AULAS DE BIOQUÍMICA

METABOLISMO DOS GLÚCIDOS (cont.)

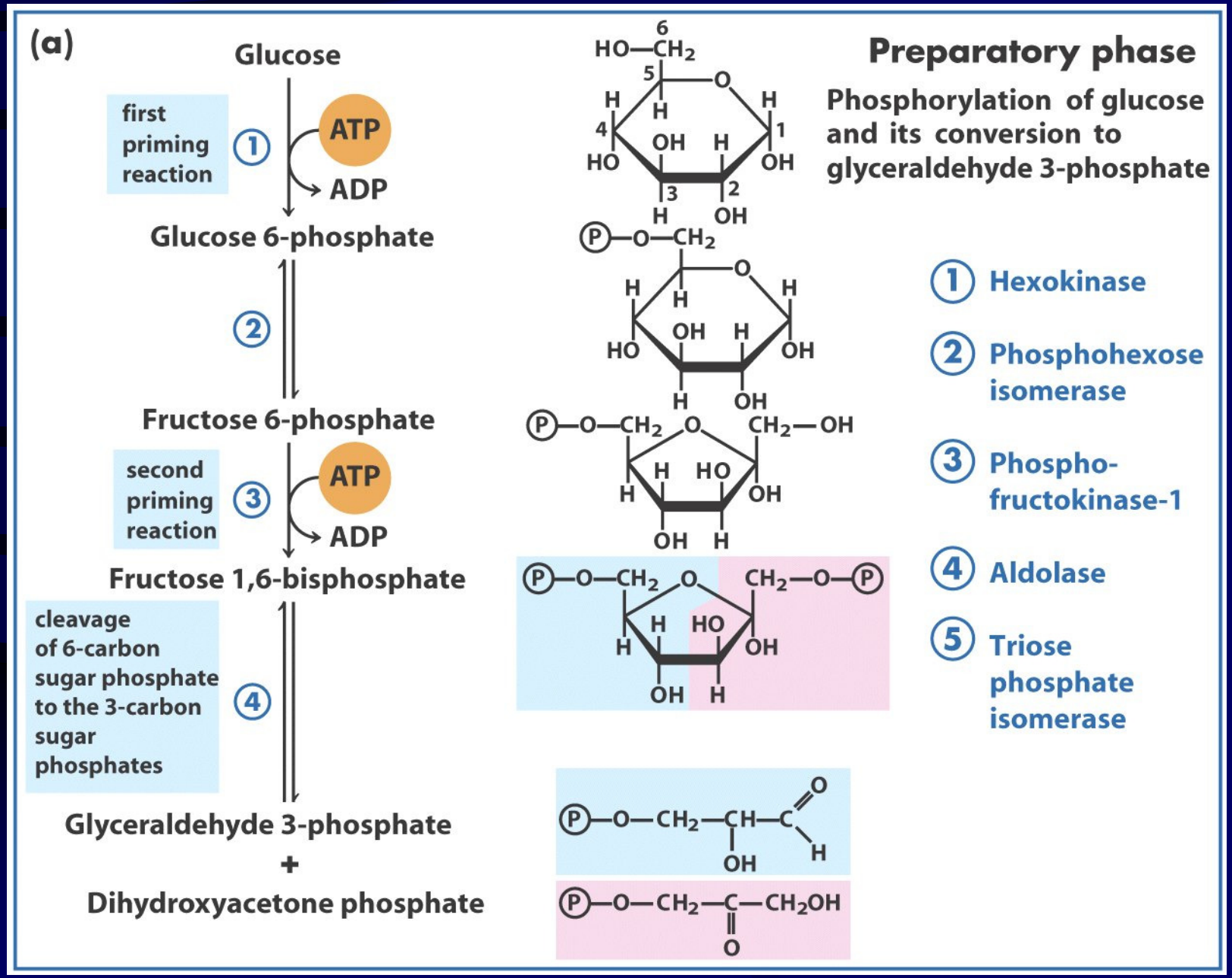
Gluconeogénese. Diferentes alternativas para a conversão de piruvato em fosfoenolpiruvato. Balanço energético. Regulação.

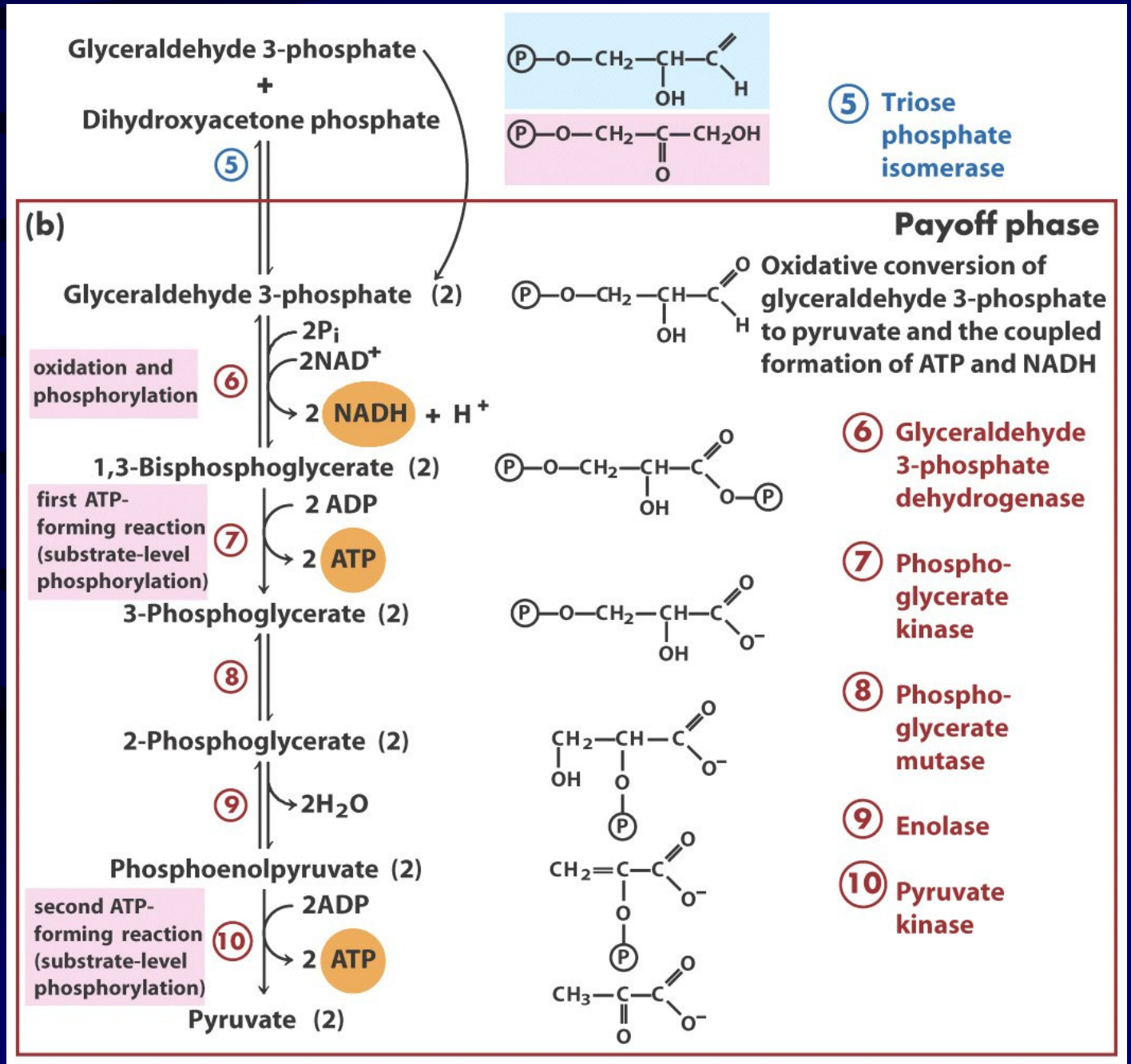
Via dos fosfatos de pentose. Reacções da fase oxidativa e da fase não oxidativa. Função nos organismos. Regulação.

Ligação entre a glicólise, gluconeogénese e a via dos fosfatos de pentose.

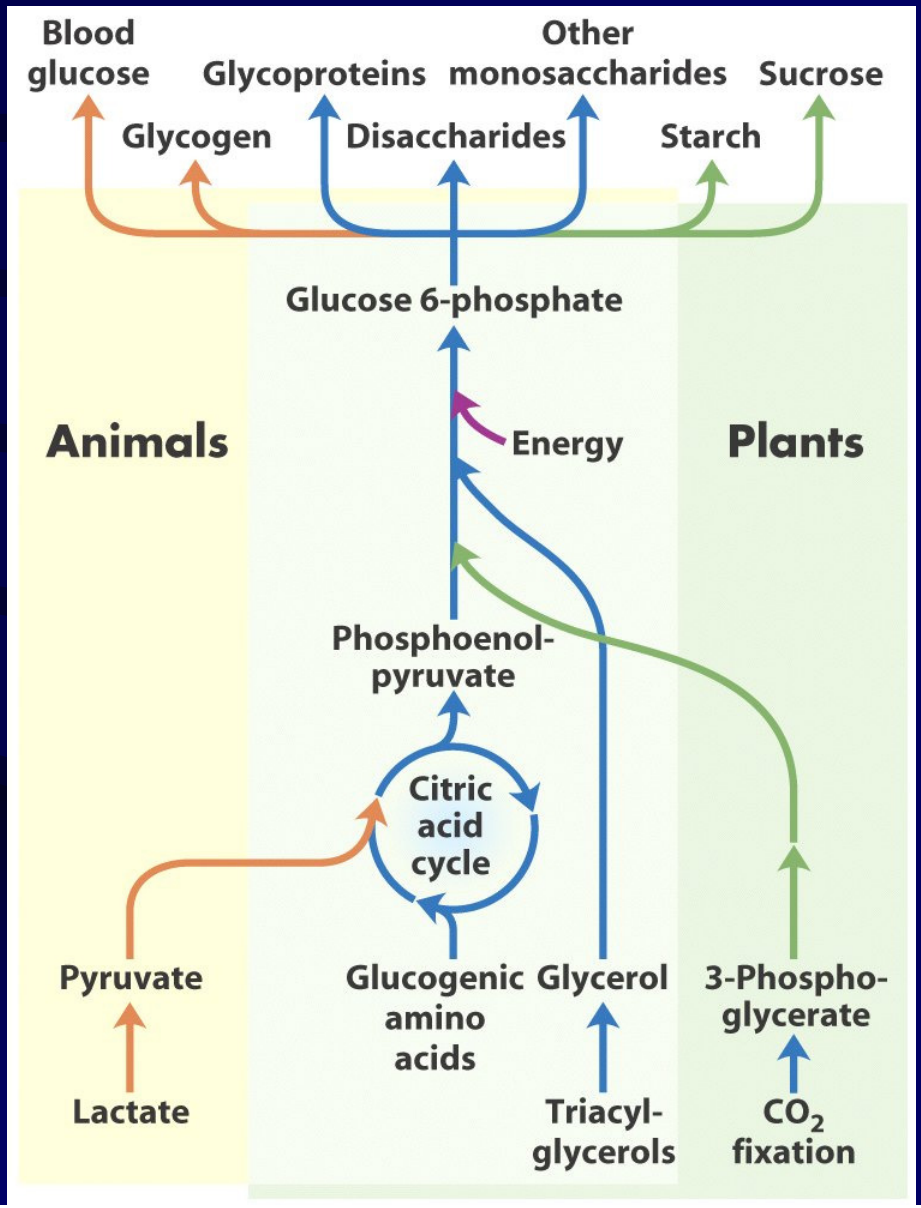
Material de estudo: diapositivos das aulas e bibliografia recomendada.

Sara Monteiro


(a)
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to the 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
1 Hexokinase
2 Phosphohexose isomerase
3 Phospho-fructokinase-1
4 Aldolase
5 Triose phosphate isomerase


Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
5 Triose phosphate isomerase
(b)
Payoff phase
Glyceraldehyde 3-phosphate (2)
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
oxidation and phosphorylation
6
2Pi
2NAD+
2 NADH + H+
6 Glyceraldehyde 3-phosphate dehydrogenase
1,3-Bisphosphoglycerate (2)
first ATP-forming reaction (substrate-level phosphorylation)
7
2 ADP
2 ATP
7 Phospho-glycerate kinase
3-Phosphoglycerate (2)
8
8 Phospho-glycerate mutase
2-Phosphoglycerate (2)
9
2H2O
9 Enolase
Phosphoenolpyruvate (2)
second ATP-forming reaction (substrate-level phosphorylation)
10
2ADP
2 ATP
10 Pyruvate kinase
Pyruvate (2)

Gluconeogénese



Gluconeogenesis

Gluconeogenesis is the process of converting noncarbohydrate precursors to glucose or glycogen.

