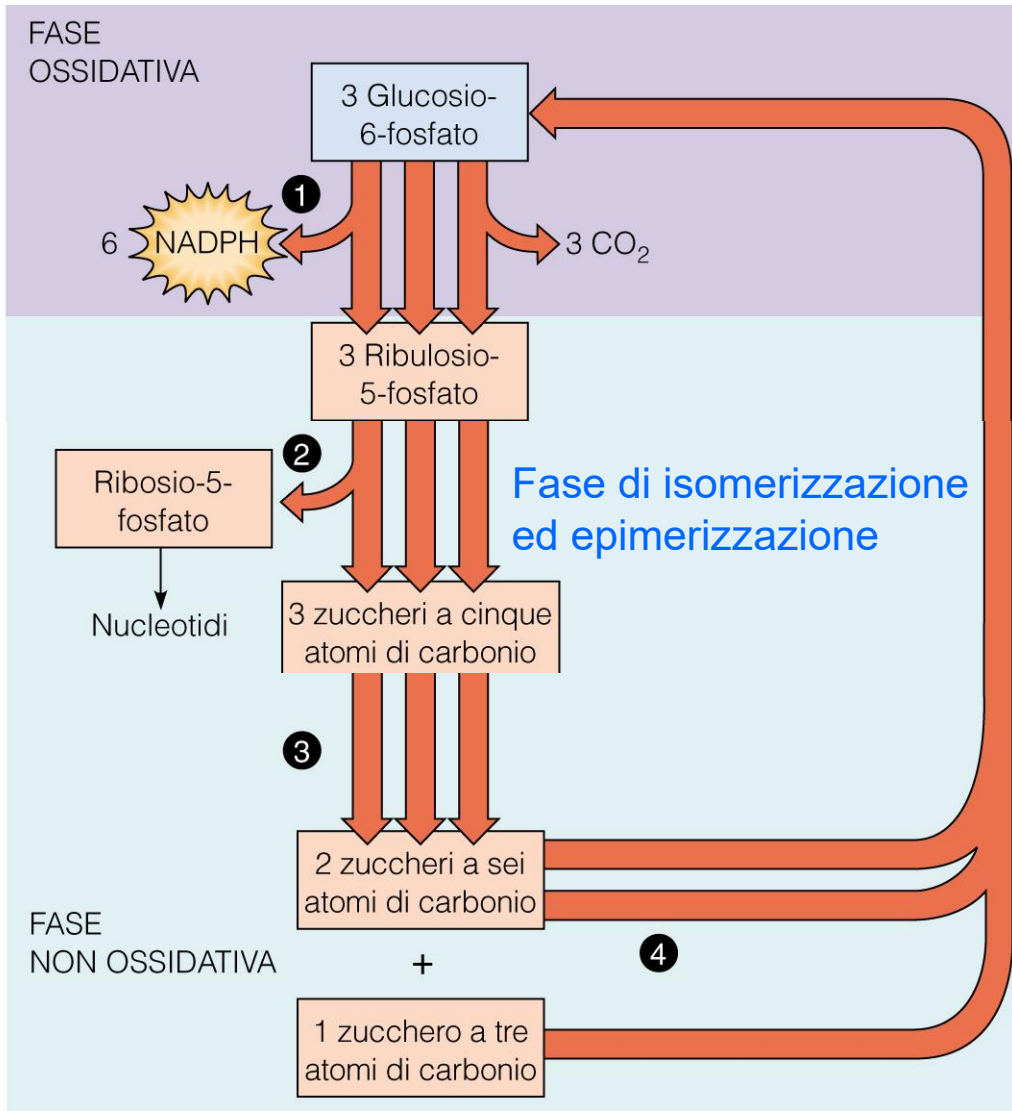
Tessuti con una intensa sintesi di colesterolo e ormoni steroidei



Fegato, ghiandole adrenali e gonadi

Richiedono **NADPH**.

VIA dei pentosio-fosfato (via del fosfogluconato o dell'esosio monofosfato)



Fase di scissione e formazione legami C-C

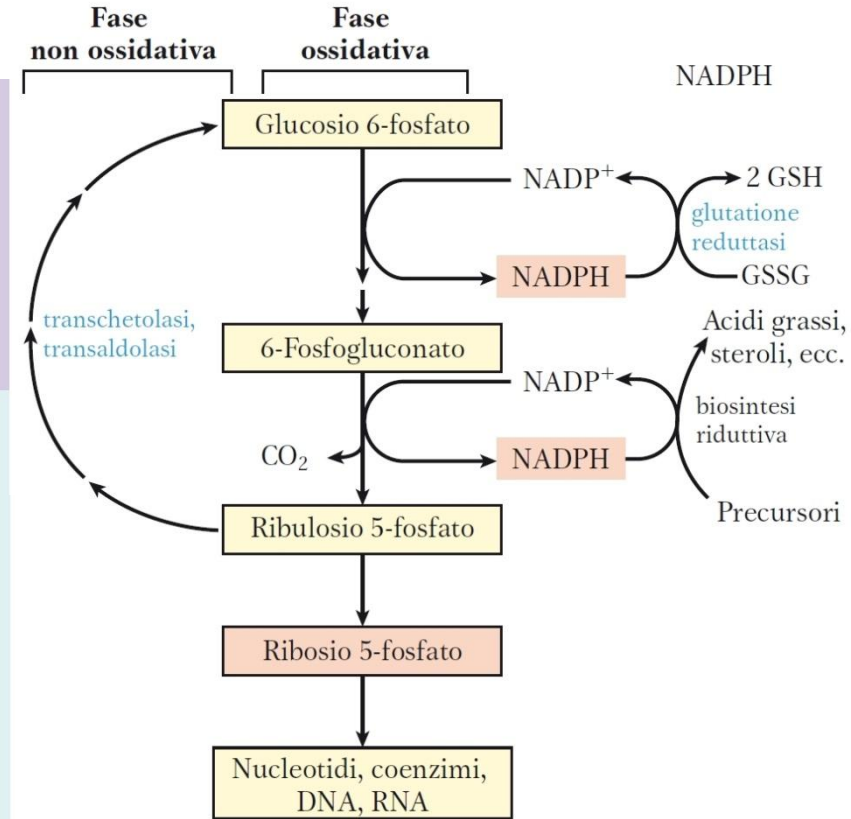


Figura 14.21 Schema generale della via del pentosio fosfato. Il NADPH che si forma nella fase ossidativa viene utilizzato per ridurre il glutatione, GSSG (vedi il Box 14.4) e per supportare le reazioni della biosintesi riduttiva. L'altro prodotto della fase ossidativa è il ribosio 5-fosfato, che serve come precursore di nucleotidi, coenzimi e acidi nucleici. Nelle cellule che non utilizzano il ribosio 5-fosfato per le reazioni di biosintesi, la fase non ossidativa ricicla sei molecole di pentosio in cinque di glucosio 6-fosfato, consentendo la produzione continua di NADPH e convertendo una molecola di glucosio 6-fosfato (in sei cicli) in CO₂.



NADPH,

Biosintesi riduttive o per contrastare gli effetti dannosi dei radicali dell'ossigeno

Eritrociti, cellule del cristallino e della cornea sono direttamente esposti all'ossigeno ai dannosi radicali liberi generati dall'ossigeno.

Eritrociti: (NADPH previene il danno ossidativo.

Un difetto genetico nella glucosio 6-fosfato deidrogenasi (primo enzima della via) può avere gravi conseguenze.



La via dei pentoso fosfati

Consiste in due fasi distinte:

- 1) Fasi ossidativa
- 2) Fase non-ossidativa

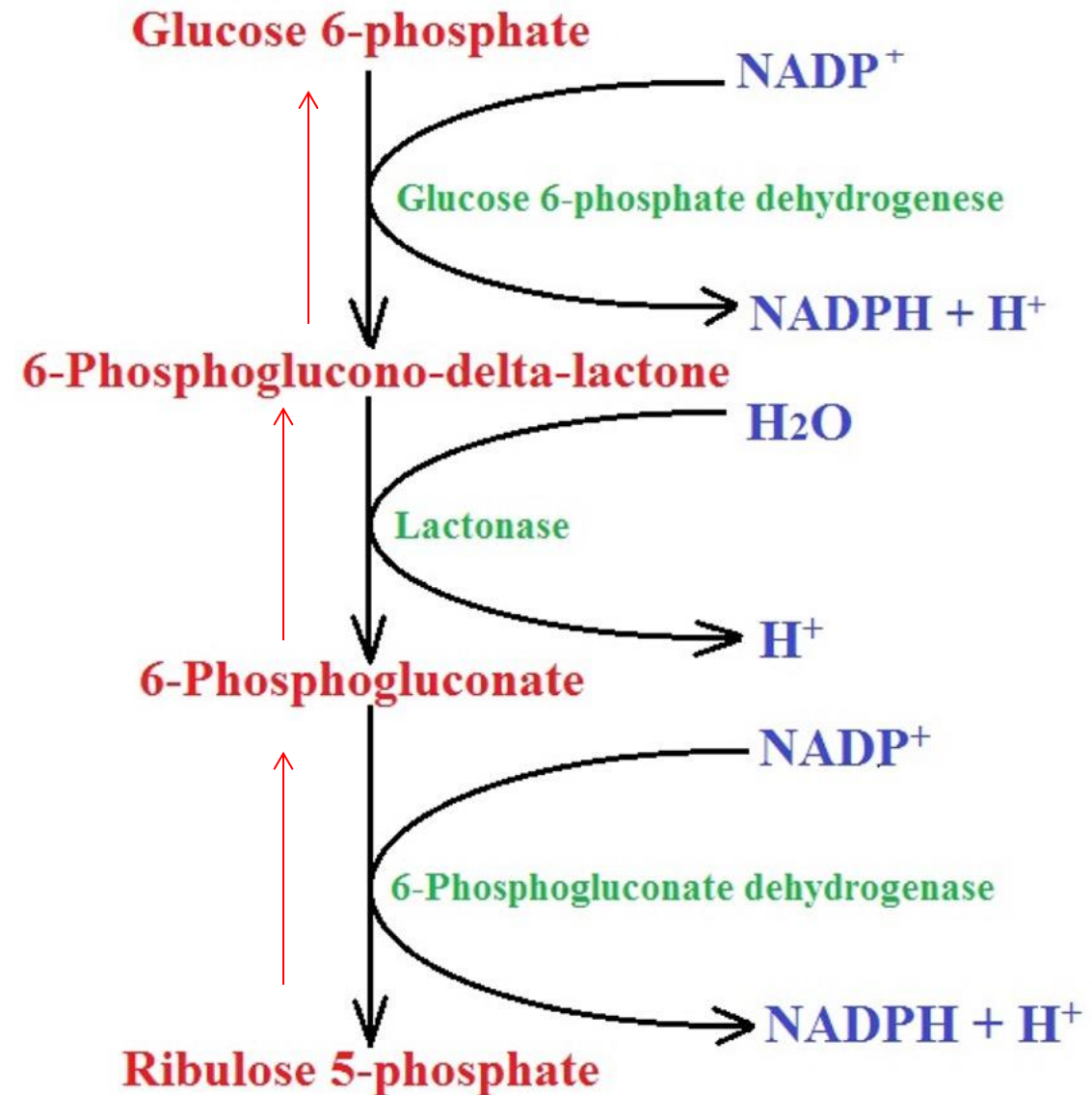


Pentose Phosphate Pathway

PHASE I (Oxidative Phase)