Some tissues, such as:
- the brain
- red blood cells,
- kidney medulla,
- lens and cornea of the eye,
- testes, and
- exercising muscle,

require a continuous supply of glucose as a metabolic fuel.

Liver glycogen, an essential postprandial source of glucose, can meet these needs for only ten to eighteen hours in the fasting state

During a prolonged fast, hepatic glycogen stores are depleted, and glucose is formed from precursors such as:

- lactate
- pyruvate
- glycerol
- glucogenic amino acids

The formation of glucose does not occur by a simple reversal of glycolysis, because the overall equilibrium of glycolysis strongly favors pyruvate formation. Instead, glucose is synthesized by a special pathway, gluconeogenesis.

- Failure of gluconeogenesis is usually fatal. **Hypoglycemia** causes brain dysfunction, which can lead to coma and death.
- Glucose is also important in maintaining the level of intermediates of the citric acid cycle even when fatty acids are the main source of acetyl CoA in the tissues.

- In addition, gluconeogenesis clears lactate produced by muscle and erythrocytes and glycerol produced by adipose tissue.

II. SUBSTRATES FOR GLUCONEOGENESIS

Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose.

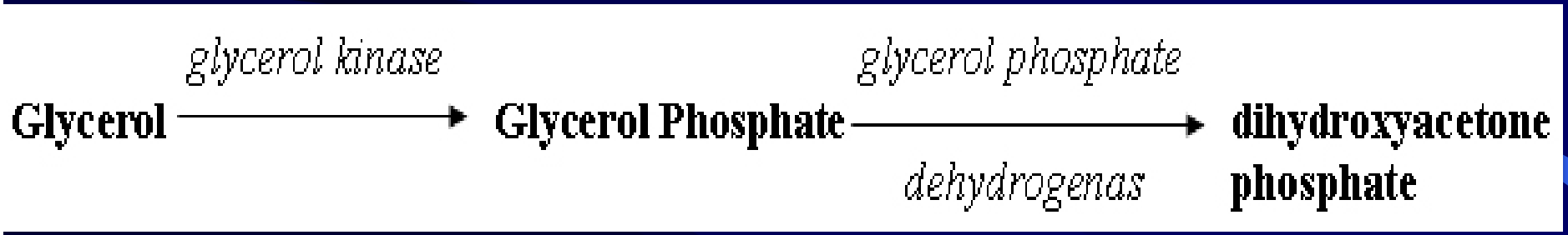
They include all the intermediates of glycolysis and the citric acid cycle. Glycerol, lactate, and the α-keto acids obtained from the deamination of glucogenic amino acids are the most important gluconeogenic precursors.

A. Glycerol

Glycerol is released during the hydrolysis of triacylglycerols in adipose tissue, and is delivered by the blood to the liver.
Glycerol is phosphorylated by glycerol kinase to **glycerol phosphate**, which is oxidized by

glycerol phosphate dehydrogenase to dihydroxyacetone phosphate - an intermediate of glycolysis.

[Note: Adipocytes cannot phosphorylate glycerol because they lack glycerol kinase.]



B. Lactate

Lactate is released into the blood by:
exercising skeletal muscle,
cells that lack mitochondria, such as RBCs
In the **Cori cycle**, blood-borne glucose is converted by exercising muscle to lactate, which diffuses into the blood. This lactate is taken up by the liver and reconverted to glucose, which is released back into the circulation.

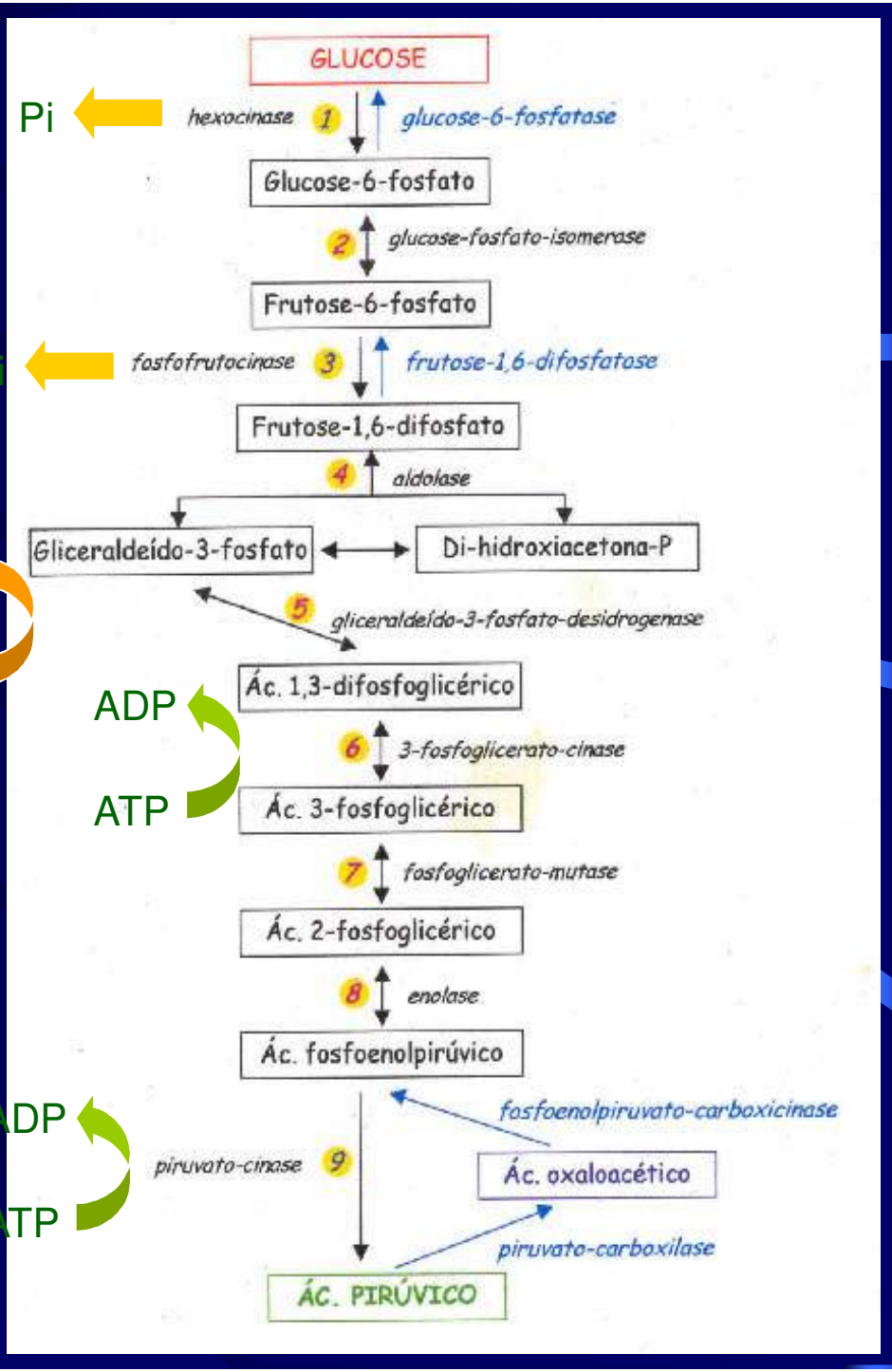
C. Amino acids

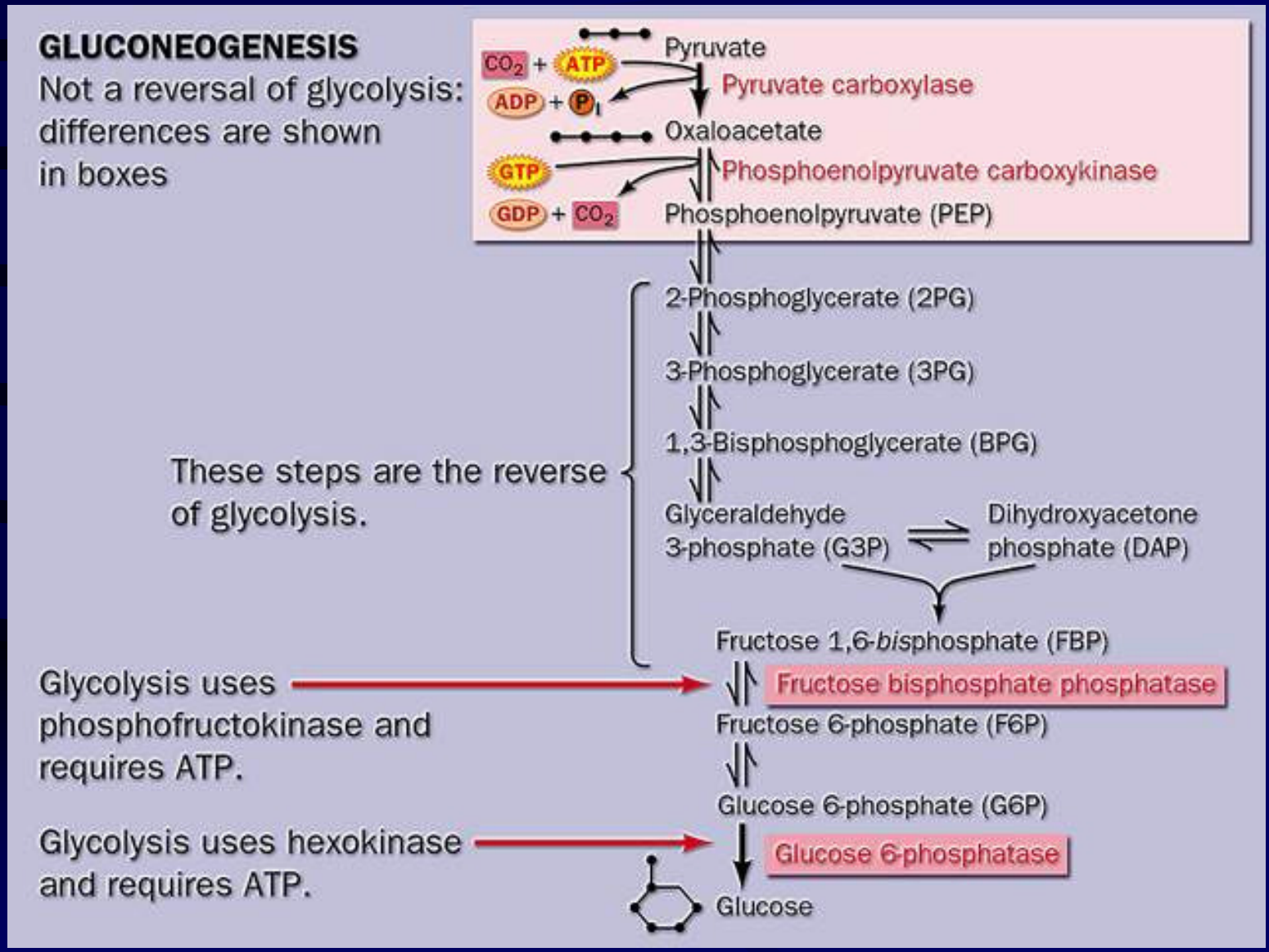
Amino acids derived from hydrolysis of tissue proteins are the major sources of glucose during a fast. α-Ketoacids, such as oxaloacetate and α-ketoglutarate, are derived from the metabolism of glucogenic amino acids. These substances can enter the citric acid cycle and form oxaloacetate - a direct precursor of phosphoenolpyruvate.

[Note: Acetyl CoA does not participate in synthesis of glucose. This is due to the irreversible nature of the pyruvate dehydrogenase reaction, which converts pyruvate to acetyl CoA.]

Gluconeogénese

Via que funciona no sentido inverso da glicólise:




GLUCONEOGENESIS
Not a reversal of glycolysis: differences are shown in boxes
CO_2 + ATP
ADP + P_i
Pyruvate
Pyruvate carboxylase
Oxaloacetate
GTP
GDP + CO_2
Phosphoenolpyruvate carboxykinase
Phosphoenolpyruvate (PEP)
2-Phosphoglycerate (2PG)
3-Phosphoglycerate (3PG)
1,3-Bisphosphoglycerate (BPG)
These steps are the reverse of glycolysis.
Glyceraldehyde 3-phosphate (G3P)
Dihydroxyacetone phosphate (DAP)
Fructose 1,6-*bis*phosphate (FBP)
Glycolysis uses phosphofructokinase and requires ATP.
Fructose bisphosphate phosphatase
Fructose 6-phosphate (F6P)
Glucose 6-phosphate (G6P)
Glycolysis uses hexokinase and requires ATP.
Glucose 6-phosphatase
Glucose

Gluconeogenesis occurs mainly in **liver**.

Gluconeogenesis occurs to a more limited extent in kidney & small intestine under some conditions.

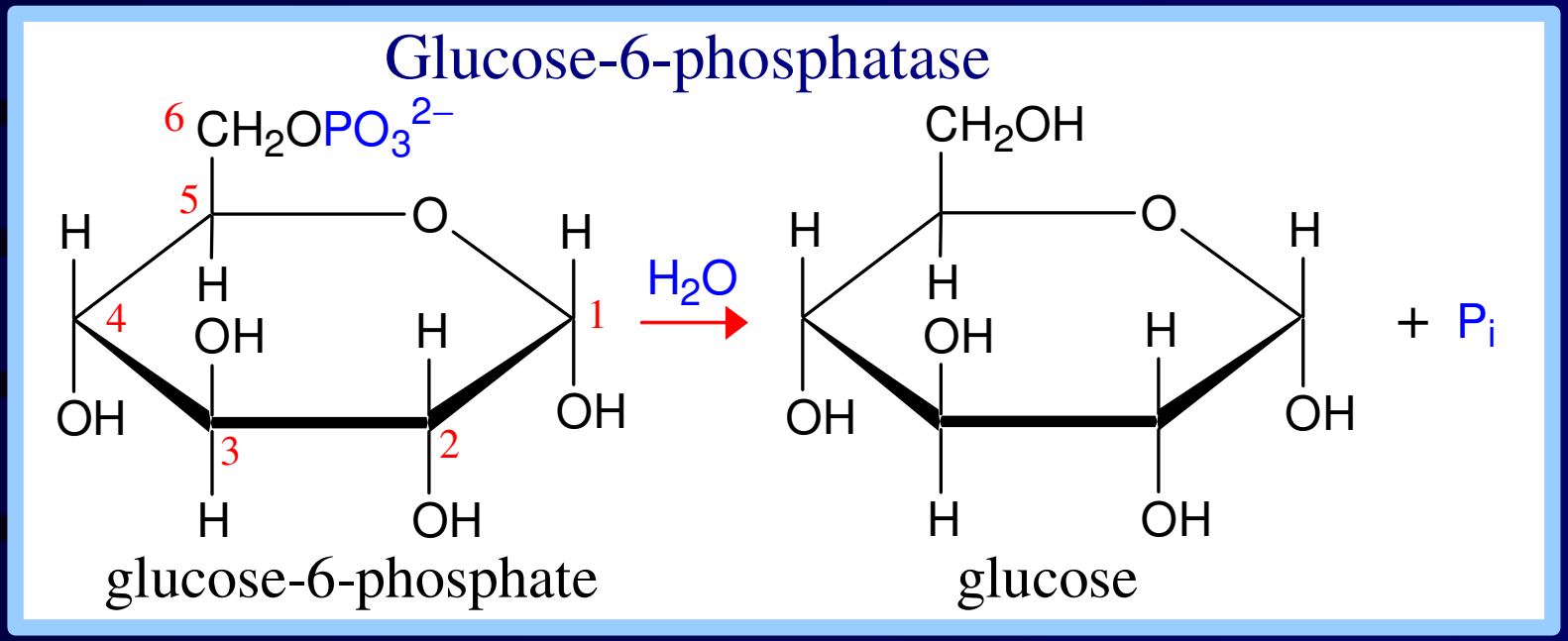
Synthesis of glucose from pyruvate utilizes many of the same enzymes as **Glycolysis**.

Three Glycolysis reactions have such a large negative ΔG that they are essentially **irreversible**.

- **Hexokinase** (or Glucokinase)
- **Phosphofructokinase**
- **Pyruvate Kinase.**

These steps must be **bypassed** in Gluconeogenesis.

Two of the bypass reactions involve simple **hydrolysis** reactions.

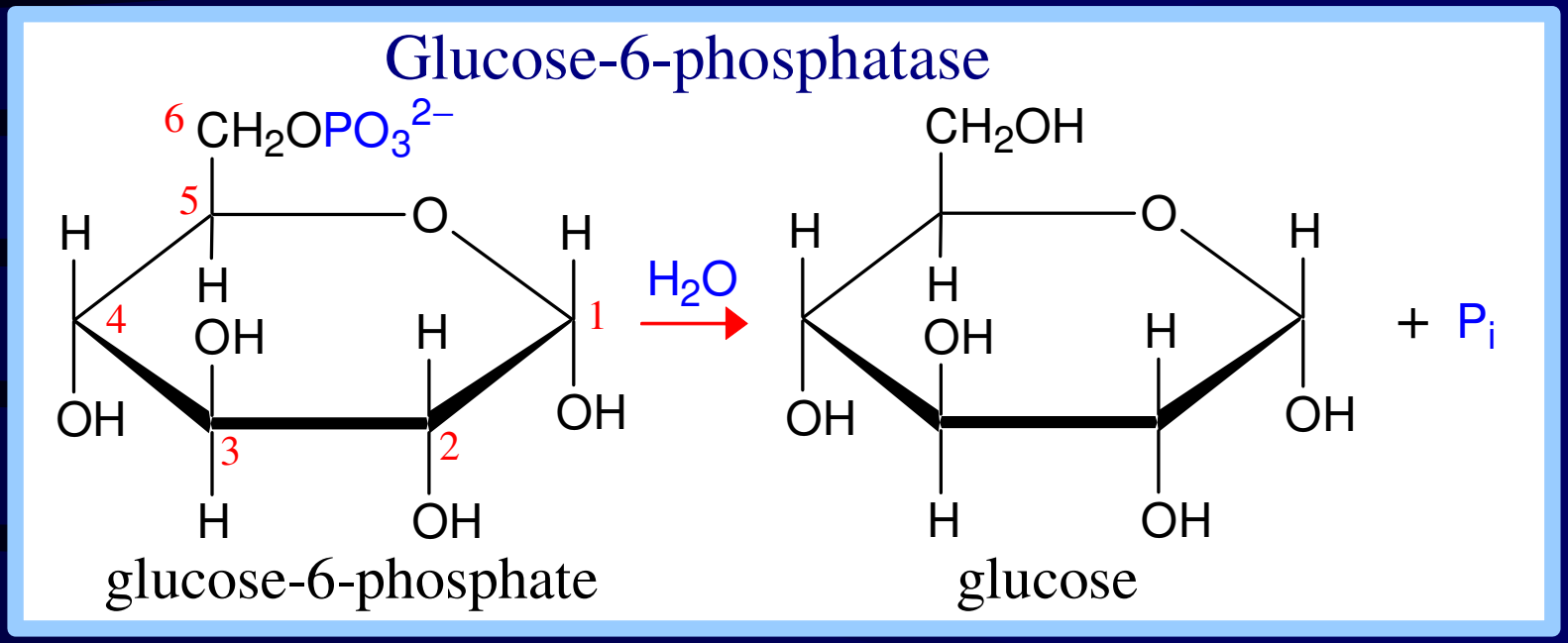


Hexokinase or Glucokinase (Glycolysis) catalyzes:

glucose + ATP → glucose-6-phosphate + ADP

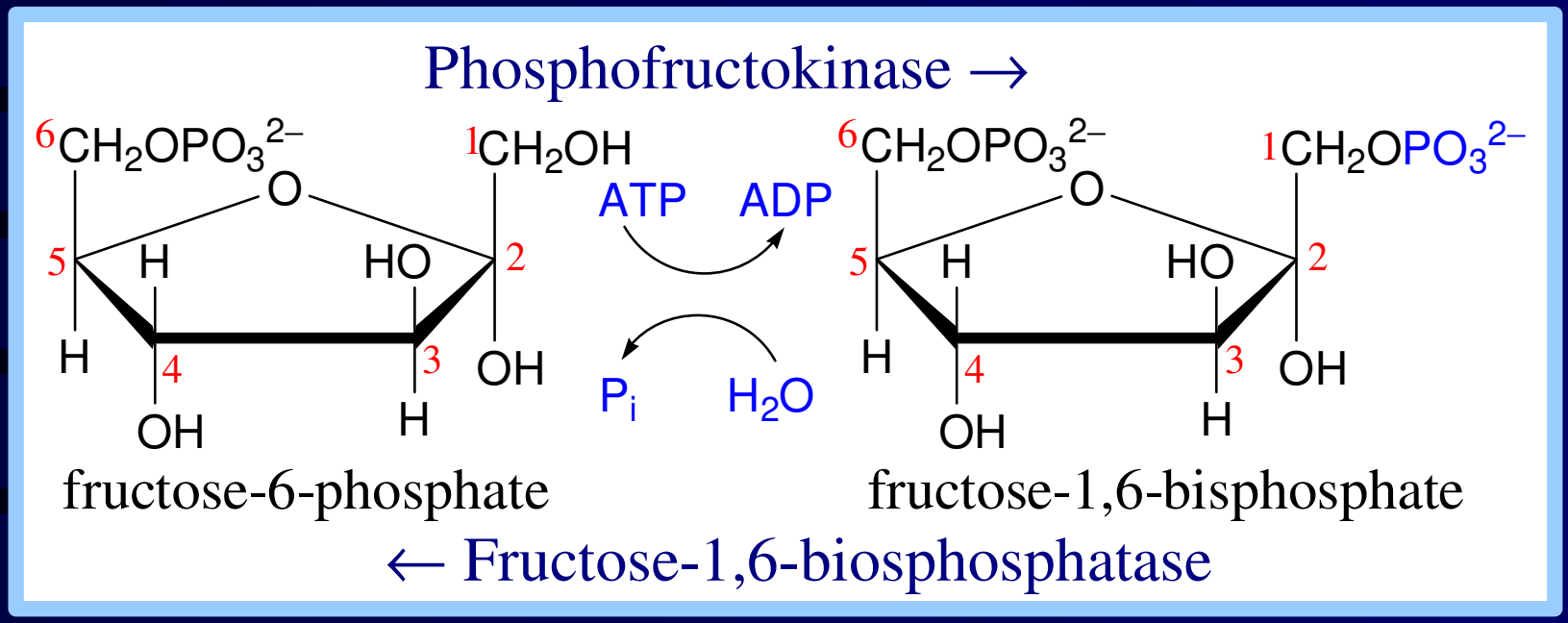
Glucose-6-Phosphatase (Gluconeogenesis) catalyzes:

glucose-6-phosphate + H_2O → glucose + P_i



Glucose-6-phosphatase enzyme is embedded in the endoplasmic reticulum (ER) membrane in liver cells.

The catalytic site is found to be exposed to the ER lumen. Another subunit may function as a translocase, providing access of substrate to the active site.



Phosphofructokinase (Glycolysis) catalyzes:

fructose-6-P + ATP → fructose-1,6-bisP + ADP

Fructose-1,6-bisphosphatase (Gluconeogenesis) catalyzes:

fructose-1,6-bisP + H_2O → fructose-6-P + P_i

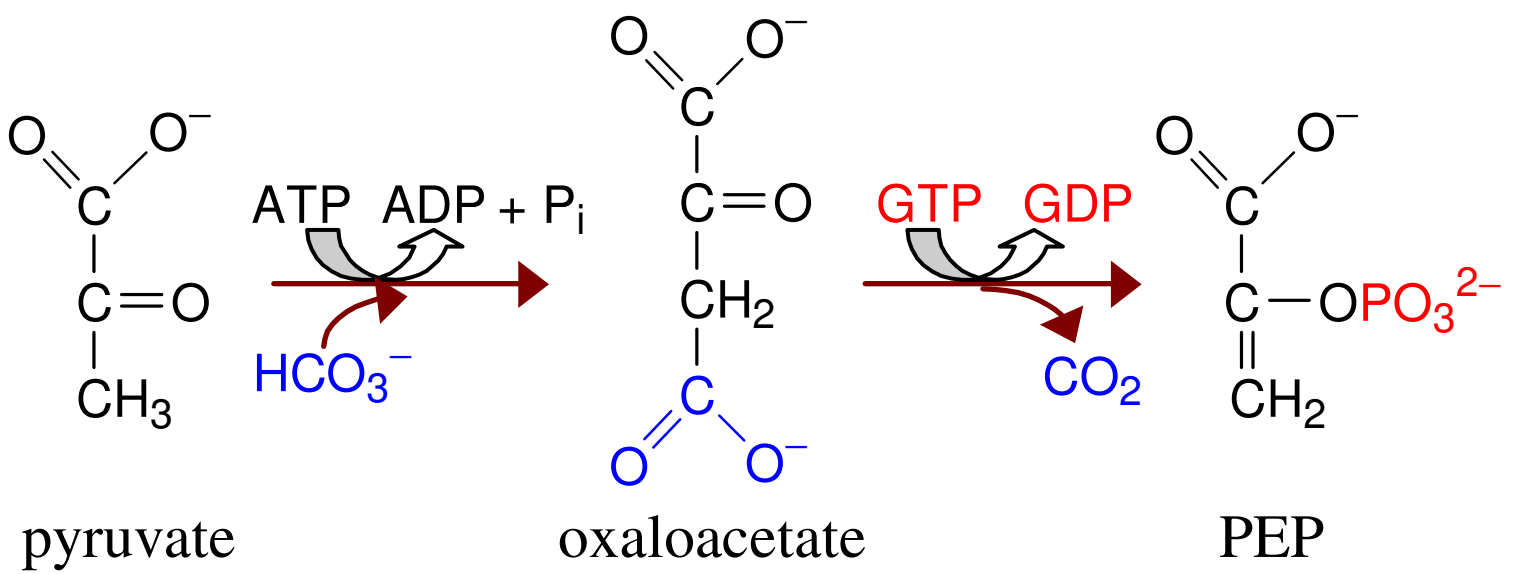
Bypass of Pyruvate Kinase:

Pyruvate Kinase (last step of Glycolysis) catalyzes:

phosphoenolpyruvate + ADP → pyruvate + ATP

For bypass of the Pyruvate Kinase reaction, cleavage of **2 ~P** bonds is required.