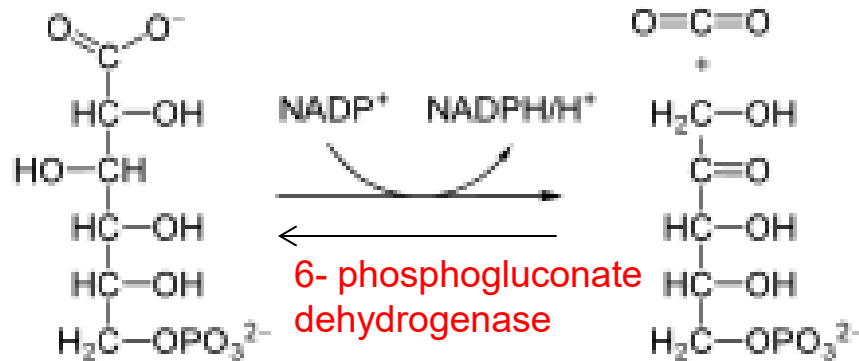
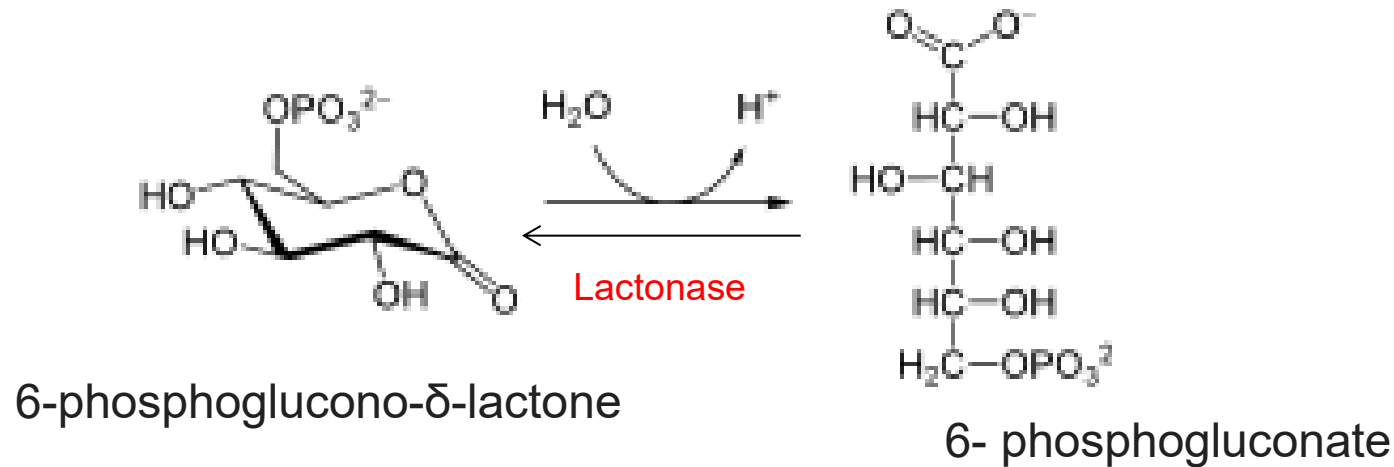
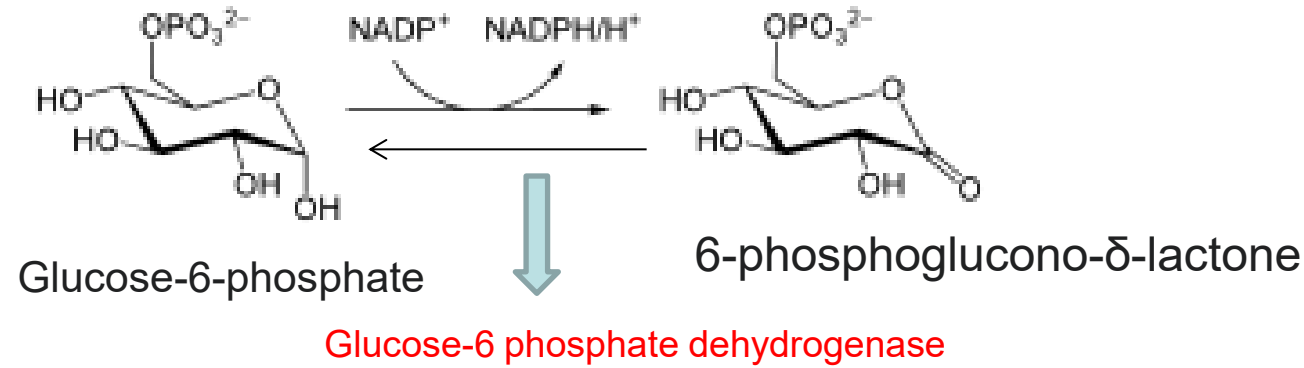
The first is the oxidative phase, in which NADPH is generated.

There are **three** reversible reactions





Oxidative phase (three reversible reactions)