- ΔG for cleavage of one ~P bond of ATP is insufficient to drive synthesis of phosphoenolpyruvate (PEP).
- PEP has a higher negative ΔG of phosphate hydrolysis than ATP.



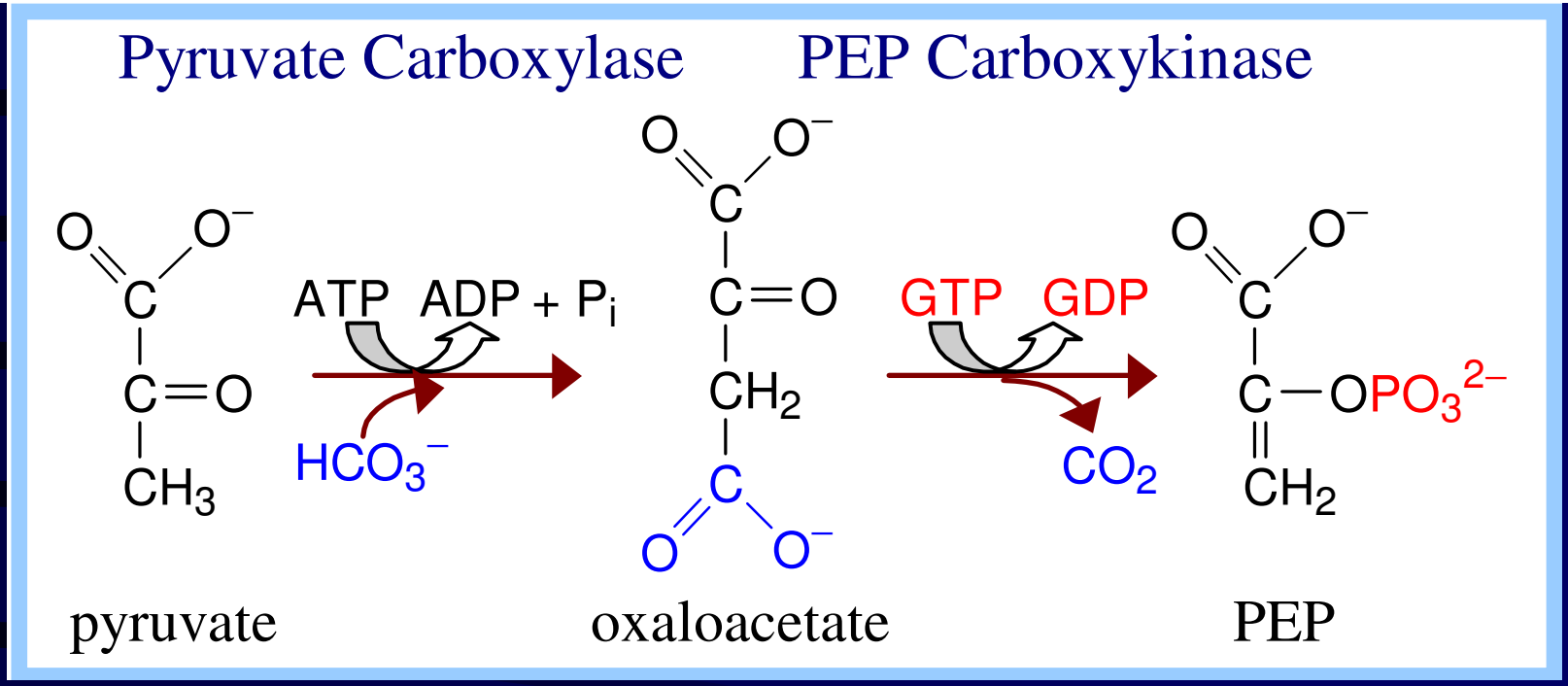
Bypass of Pyruvate Kinase (2 enzymes):

Pyruvate Carboxylase (Gluconeogenesis) catalyzes:

pyruvate + HCO_3^- + ATP → oxaloacetate + ADP + P_i

PEP Carboxykinase (Gluconeogenesis) catalyzes:

oxaloacetate + GTP → PEP + GDP + CO_2



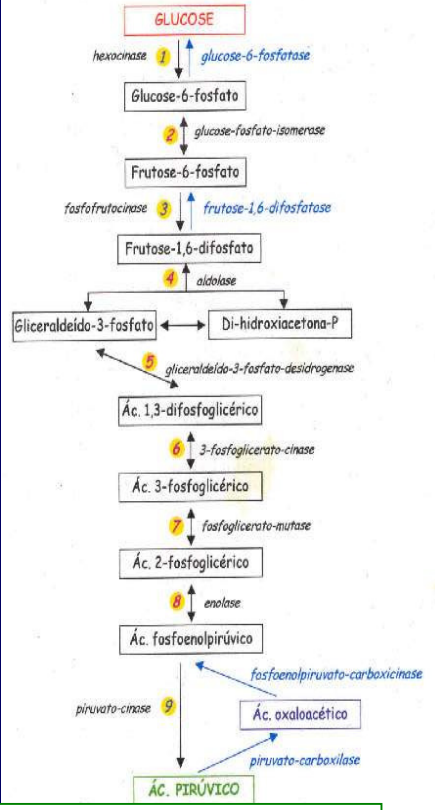
Contributing to spontaneity of the 2-step process:

Free energy of one **~P** bond of **ATP** is conserved in the carboxylation reaction.

Spontaneous decarboxylation contributes to spontaneity of the 2nd reaction.

Cleavage of a second **~P** bond of **GTP** also contributes to driving synthesis of PEP.

1ª reacção da gluconeogénese:



➢ Reacção global:

ác. pirúvico + ATP + GTP ⟶ ác. fosfoenolpirúvico + ADP + GDP + Pi

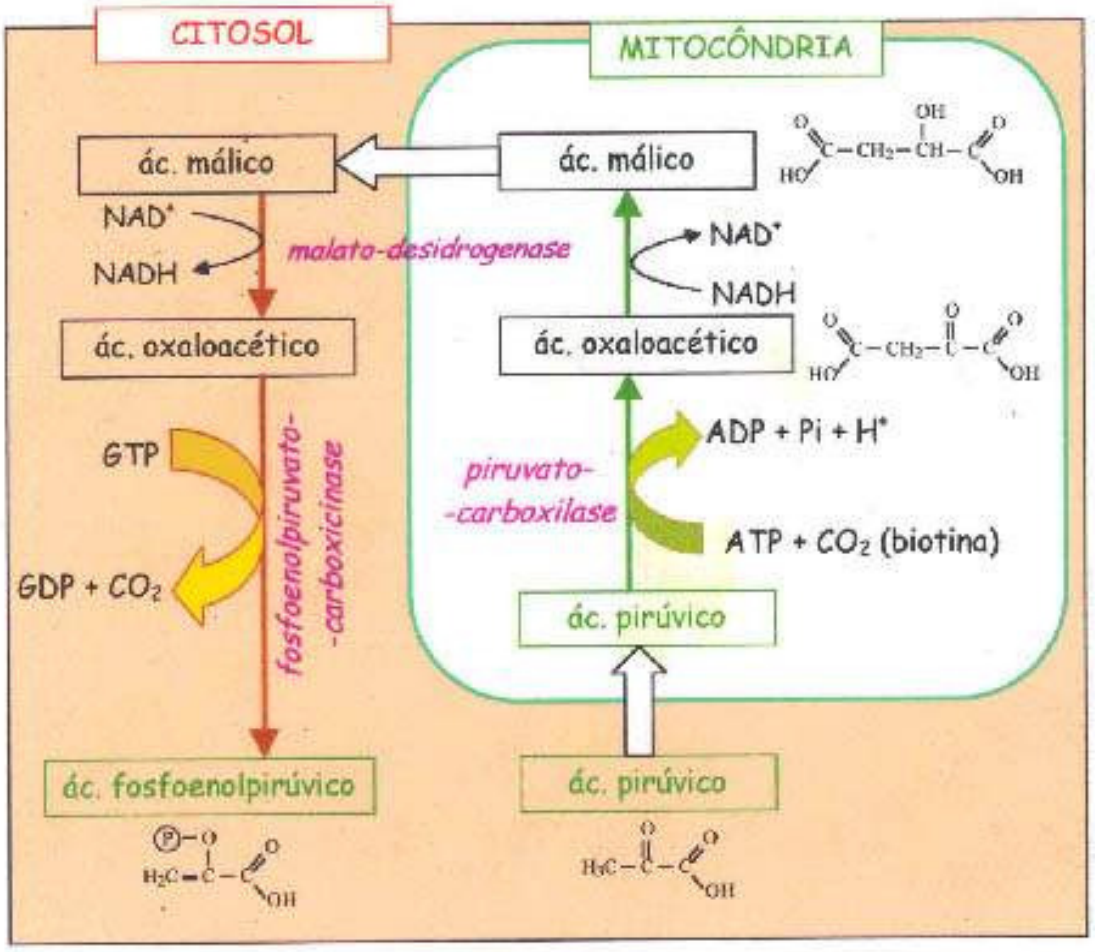
Na 1ª reacção da gluconeogénese ocorre sempre uma passagem pela mitocôndria

- ocorre na Mitocôndria e no citosol
- ocorre só na mitocôndria

1ª reacção da gluconeogénese:

- via que ocorre na mitocondria e citosol:

há transferência de NADH mitocondrial para o citosol

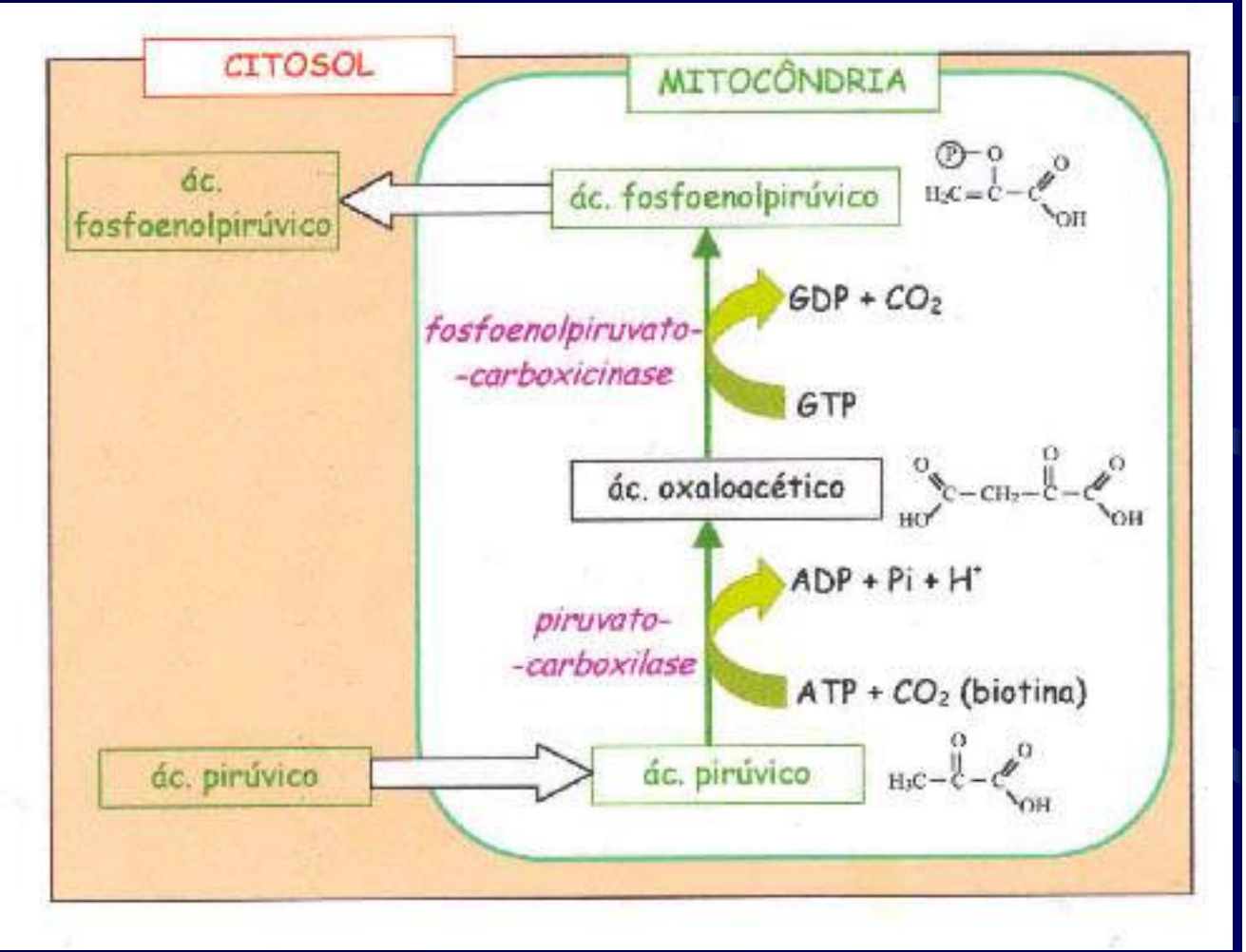


- piruvato-carboxilase: só existe na mitocôndria
- fosfoenolpiruvato-carboxicinase: existe na mitocôndria e no citosol
- esta via serve para "transferir" NADH para o citosol (que vai ser necessário mais à frente na Neoglucogénese).

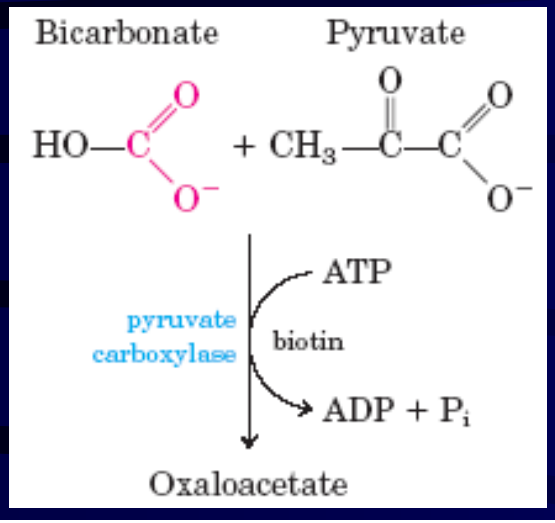
1ª reacção da Gluconeogénese:

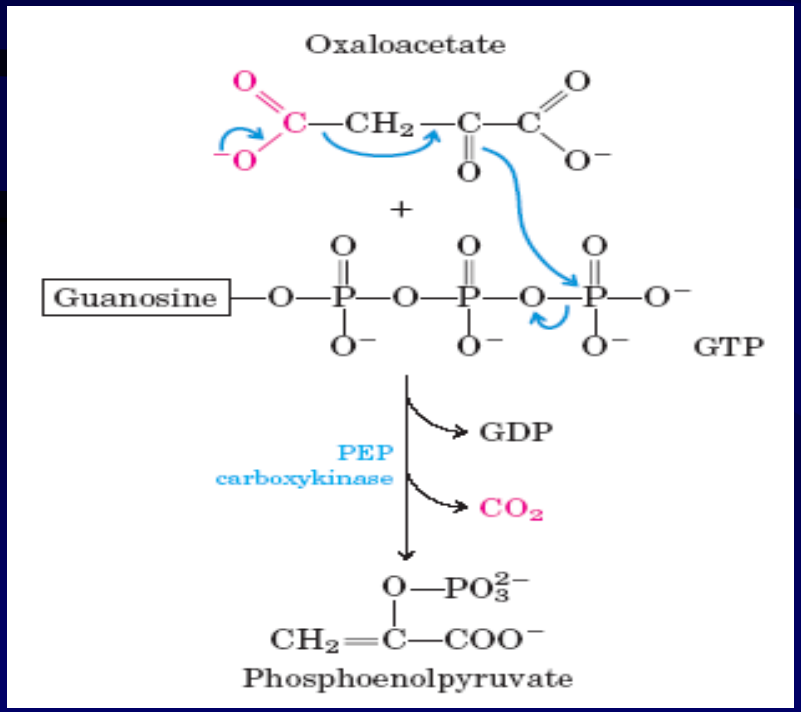
- via que ocorre só no mitocôndrio:

- não há transferência de NADH mitocondrial para o citosol

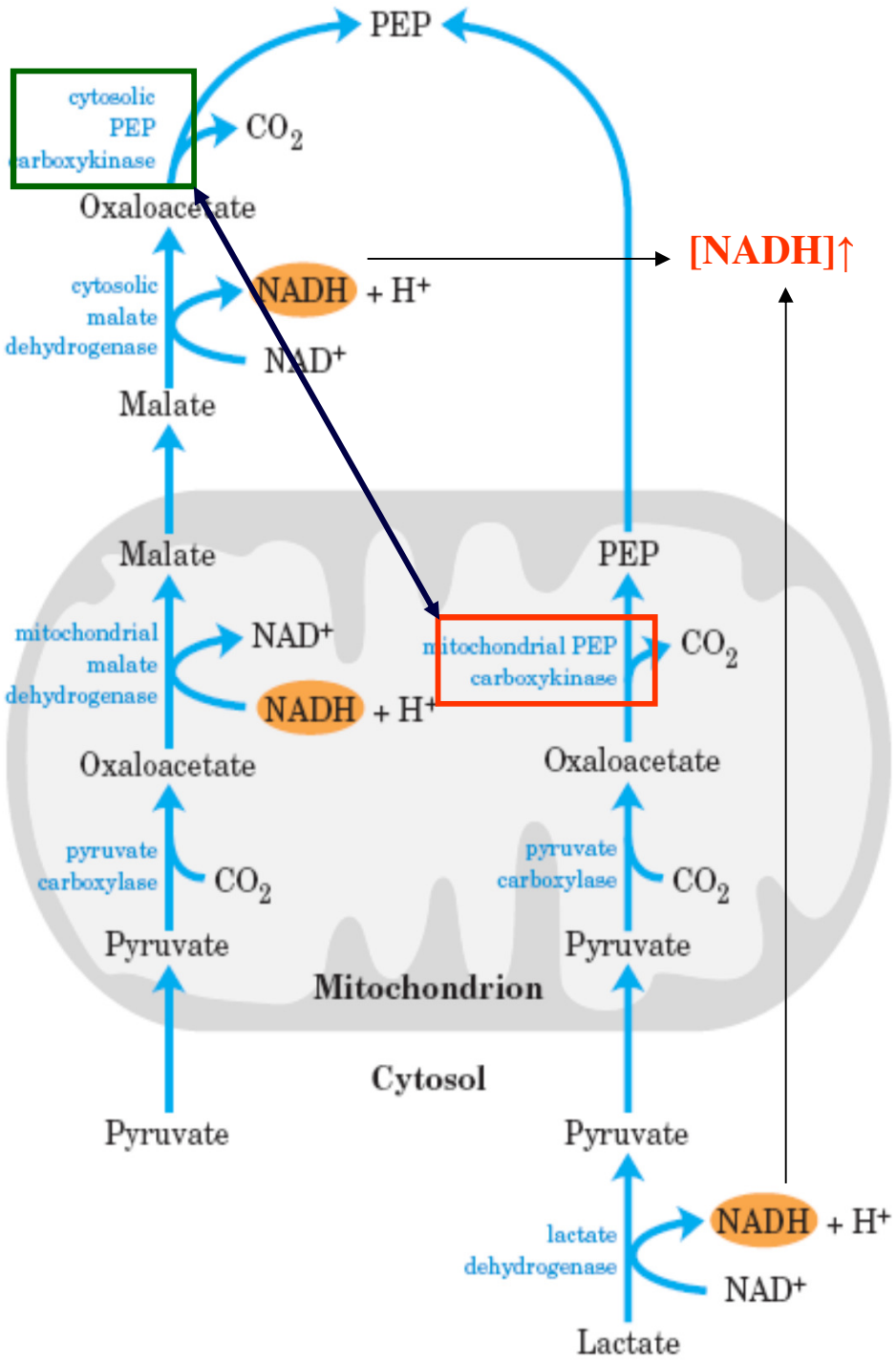


First bypass: Phosphorylation of pyruvate to PEP in two routes in cytosol and mitochondria





- the predominant path (use pyruvate or alanine) -Pyruvate is transported from the cytosol into mitochondria or is generated from alanine within mitochondria by transamination, (the -amino group is removed from alanine and leaving pyruvate), then **Pyruvate carboxylase** (coenzyme **biotin**) converts the pyruvate to oxaloacetate (OAA).
- the mitochondrial membrane has no OAA transporter, OAA is reduced to malate by mitochondrial **malate dehydrogenase,** Malate leaves the mitochondrion through a malate transporter (inner mito mm.) and is reoxidized to OAA.
- In the cytosol, OAA is converted to PEP by PEP carboxykinase.
- 2 ATPs are required for (pyruvate to PEP).
- CO_2 added to pyruvate in the pyruvate carboxylase step is the same molecule that is lost in the PEP carboxykinase reaction (activation)
- second pathway, (lactate) as precursor.



Alternative paths from pyruvate to PEP

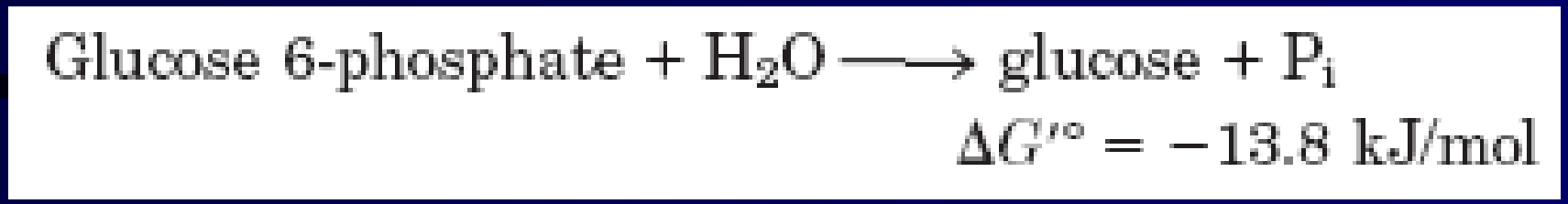
- Conversion of lactate to pyruvate in the cytosol of hepatocytes yields NADH - no need to export reducing equivalents.
- Pyruvate in mitochondria is converted to OAA - converted directly to PEP by a mitochondrial isozyme of PEP carboxykinase, and the PEP is transported out of the mitochondrion to continue on the gluconeogenic path.
- The mitochondrial and cytosolic isozymes of PEP carboxykinase are encoded by separate genes in the nuclear chromosomes, providing another example of two distinct enzymes catalyzing the same reaction but having different cellular locations or metabolic roles (i.e. isozymes of hexokinase).

Second Bypass: Conversion of Fructose 1,6 - BisP to Fructose 6P

$$\text{Fructose 1,6-bisphosphate} + H_2O \longrightarrow \text{fructose 6-phosphate} + P_i$$
$$\Delta G'^{\circ} = -16.3 \text{ kJ/mol}$$

- the generation of fructose 6-phosphate from fructose 1,6-bisphosphate is catalyzed Mg^{2+} -dependent **fructose 1,6- bisphosphatase (FBPase-1),** which promotes the *hydrolysis* of the C-1 phosphate.