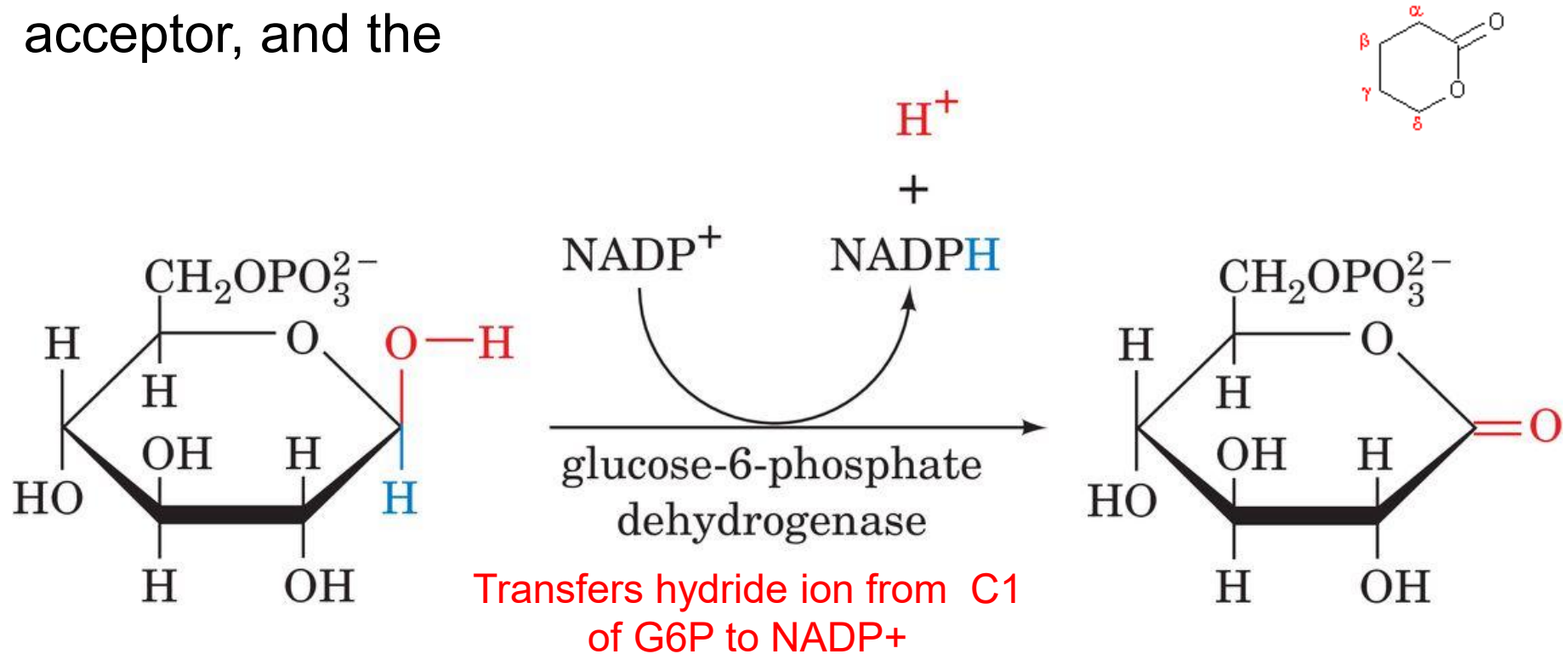




G6PD Generates 1st NADPH

NADP⁺ is the electron acceptor, and the

Oxidative reaction produces **NADPH**



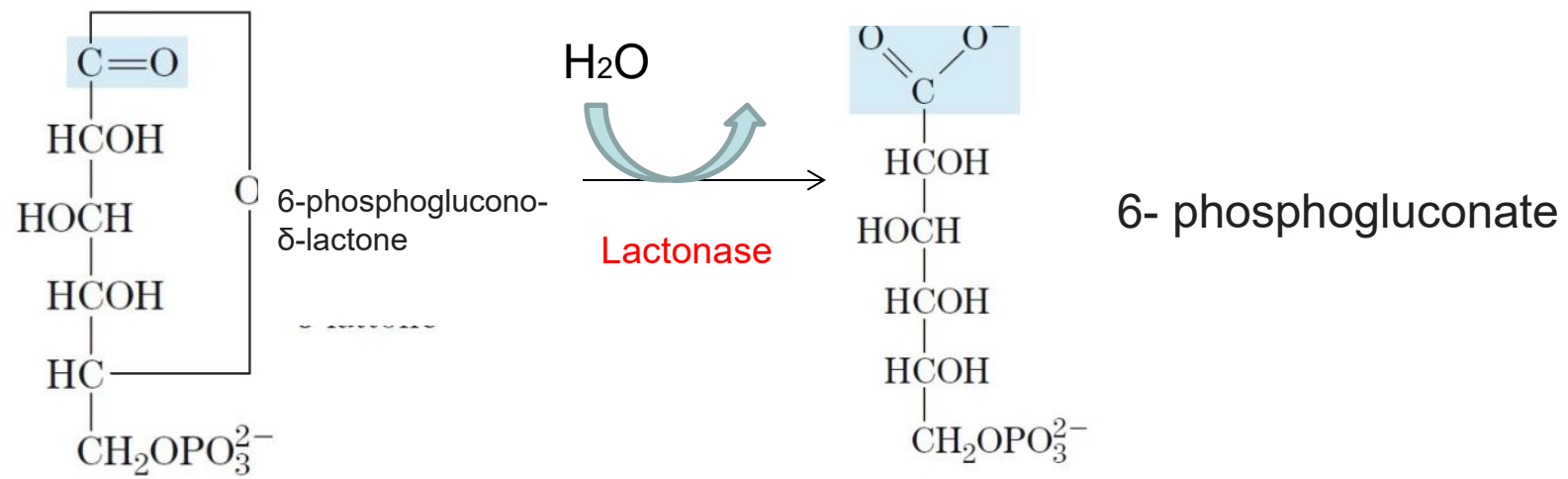
Glucose-6-phosphate

6-phosphoglucono- δ -lactone

(cyclic ester)

Derived from glycolysis or glycogenolysis





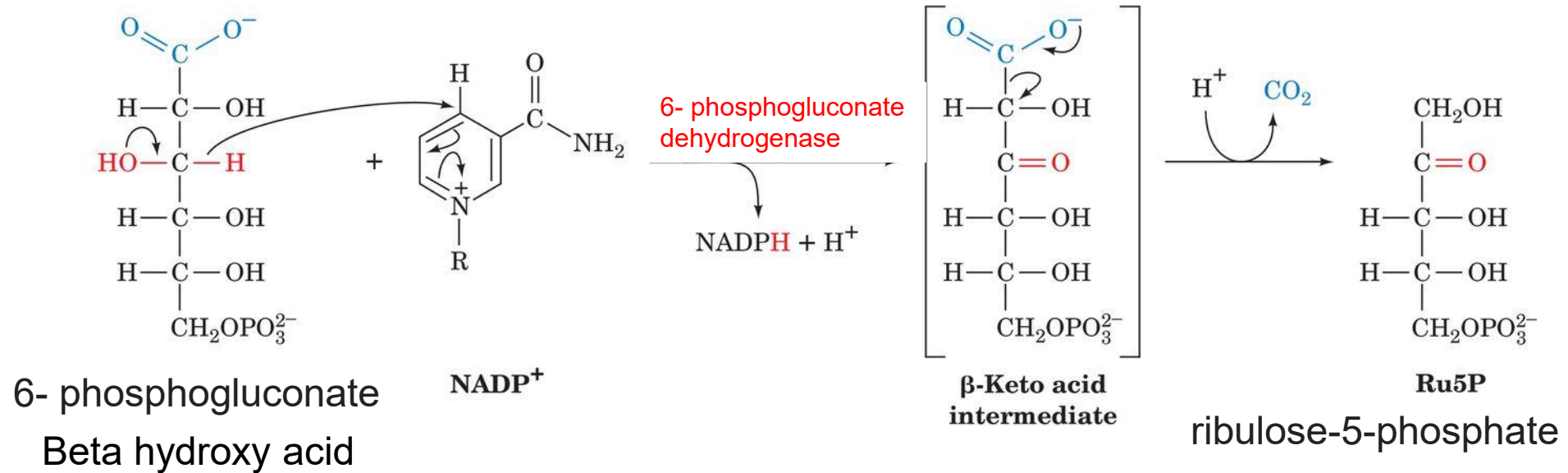
The lactone is hydrolyzed to the free acid 6-phosphogluconate by a specific **lactonase**,



Generates 2nd NADPH

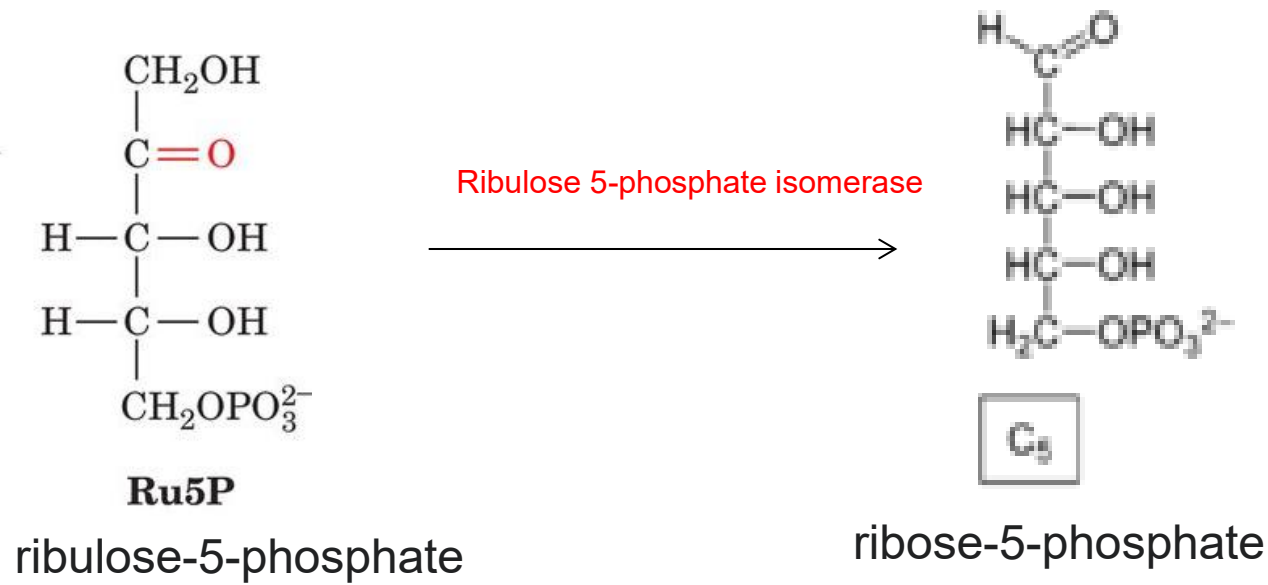
Oxidative decarboxylation

Ribulose 5-phosphate is important in the regulation of glycolysis and gluconeogenesis



6-phosphogluconate undergoes oxidation and decarboxylation by **6-phosphogluconate dehydrogenase**

to form the **ketopentose** ribulose 5-phosphate; the reaction generates a second molecule of NADPH.



Phosphopentose isomerase converts

ribulose 5-phosphate to its aldose isomer, **ribose 5-phosphate**.

In some tissues, the pentose phosphate pathway ends at this point.



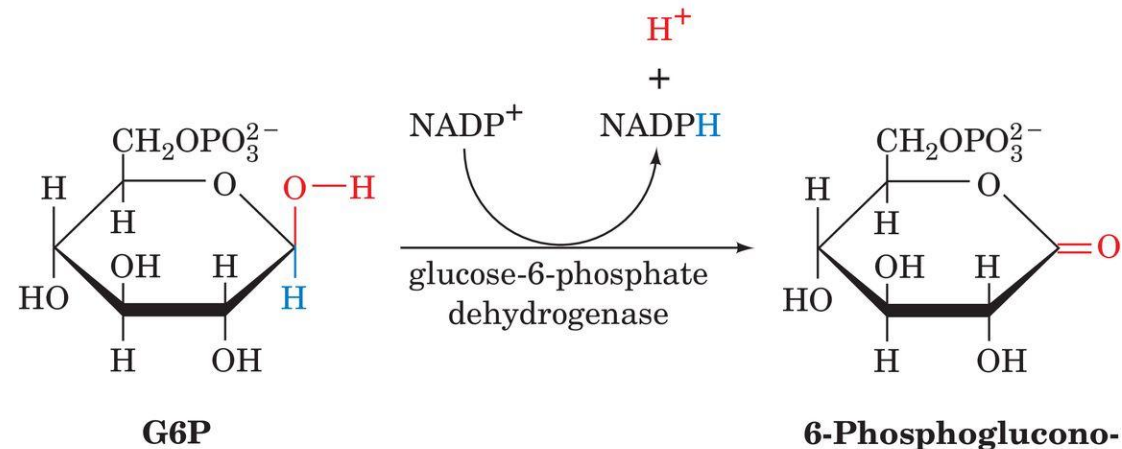


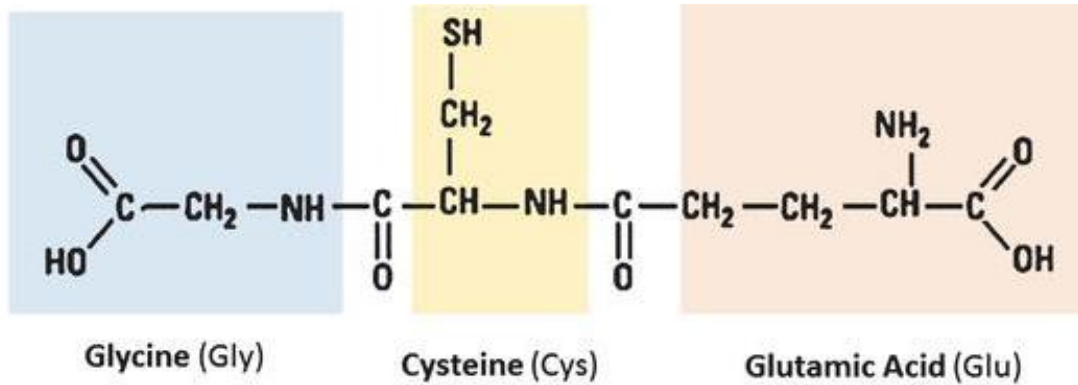
Glucose 6-phosphate dehydrogenase (G6PD) deficiency

Glucose 6-phosphate dehydrogenase catalyzes **the first step** in the pentose phosphate pathway which produces **NADPH**.