Third Bypass: Conversion of Glucose 6P to Glucose

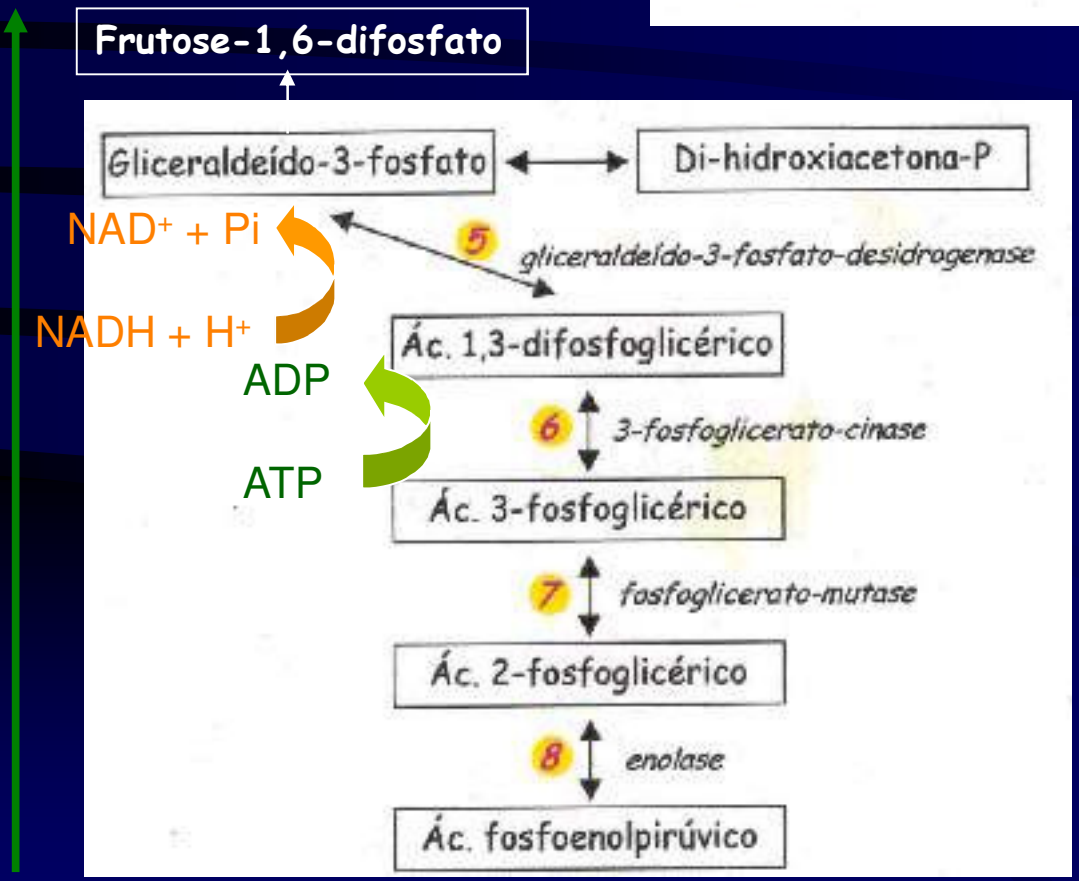


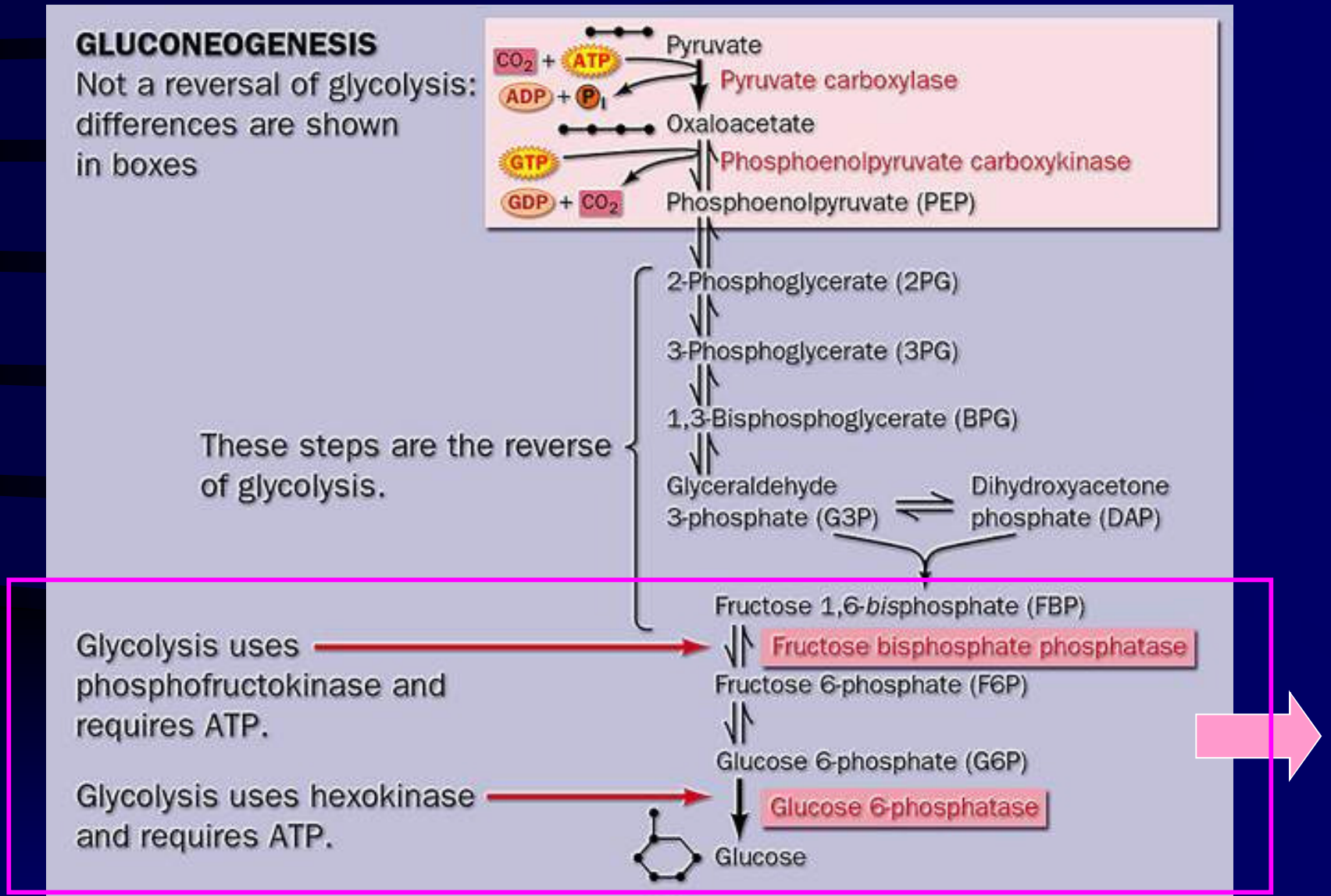
- catalyzed by **glucose 6-phosphatase –** Mg^{2+} -activated enzyme is found on the lumenal side of the endoplasmic reticulum (ER) of hepatocytes and renal cells
- Muscle and brain tissue do not contain **glucose 6-phosphatase** and so cannot carry out gluconeogenesis.
- Glucose produced by gluconeogenesis in the liver or kidney or ingested in the diet is delivered to brain and muscle through the bloodstream.

Reacções seguintes da gluconeogénese:

- Do ác. fosfoenolpirúvico à frutose-1,6-difosfato:

ác. fosfoenolpirúvico ⟷ ác. 2-fosfoglicérico
ác. 2-fosfoglicérico ⟷ ác. 3-fosfoglicérico
ác. 3-fosfoglicérico + ATP ⟷ ác. 1,3-difosfoglicérico + ADP
ác. 1,3-difosfoglicérico + NADH ⟷ gliceraldeído-3-fosfato + + NAD^+
gliceraldeído-3-fosfato ⟷ frutose-1,6-difosfato




GLUCONEOGENESIS
Not a reversal of glycolysis: differences are shown in boxes
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CO_2 + ATP
Pyruvate carboxylase
ADP + P_i
Oxaloacetate
GTP
Phosphoenolpyruvate carboxykinase
GDP + CO_2
Phosphoenolpyruvate (PEP)
2-Phosphoglycerate (2PG)
3-Phosphoglycerate (3PG)
1,3-Bisphosphoglycerate (BPG)
These steps are the reverse of glycolysis.
Glyceraldehyde 3-phosphate (G3P)
Dihydroxyacetone phosphate (DAP)
Fructose 1,6-bisphosphate (FBP)
Glycolysis uses phosphofructokinase and requires ATP.
Fructose bisphosphate phosphatase
Fructose 6-phosphate (F6P)
Glucose 6-phosphate (G6P)
Glycolysis uses hexokinase and requires ATP.
Glucose 6-phosphatase
Glucose

Últimas reacções da gluconeogénese e formação da glucose:

Frutose-1,6-bisfosfato —(*Frutose-1,6-bifosfatase*)→ Frutose-6-fosfato + Pi

Frutose-6-fosfato —(*Glucose-fosfato-isomerase*)→ Glucose-6-fosfato

Glucose-6-fosfato —(*Glucose-6-fosfase*)→ Glucose

↓

Pode ser convertida posteriormente em glúcidos de reserva ou estruturais

Resumo da gluconeogénese:

-A maioria das reacções ocorre no sentido inverso às da glicólise, catalisadas pelas mesmas enzimas; em 3 reacções há diferenças:

Piruvato ⟷ Fosfoenolpiruvato

Gluconeogénese: piruvato-carboxilase e fosfoenolpiruvato-carboxicinase
Glicólise: piruvato-cinase

Frutose-1,6-bisfosfato ⟷ Frutose-6-fosfato

Gluconeogénese: frutose-1,6-difosfatase
Glicólise: fosfofrutocinase

Glucose-6-fosfato ⟷ Glucose

Neoglucogénese: glucose-6-fosfatase
Glicólise: hexocinase

Reacção global:

$$2\ \text{Piruvato} + 4\ \text{ATP} + 2\ \text{GTP} + 2\ \text{NADH} + 2\ H^+ + 6\ H_2O \leftrightarrow$$
$$\leftrightarrow \text{Glucose} + 4\ \text{ADP} + 2\ \text{GDP} + 2\ NAD^+ + 6\ \text{Pi}$$

Glycolysis & **Gluconeogenesis** are **both spontaneous**. If both pathways were simultaneously active in a cell, it would constitute a "**futile cycle**" that would waste energy.

Glycolysis:

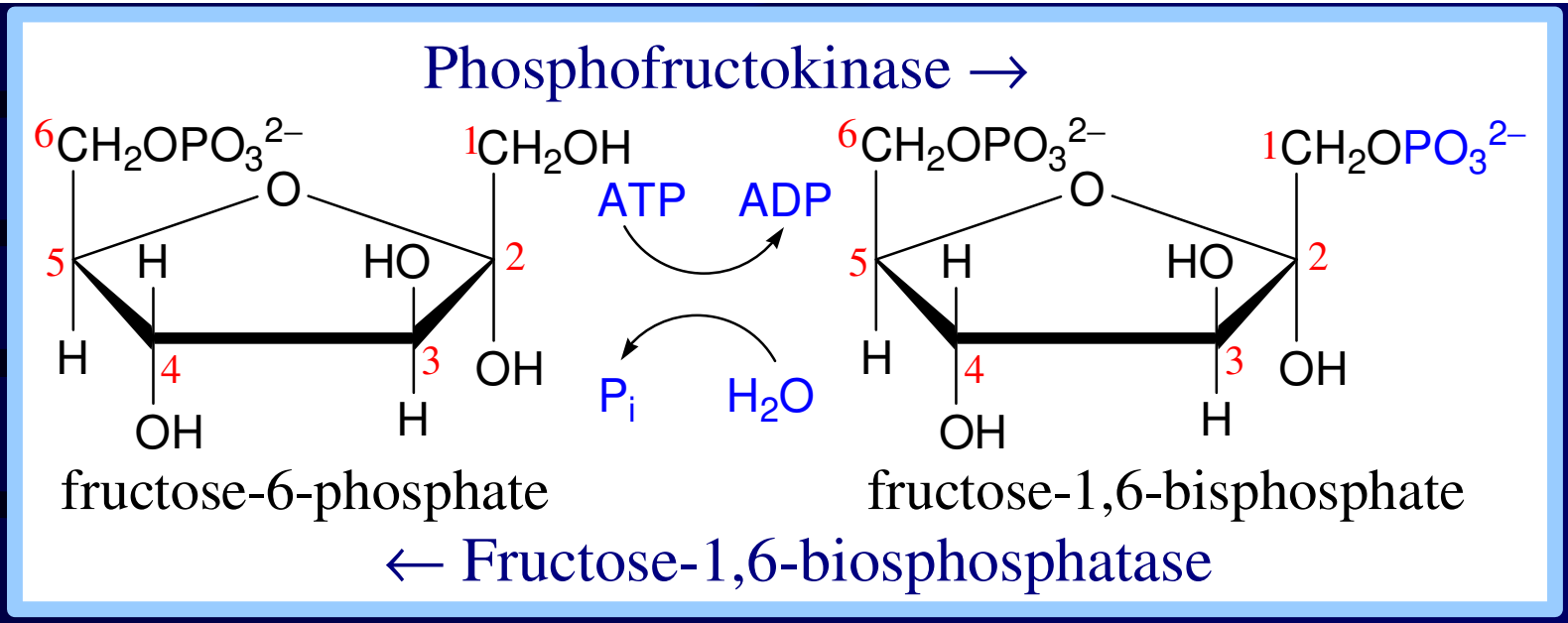
glucose + 2 NAD^+ + 2 ADP + 2 P_i →
2 pyruvate + 2 NADH + 2 ATP

Gluconeogenesis:

2 pyruvate + 2 NADH + 4 ATP + 2 GTP →
glucose + 2 NAD^+ + 4 ADP + 2 GDP + 6 P_i

Questions:

1. **Glycolysis** yields **how many ~P** ?
2. **Gluconeogenesis** expends **how many ~P** ?
3. A **futile cycle** of both pathways would waste **how many ~P per cycle** ?