Essential in many biosynthetic pathways, also protects cells from **oxidative damage by hydrogen peroxide (H_2O_2)** and **superoxide free radicals**,

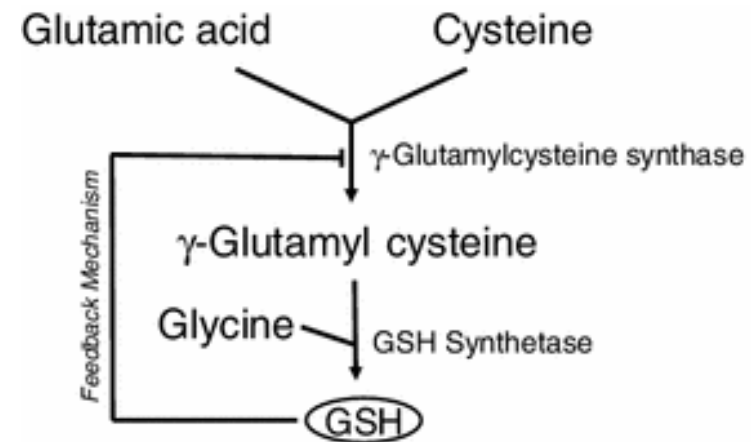
Highly reactive oxidants generated as metabolic byproducts and through the actions of **drugs** such as **primaquine** and natural products such as **divicine** (the toxic ingredient of fava beans)





Glutathione (GSH) is a tripeptide that performs various functions, such as antioxidant, reducing the -SH groups of proteins, reducing the ferrous ion of Hb and cofactor of some enzymes

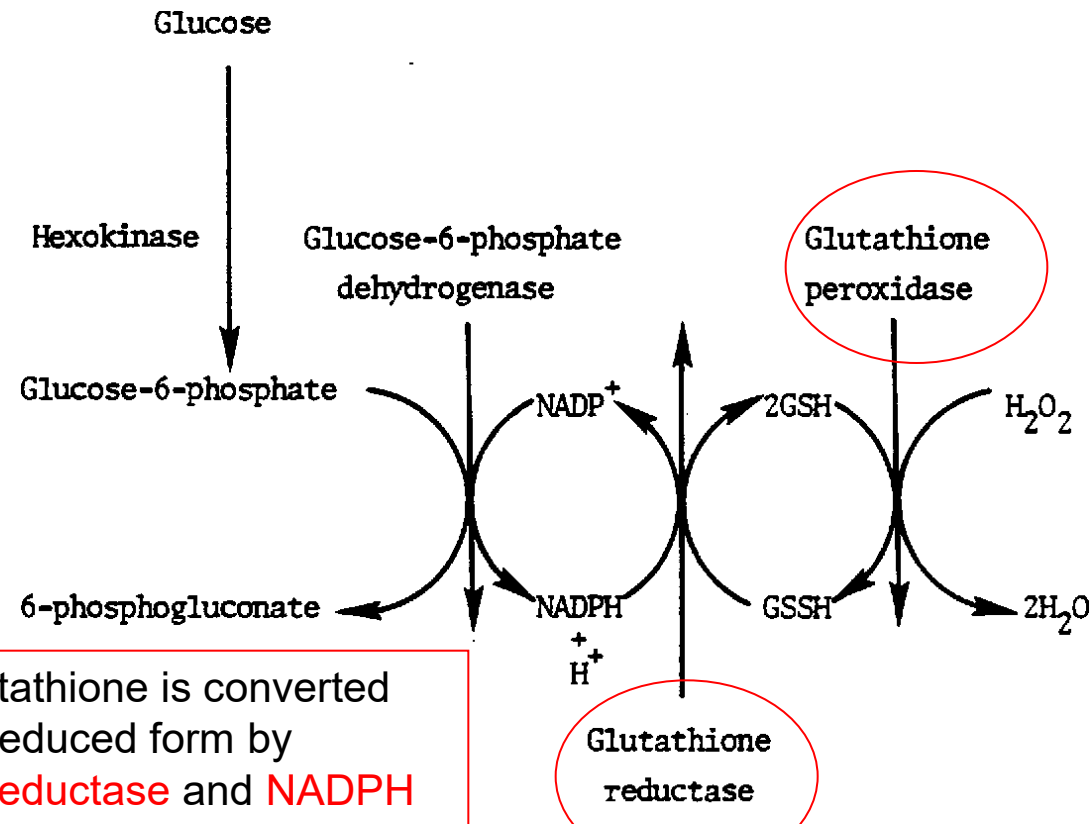
It is synthesized from glutamate





Glucose 6-phosphate dehydrogenase (G6PD) deficiency

In G6PD-deficient individuals, the **NADPH** production is **diminished** and detoxification of H_2O_2 is inhibited.

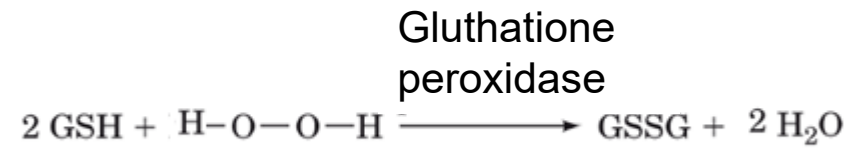


Oxidized glutathione is converted back to the reduced form by **glutathione reductase** and **NADPH**

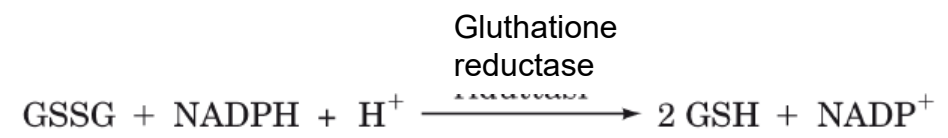
During normal detoxification, H_2O_2 is converted to H_2O in a reaction which **glutathione** is oxidated by **glutathione peroxidase**



Reduced intra erythrocyte glutathione counteracts the action of ROS

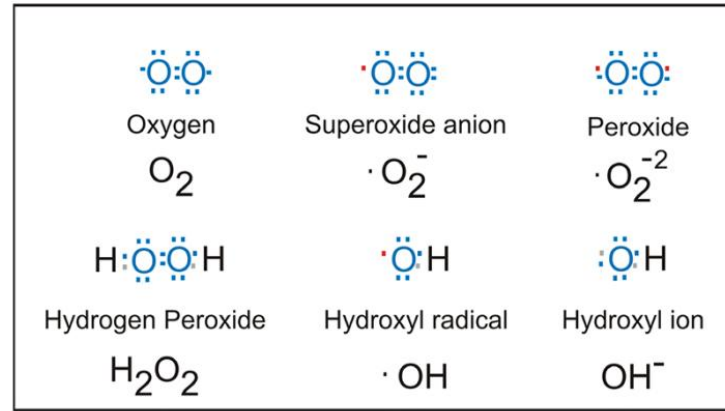


Glutathione is oxidated by glutathione peroxidase



Oxidized glutathione is converted back to the reduced form by glutathione reductase and NADPH

ROS (Reactive oxygen species)



Oxidizing molecules

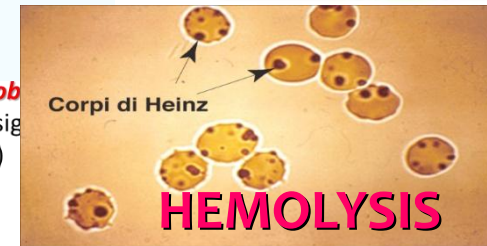
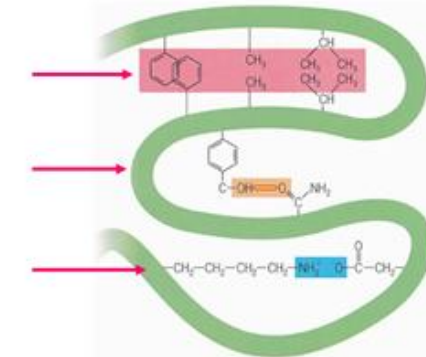
The absence of intra erythrocyte glutathione causes damage to the erythrocytes



Damage to cellular membrane

Oxidation of heme iron with the formation of methemoglobin

Destruction of the tertiary structure of the Hb



It is therefore vital for the integrity of the erythrocytes to have a continuous supply of NADPH + H⁺.