To prevent the waste of a futile cycle, Glycolysis & Gluconeogenesis are **reciprocally regulated**.

Local Control includes reciprocal allosteric regulation by **adenine nucleotides**.

- **Phosphofructokinase** (Glycolysis) is inhibited by ATP and stimulated by AMP.
- **Fructose-1,6-bisphosphatase** (Gluconeogenesis) is inhibited by AMP.

The **opposite effects of adenine nucleotides** on

- **Phosphofructokinase** (Glycolysis)
- **Fructose-1,6-bisphosphatase** (Gluconeogenesis)

insures that when cellular ATP is high (AMP would then be low), glucose is not degraded to make ATP.

When ATP is high it is more useful to the cell to store glucose as glycogen.

When ATP is low (AMP would then be high), the cell does not expend energy in synthesizing glucose.

Global Control in **liver** cells includes reciprocal effects of a **cyclic AMP cascade**, triggered by the hormone glucagon when blood glucose is low.

Phosphorylation of enzymes & regulatory proteins in liver by Protein Kinase A (cAMP Dependent Protein Kinase) results in

- **inhibition of glycolysis**
- **stimulation of gluconeogenesis**,

making glucose available for release to the blood.

Enzymes relevant to these pathways that are **phosphorylated** by Protein Kinase A include:

- **Pyruvate Kinase**, a glycolysis enzyme that is **inhibited** when phosphorylated.
- **CREB** (cAMP response element binding protein) which activates, through other factors, transcription of the gene for **PEP Carboxykinase**, leading to **increased gluconeogenesis**.
- A **bi-functional enzyme** that makes and degrades an allosteric regulator, **fructose-2,6-bisphosphate**.

Reciprocal regulation by fructose-2,6-bisphosphate:

- **Fructose-2,6-bisphosphate stimulates Glycolysis**.

  Fructose-2,6-bisphosphate allosterically **activates** the Glycolysis enzyme **Phosphofructokinase**.

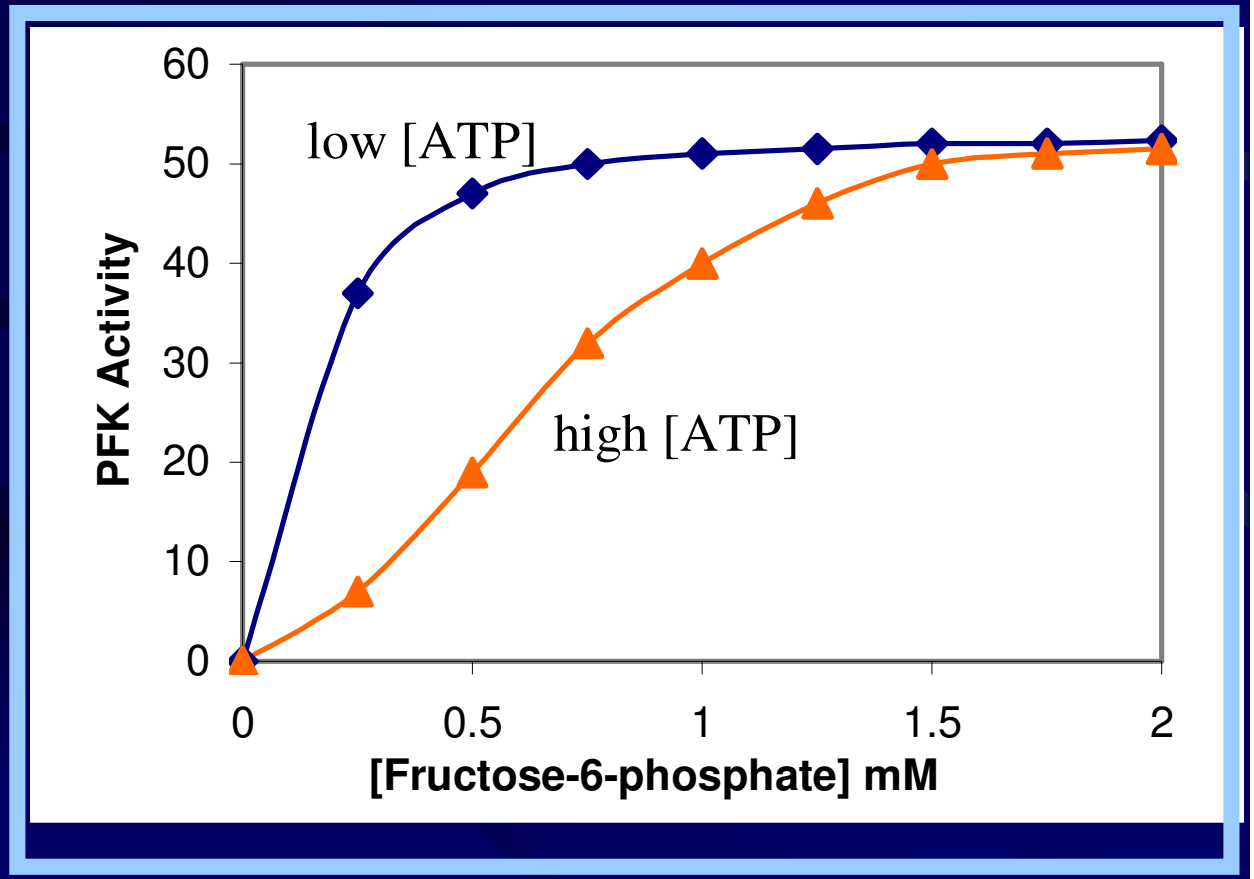
  Fructose-2,6-bisphosphate also **activates transcription** of the gene for **Glucokinase**, the liver variant of Hexokinase that phosphorylates glucose to glucose-6-phosphate, the input to Glycolysis.

- **Fructose-2,6-bisphosphate** allosterically **inhibits** the **gluconeogenesis** enzyme **Fructose-1,6-bisphosphatase**.

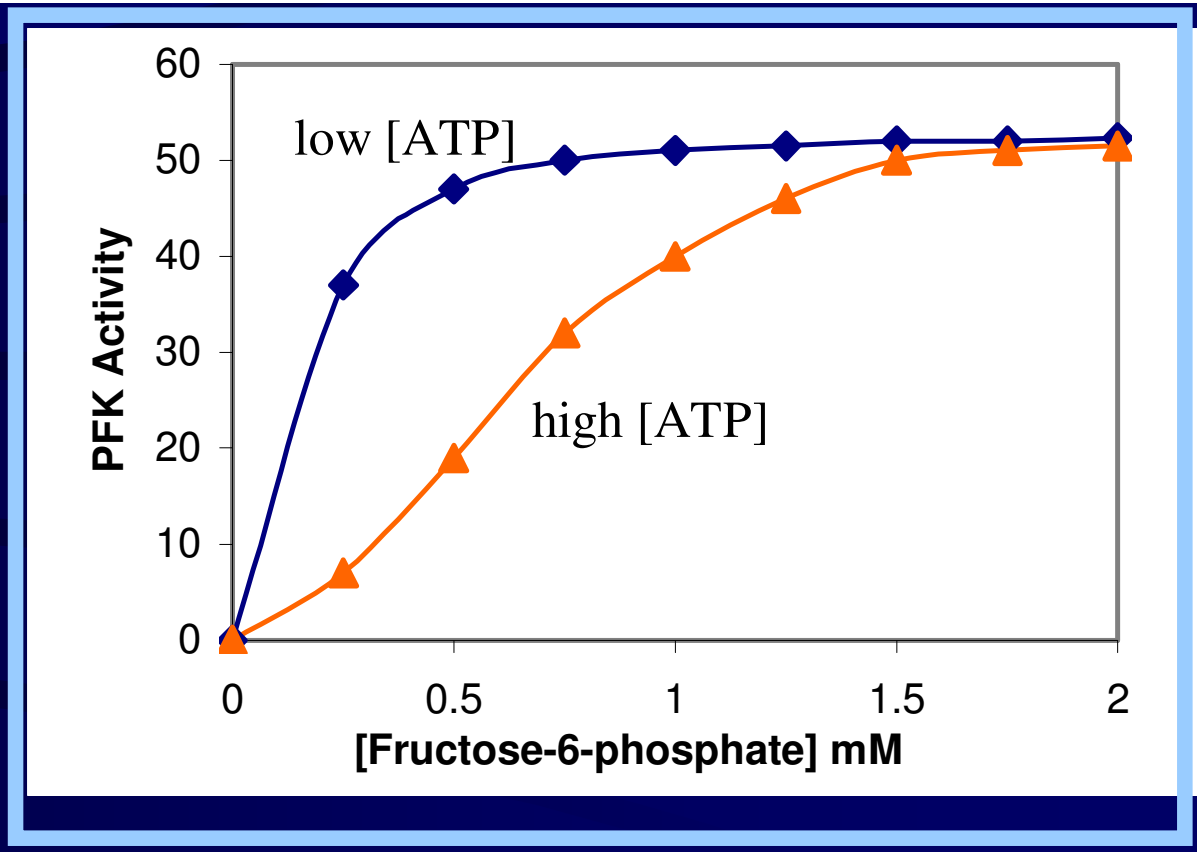
Recall that **Phosphofructokinase**, the rate-limiting step of Glycolysis, is **allosterically inhibited by ATP**.

At high concentration, ATP binds at a low-affinity **regulatory site**, promoting the tense conformation.

Sigmoidal dependence of reaction rate on [fructose-6-phosphate] is observed at high [ATP].



PFK activity in the presence of the **globally controlled** allosteric regulator **fructose-2,6-bisphosphate** is similar to that at low ATP.



Fructose-2,6-bisphosphate promotes the **relaxed** state, activating Phosphofructokinase even at high [ATP].

Thus **activation by fructose-2,6-bisphosphate**, whose concentration fluctuates in response to external hormonal signals, **supersedes local control** by [ATP].

Gluconeogenesis: Fact Sheet

- Occurs in liver and kidney
- Expensive but necessary
- 3 steps are NOT the reverse of gycolysis
  - Pyruvate → PEP (through carboxylation to oxaloacetate; then decarboxylation and phosphorylation (from GTP) to PEP)
  - F-1,6-BP → F-6-P
  - G-6-P → glucose
- Acetyl CoA is an positive effector
- Fatty acids CANNOT be converted to glucose (only to acetyl CoA)
- Glucogenic amino acids and other metabolites can be converted to glucose via gluconeogenesis

Regulação da neoglucogénese na célula (intracelular):

-Regulada pelas necessidades em glucose nas células, através dos níveis de ATP, ADP, AMP, etc., no interior da célula.