Cellular damage results: lipid peroxidation leading to breakdown of erythrocyte membranes and oxidation of proteins and DNA

Glucose 6-phosphate dehydrogenase (G6PD) deficiency

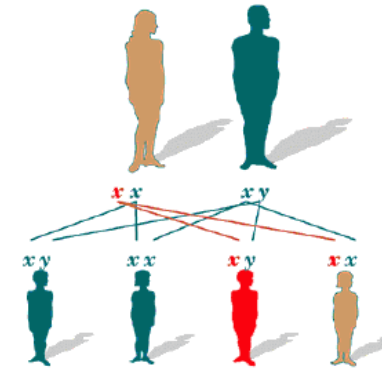
G6PD is expressed in all tissues, but its deficiency occurs essentially in erythrocytes

MUTATION IN THE GENE G6PD

400 variant

GENE is located on the X chromosome

- ☐ Enzyme is not expressed
- ☐ the enzyme is expressed but is labile (half-life of a few days)



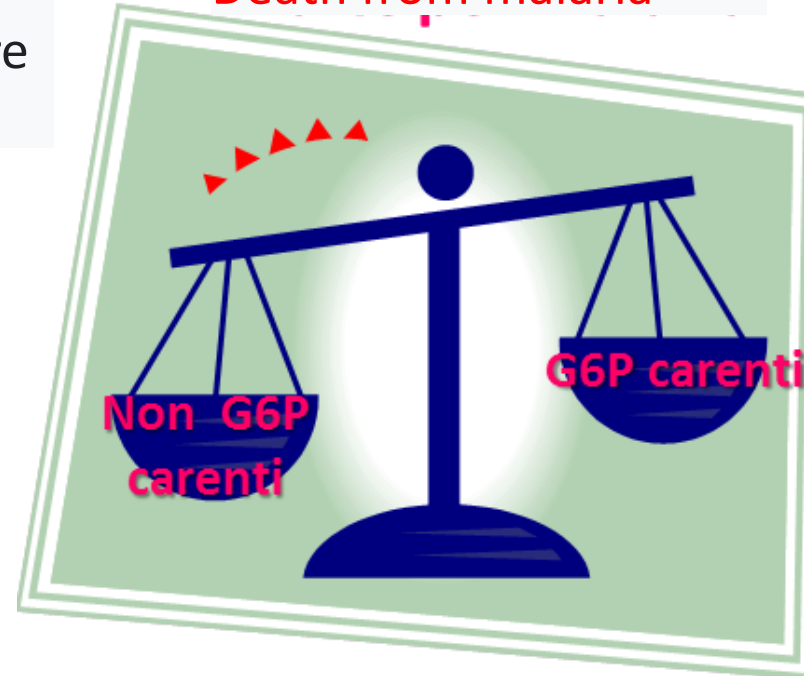
Glucose 6-phosphate dehydrogenase (G6PD) deficiency



The maximum incidence (about 30%) is observable in areas where malaria was endemic



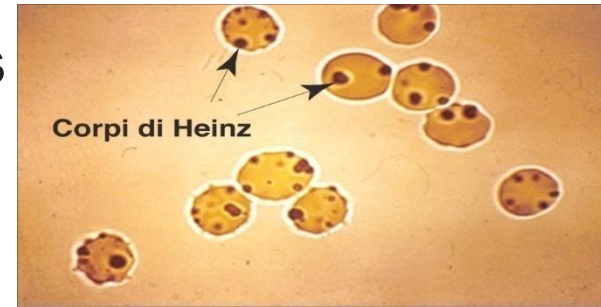
Death from malaria



Oxidative stress, induced by a condition of **G6PD deficiency**, has created an unfavorable environment at the erythrocyte level for the establishment of the protozoan *Plasmodium falciparum*

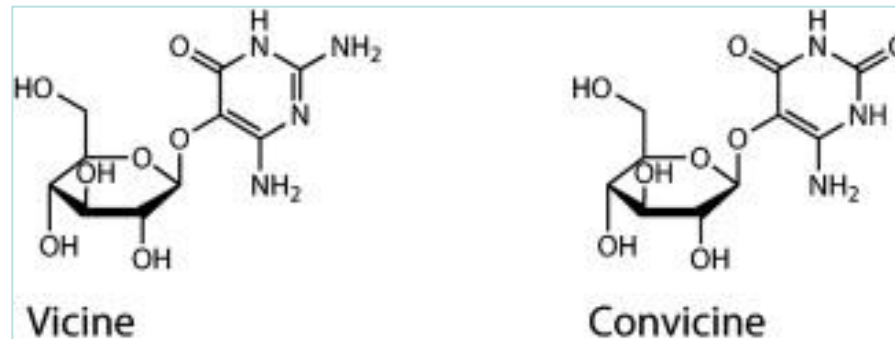
Factors triggering hemolysis in G6PD deficient subjects

Ingestion of fava beans



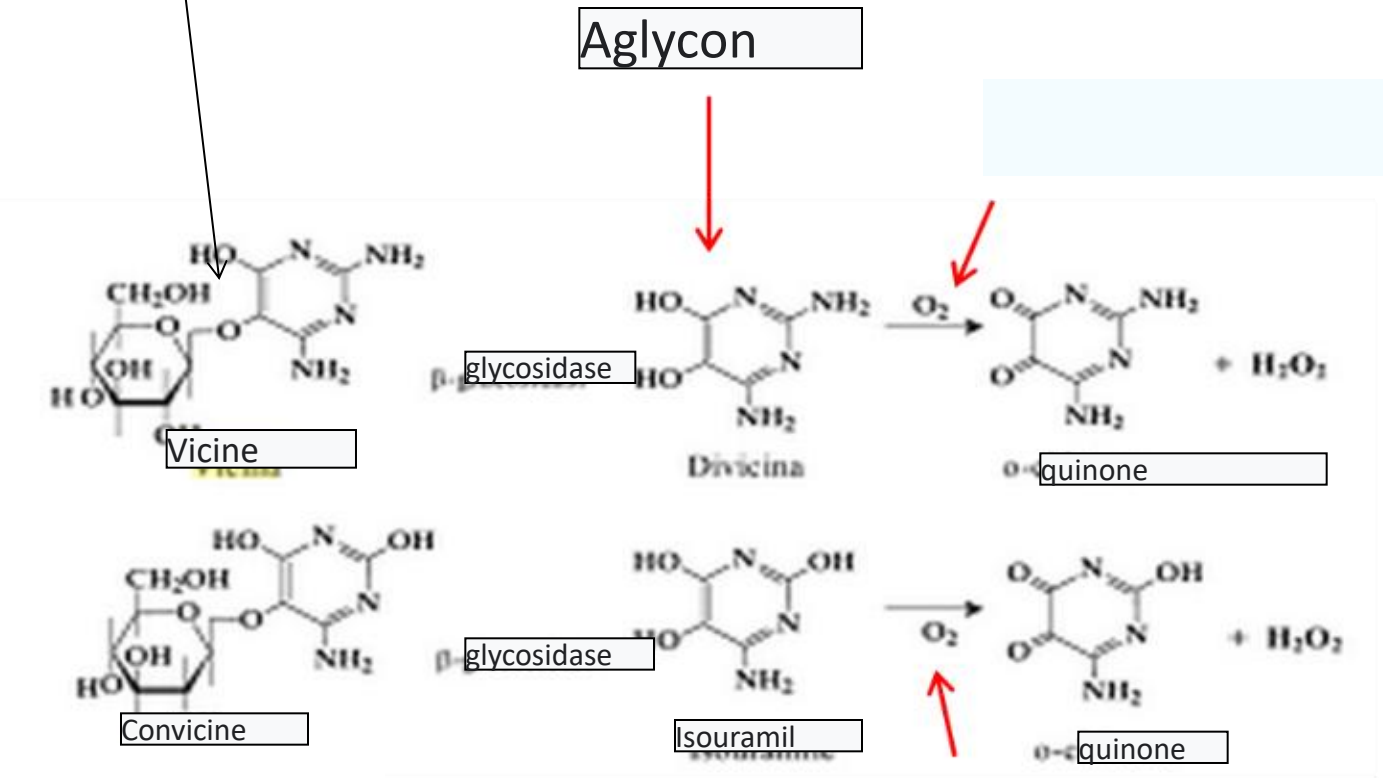
They contain the vicine and the convicine, able to promote oxidative stress

Vicine and Convicine are pyrimidine glycosides.



Vicine and Convicine

By hydrolysis of the β -glucosidic bond between glucose and the hydroxyl group at C-5 on the pyrimidine ring, generate the **aglycones divicine** (2,6-diamino-4,5-dihydroxypyrimidine) and **isouramil** (6-amino-2,4,5-trihydroxypyrimidine), respectively.



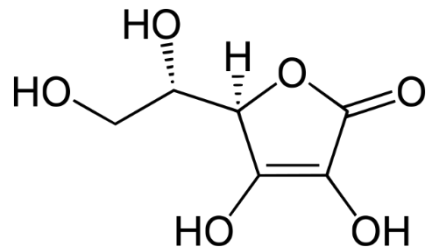
SCIENTIFIC REPORTS

OPEN Degradation of vicine, convicine and their aglycones during fermentation of faba bean flour

Received: 07 March 2016
Accepted: 08 August 2016
Published: 31 August 2016
Carlo Giuseppe Rizzello¹, Ilario Losito^{2,3}, Laura Facchini³, Kati Katina⁴, Francesco Palmisano⁵, Marco Gobetti⁶ & Rossana Coda⁶

Factors triggering hemolysis in G6PD deficient subjects

Some drugs can promote oxidative stress



Ascorbic acid

Controindicato

- Acido nalidissico
- Nitrofurantoina
- Metamizolo sodico
- Rasburicase
- Sulfadiazina (via orale)
- Sulfametossazolo (via orale e parenterale)
- Sulfasalazina
- Trimetoprim (via orale e parenterale)

Sconsigliato (eccetto particolari situazioni) a causa dell'osservazione di casi di emolisi acuta

- Ciprofloxacina (via orale e parenterale)
- Cloroquina
- Dimercapolo
- Fitomenadione (vitamina K1)
- Glibenclamide
- Levofloxacina (via orale e parenterale)
- Norfloxacina (via orale)
- Spiramicina (via orale e parenterale)
- Sulfadiazina (via orale)

Sconsigliato (eccetto particolari situazioni) a causa dell'appartenenza ad una classe farmacologica a rischio, o a causa di un rischio potenziale di emolisi

- Acido pipemidico
- Chinina
- Enoxacina
- Fenazone (via auricolare)
- Gliclazide
- Glimepiride
- Glipizide
- Idrossiclorochina
- Lomefloxacina
- Moxifloxacina
- Ofloxacina (via orale e parenterale)
- Pefloxacina (via orale e parenterale)
- Prilocaina
- Sulfacetamide

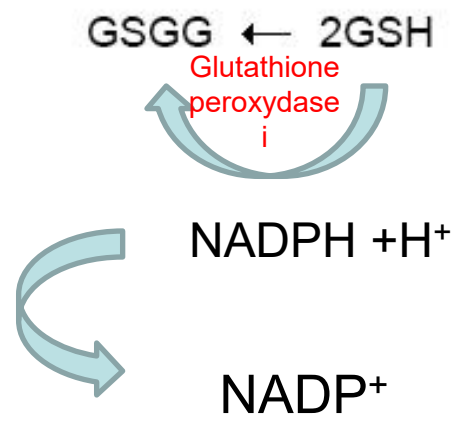
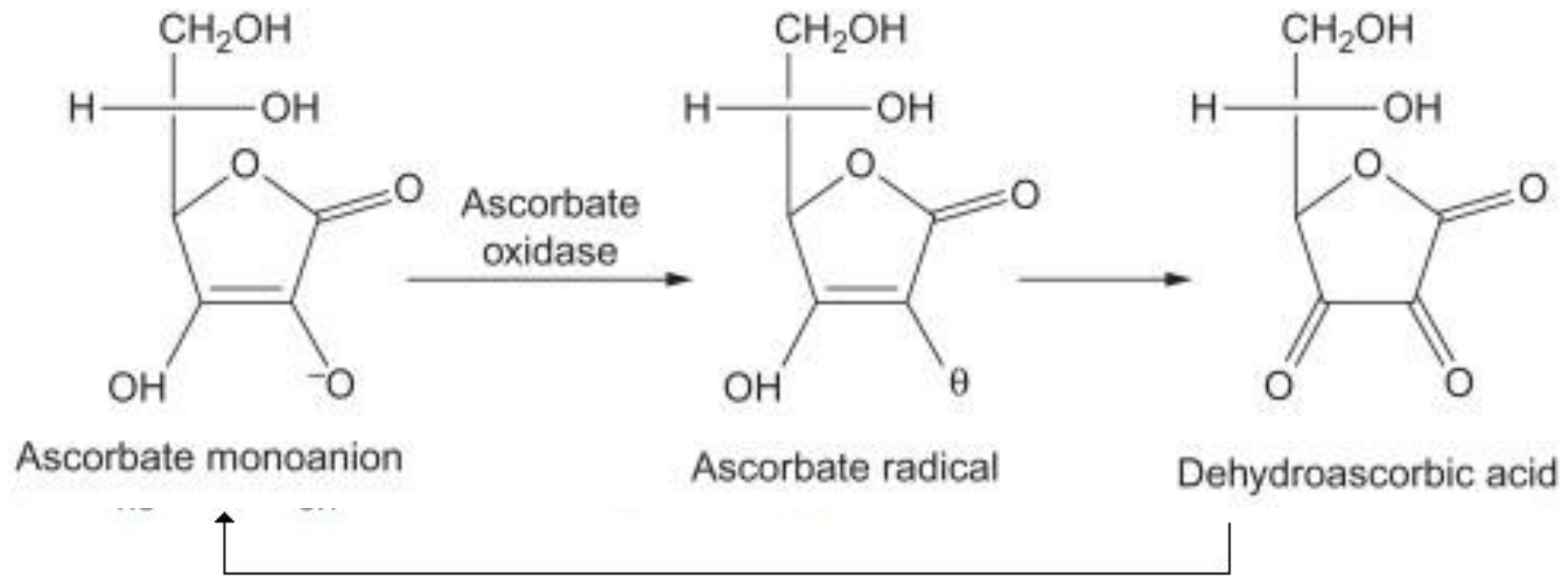
Sconsigliato a dosi elevate

- Acido acetilsalicilico
- **Acido ascorbico**
- Paracetamolo

Possibile utilizzo dopo l'analisi dei dati disponibili (letteratura e farmacovigilanza)

- Blu di metilene (via topica)*
- Bupivacaina*
- Chinidina*
- Ciprofloxacina (via oftalmica e auricolare)*
- Cloramfenicolo (via oftalmica)*
- Colchicina*
- Dietilamina*
- Diidrochinidina*
- Dimenidrinato*
- Doxorubicina*
- Fenazone (via auricolare)*
- Fenilbutazone*
- Fenitoina*
- Glicole propilenico*
- Isoniazide (via orale e parenterale)*
- Levodopa*
- Meflochina*
- Monossido di azoto*
- Nitroprussiato*
- Norfloxacina (via oftalmica)*
- Ofloxacina (via oftalmica e auricolare)*
- Pirimetamina*
- Proguanile*
- Streptomina*
- Tiamfenicolo*
- Triesilfenidile*

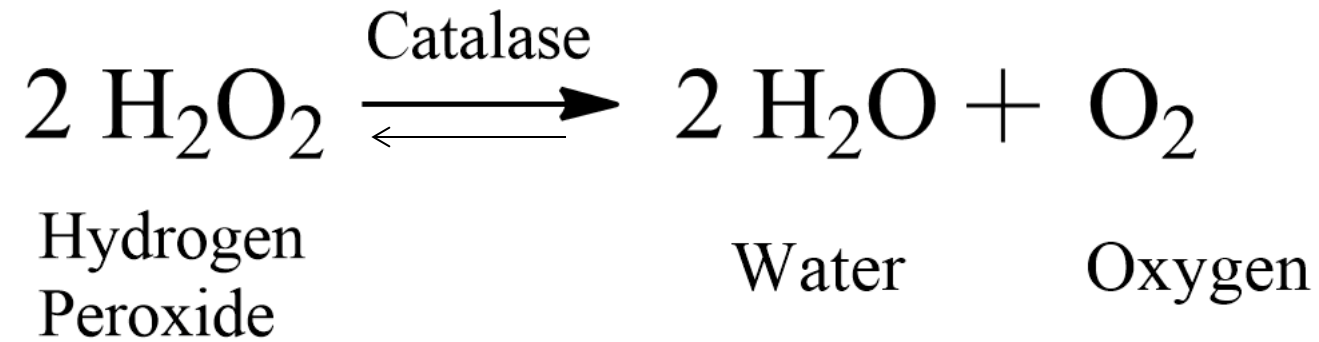
Ascorbic acid

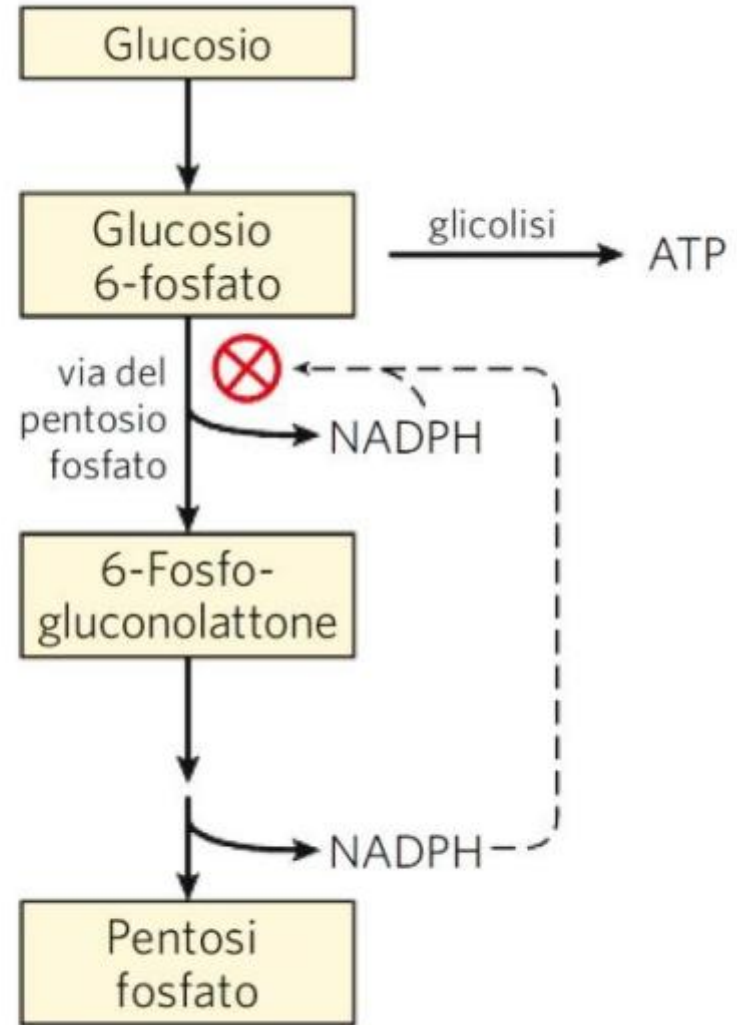


Ascorbic acid is oxidized to dehydroascorbic acid (DHA) and converted back to ascorbic acid at the expense of glutathione.

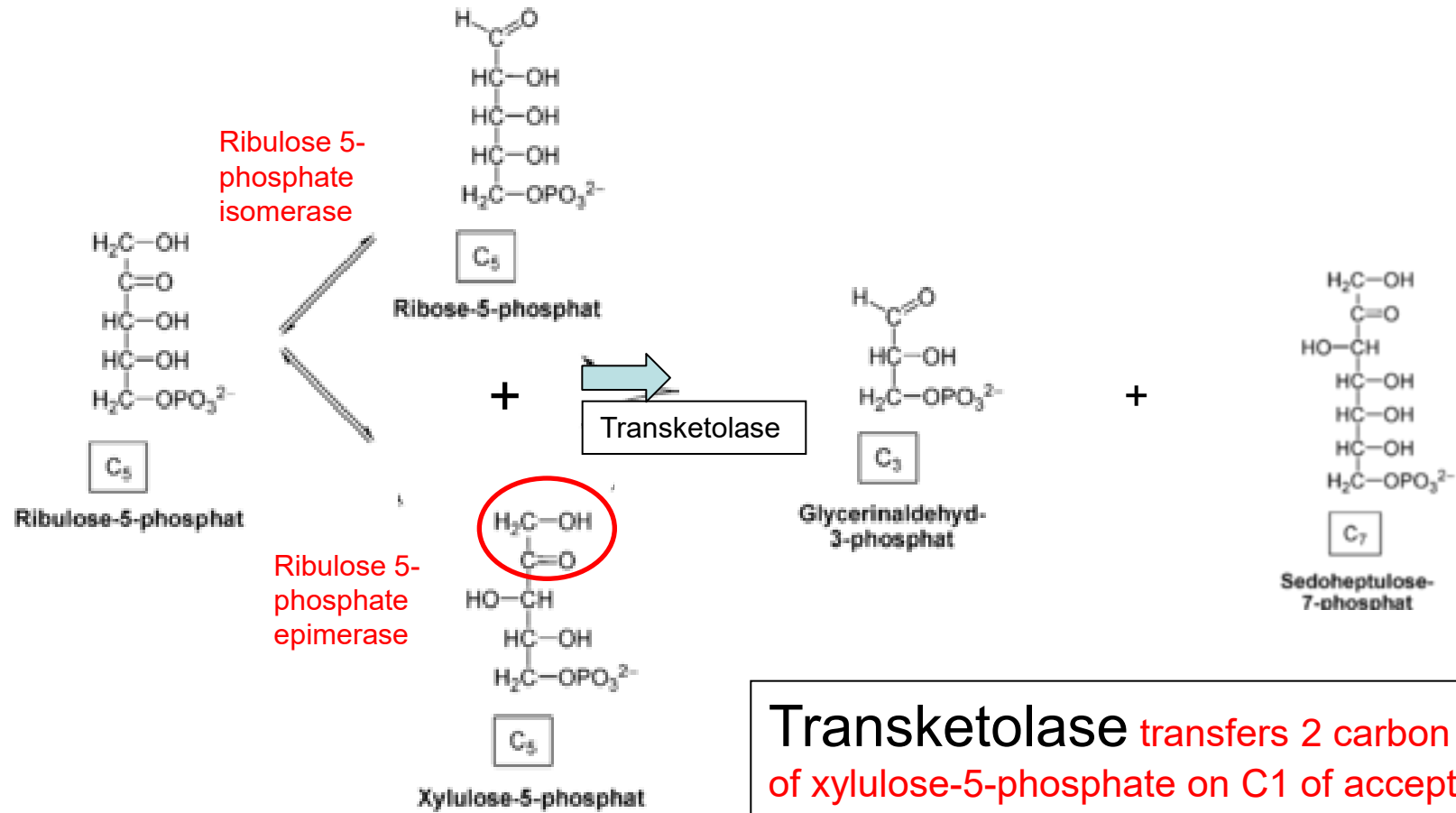


H₂O₂ is also broken down to H₂O and O₂ by **catalase**, which also requires NADPH.



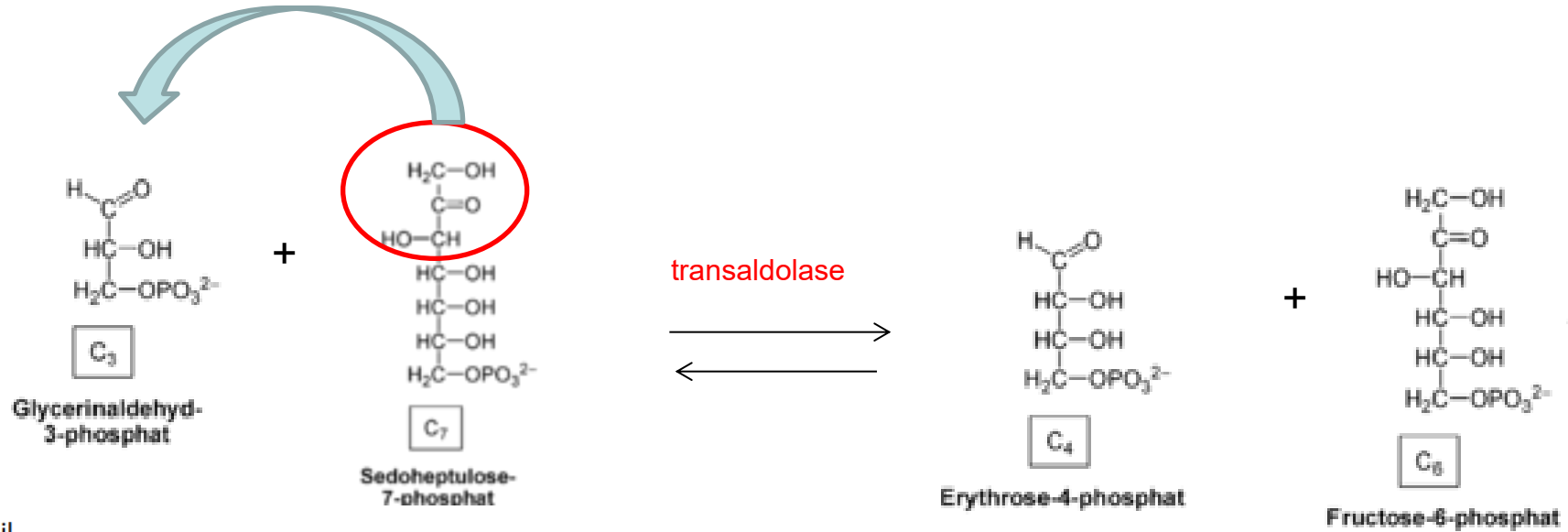


PHASE II: Non-oxidative phase

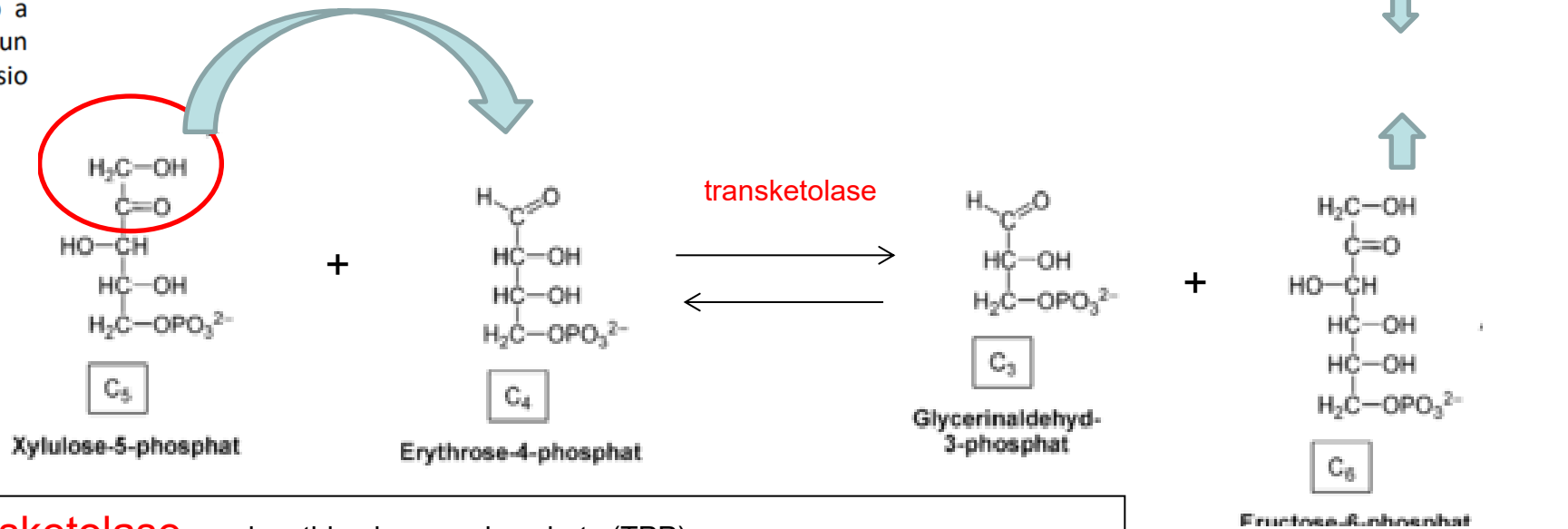


Transketolase transfers 2 carbon atoms of xylulose-5-phosphate on C1 of acceptor aldose (ribose 5-phosphate)

La transaldolasi trasferisce una unità a tre atomi di carbonio

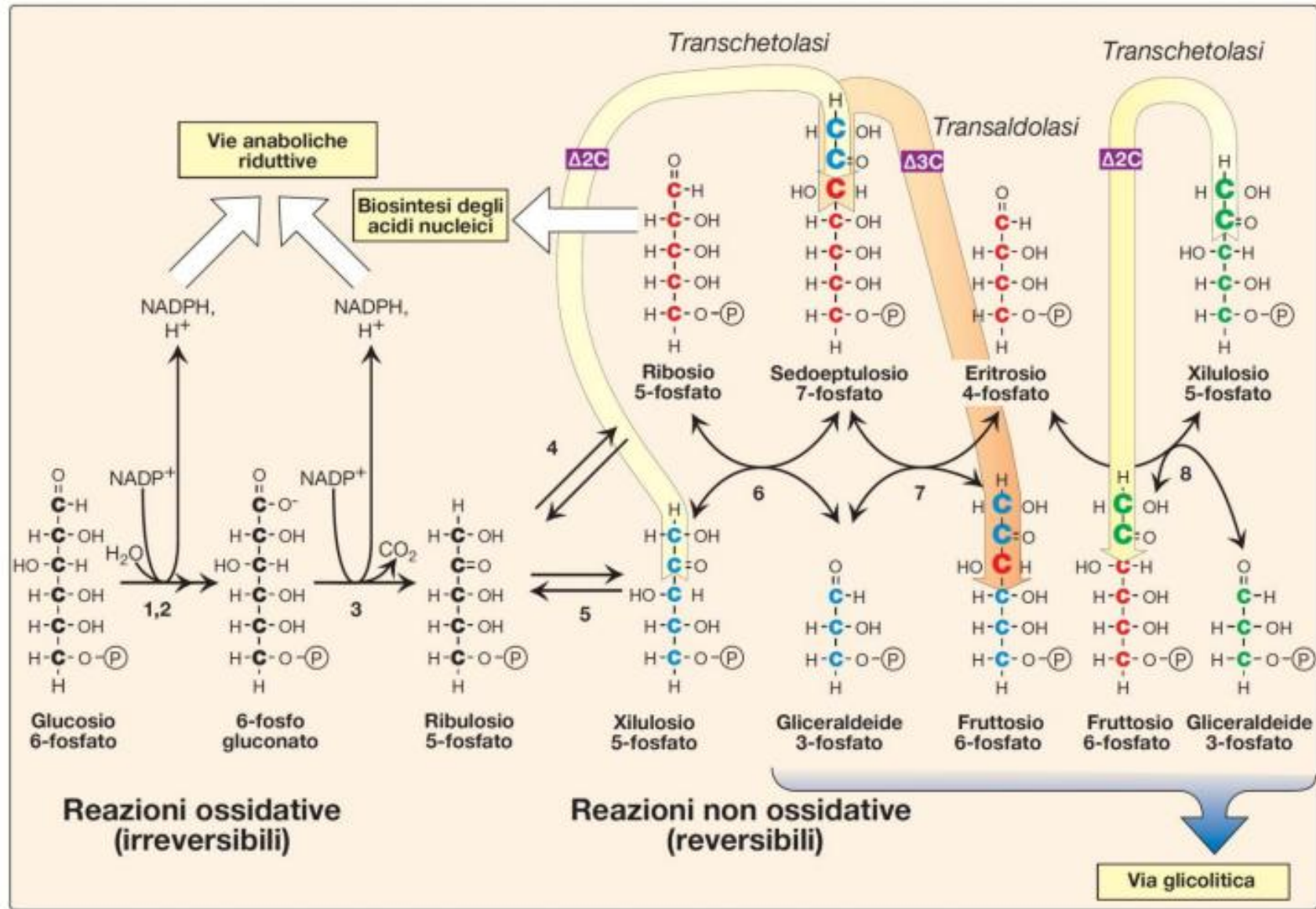


La transchetolasi catalizza il trasferimento di un frammento a due atomi di carbonio da un chetoso donatore a un aldoso accettore.



Transketolase requires thiaminepyrophosphate (TPP) as coenzyme

Form 2 fructose 6-phosphate





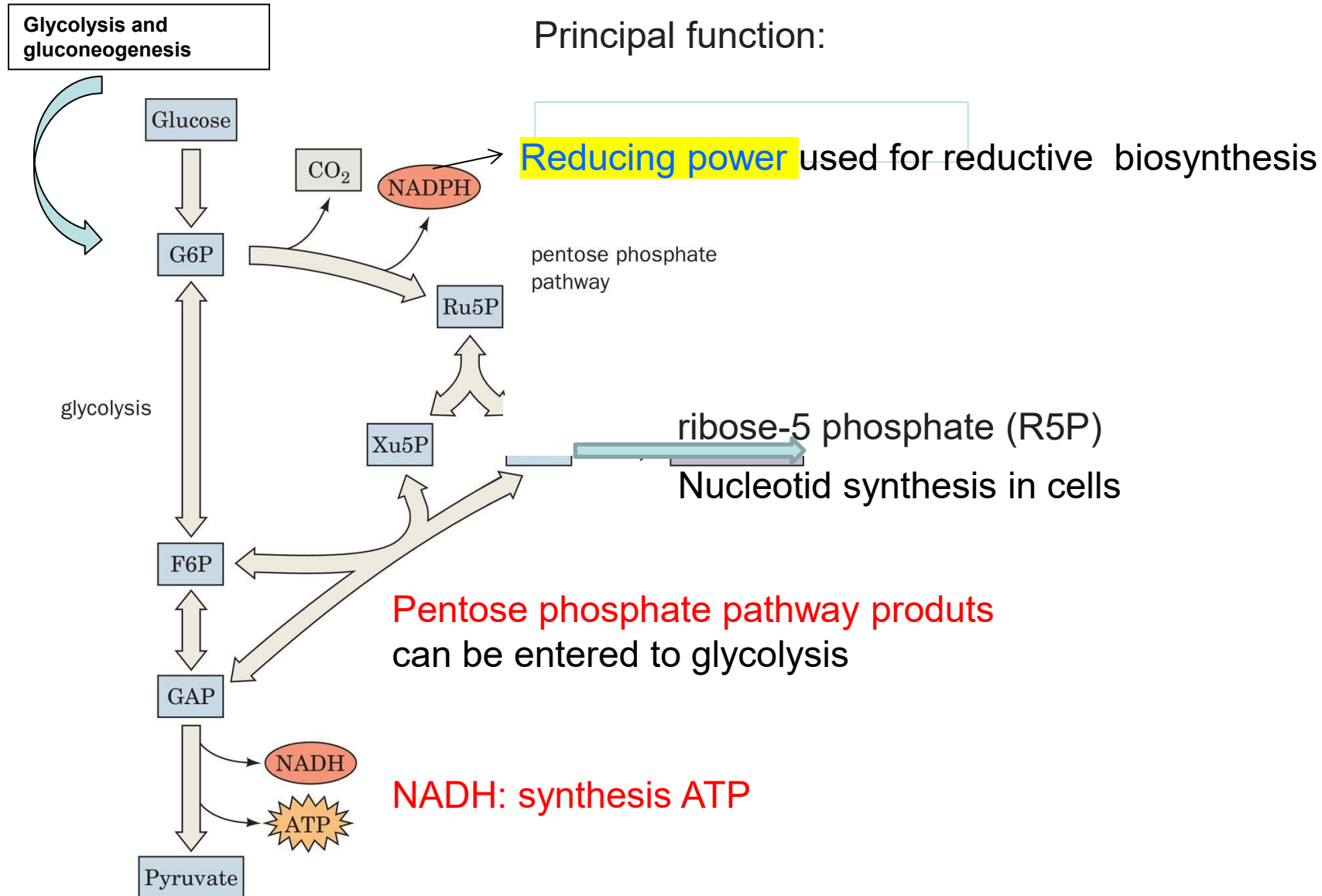
Principal products of this way are **NADPH and R5P**:
transketolases and transaldolases are activated to produce metabolic intermediates of glycolysis (**GAP**)

Generates reducing equivalents (**NADPH**)

- **Produces ribose 5-phosphate**
- Nucleotid and nucleic acid

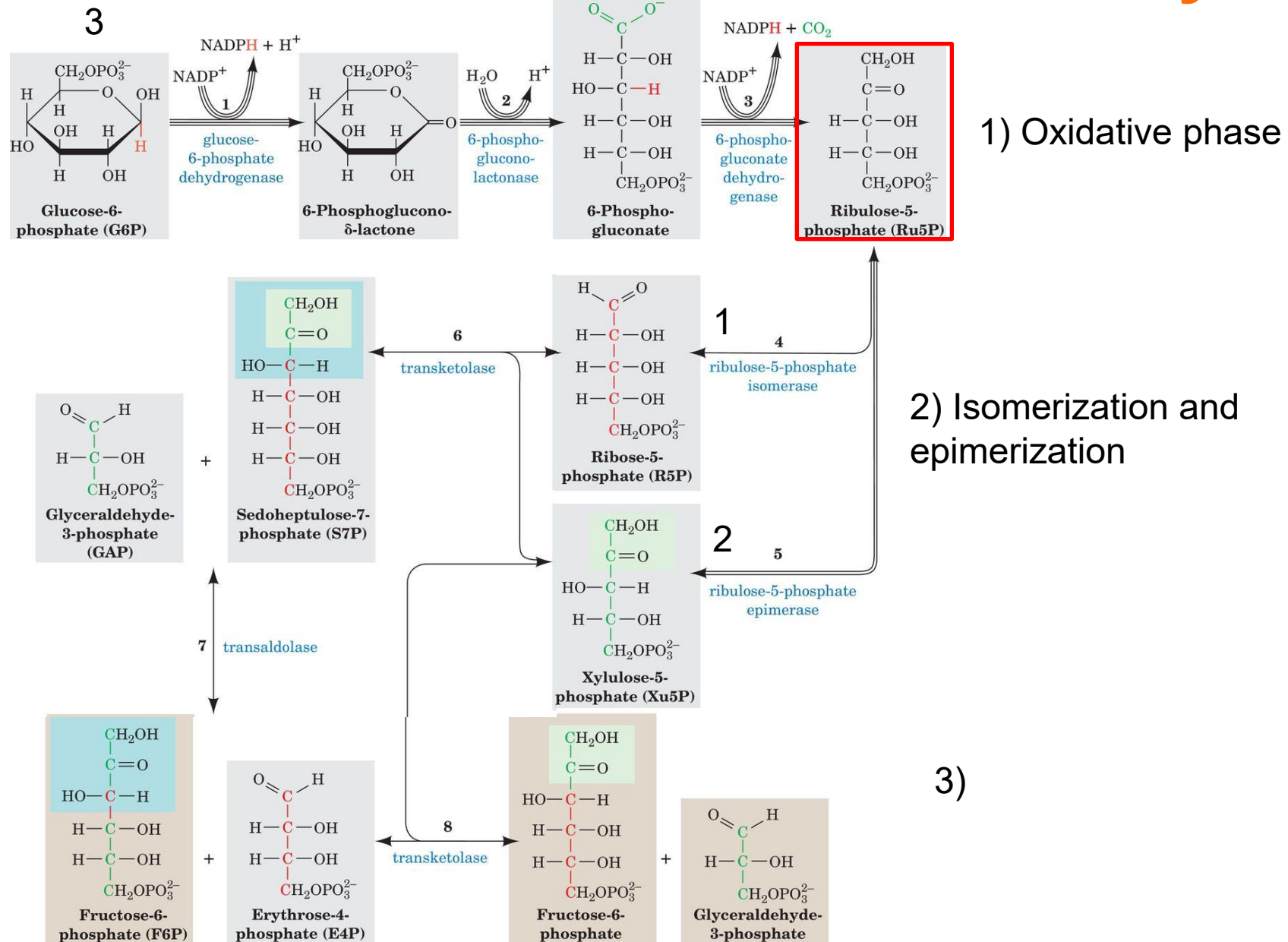
- **Produces erythrose 4-phosphate**
- Precursor of aminoacid and vitamin B6 synthesis .

Relationship Between Glycolysis & Pentose Phosphate Pathway





Pentose Phosphate Pathway





Wernicke-Korsakoff Syndrome Is Exacerbated by a Defect in Transketolase

Transketolase requires thiaminepyrophosphate (TPP) as coenzyme

Wernicke-Korsakoff syndrome is a disorder caused by a severe deficiency of **thiamine**, a component of TPP.

The syndrome is more common among **people with alcoholism** than in the general population, because chronic, heavy alcohol consumption interferes with the **intestinal absorption of thiamine**.

The syndrome can be exacerbated **by a mutation in the gene for transketolase** that results in an enzyme with a lowered affinity for TPP—an affinity one-tenth that of the normal enzyme.



The result is a slowing down of the whole pentose phosphate pathway.

Wernicke-Korsakoff syndrome symptoms:

Severe memory loss

Mental confusion

Partial paralysis