Destinos da glucose formada na neoglucogénese:

-Síntese de reservas glucídicas → amido nas plantas

→ glicogénio nos animais

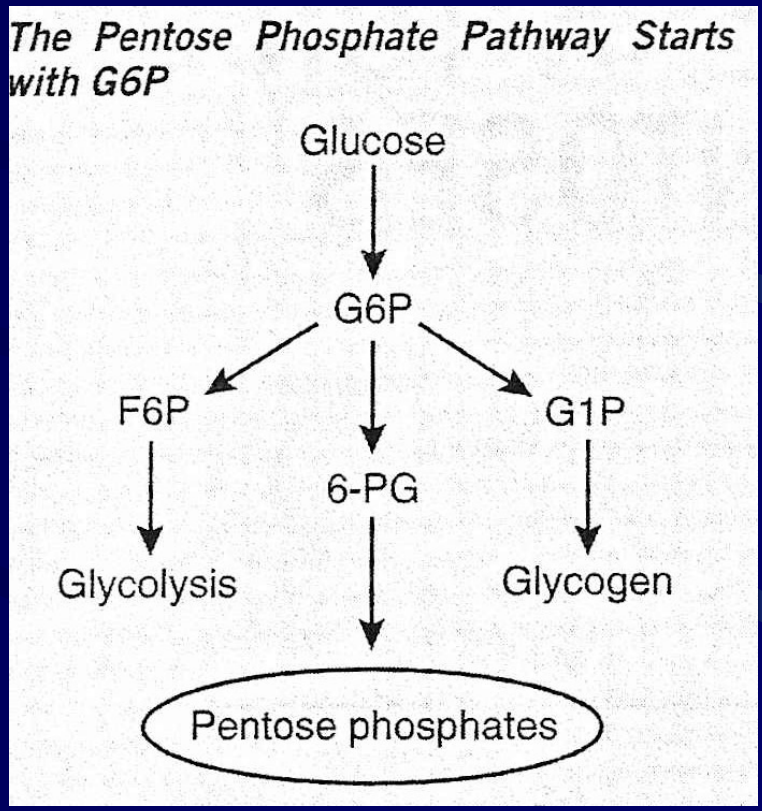
-Percursor de intermediários de outras vias metabólicas

↓

via dos fosfatos de pentose

Overview

- Function
  - NADPH production
    - Reducing power carrier
      - Synthetic pathways
    - Role as cellular antioxidants
  - Ribose synthesis
    - Nucleic acids and nucleotides

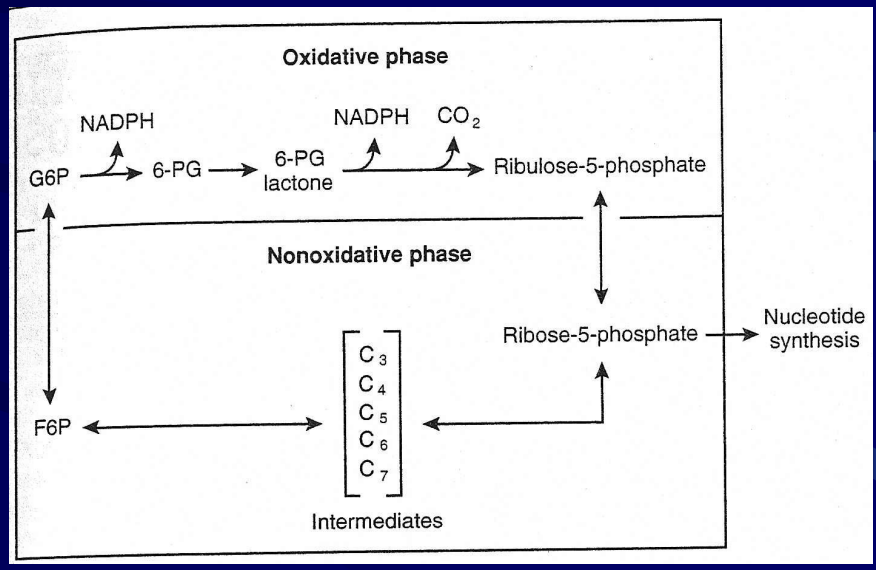


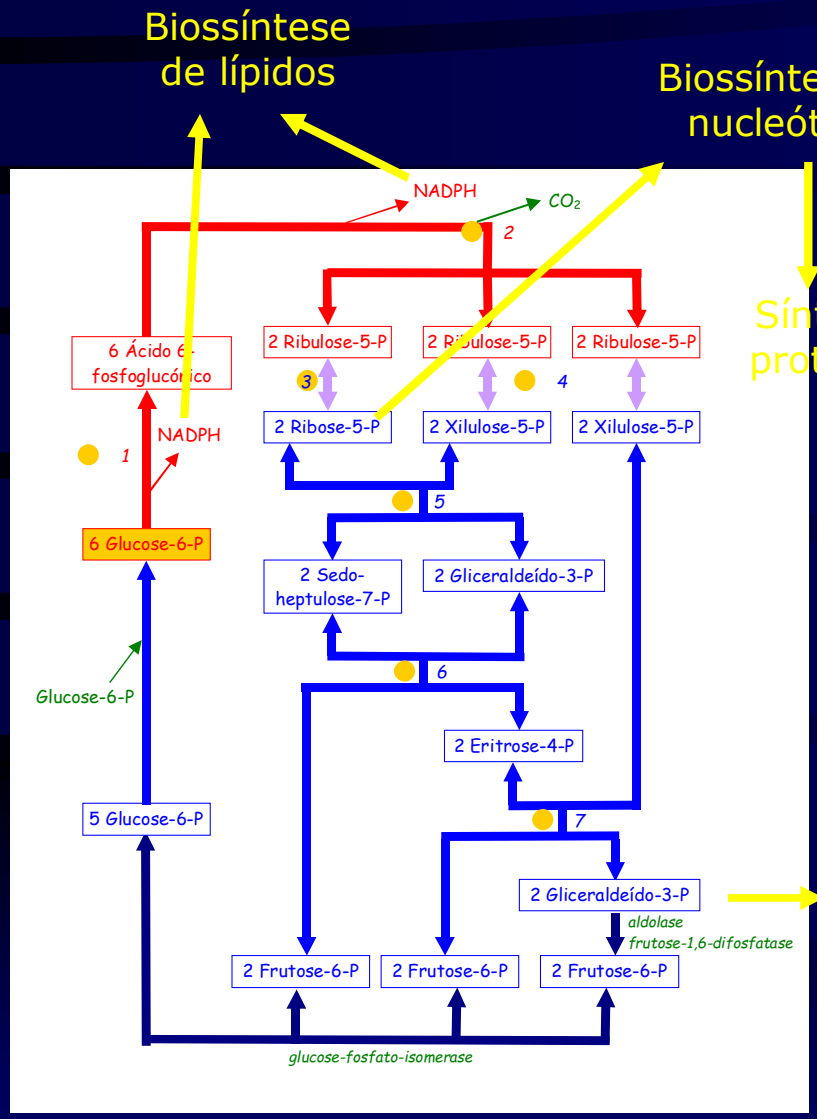
Characteristics: Tissue Distribution

- Demand for NADPH
  - Biosynthetic pathways
    - FA synthesis (liver, adipose, mammary)
    - Cholesterol synthesis (liver)
    - Steroid hormone synthesis (adrenal, ovaries, testes)
  - Detoxification (Cytochrome P-450 System) – liver
  - Reduced glutathione as an antioxidant (RBC)
  - Generation of superoxide (neutrophils)

Characteristics:
Oxidative and Non-oxidative Phases

- Oxidative phases
  - Reactions producing NADPH
  - Irreversible
- Non-oxidative phases
  - Produces ribose-5-P
  - Reversible reactions feed to glycolysis





A via dos fosfatos de pentose estabelece uma interligação entre o metabolismo dos glúcidos, proteico e lipídico

Glicólise, gluconeogénese e via dos fosfatos de pentose permitem ajustar às necessidades celulares, os teores de NADPH, ATP, ribose-5-P, ácido pirúvico, glucose.

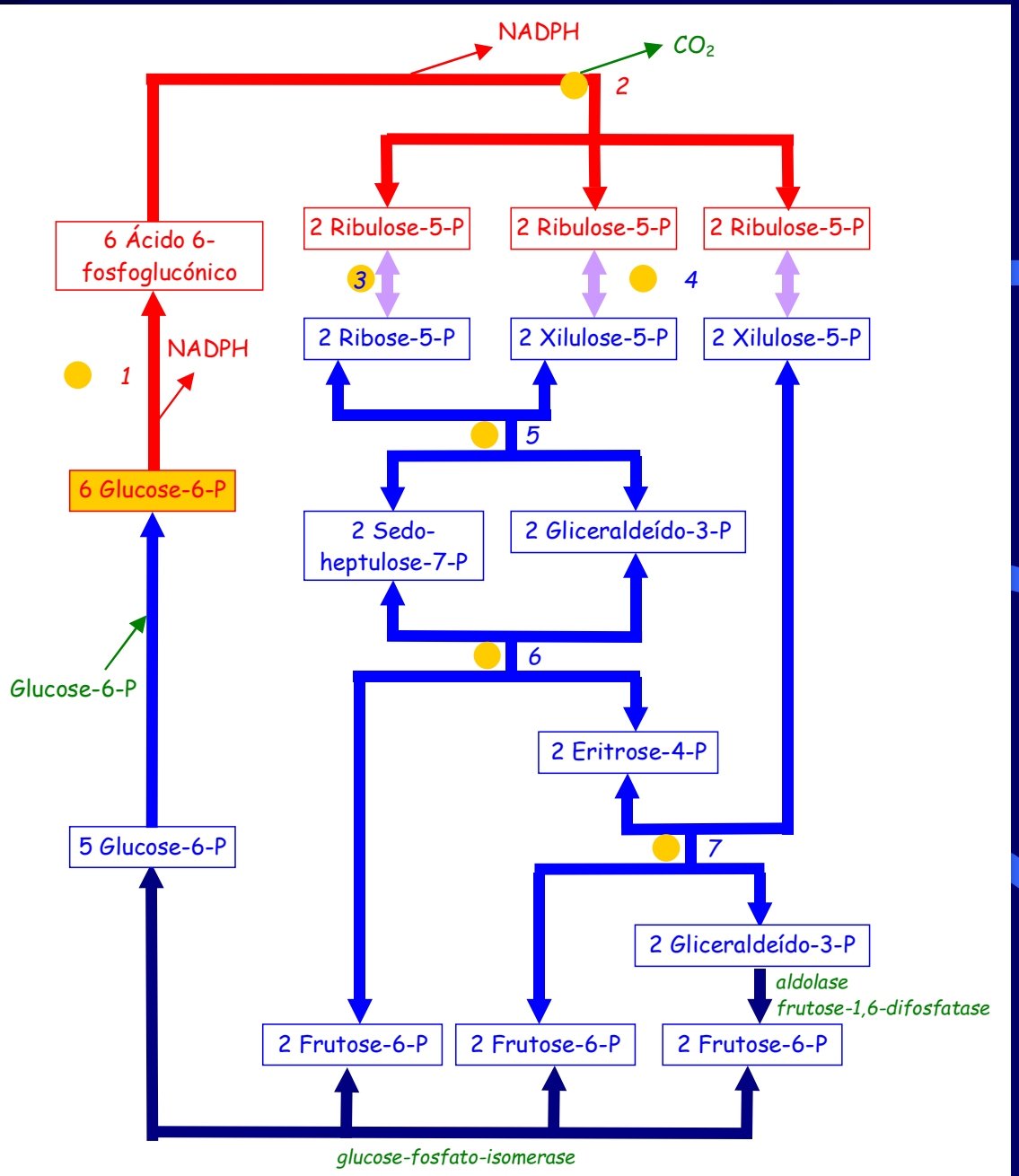
Esquema geral da via dos fosfatos de pentose:

Fase oxidante

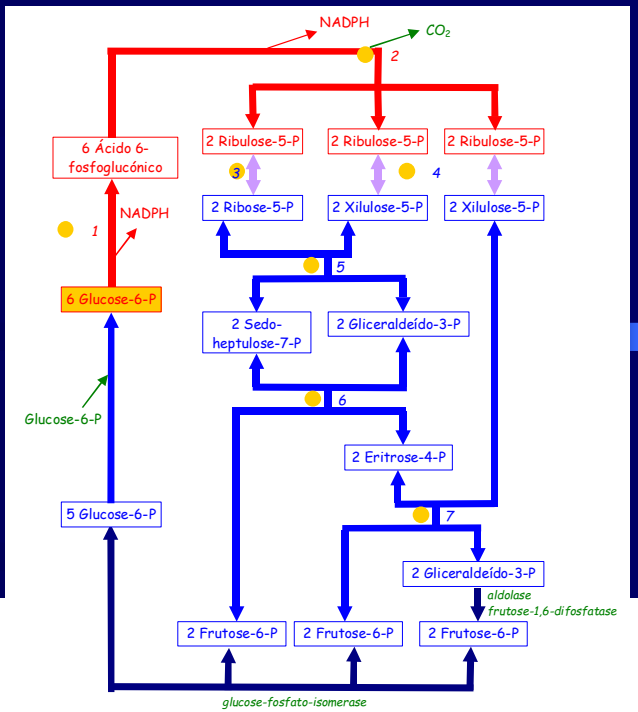
Fase não oxidante

Ocorre no fígado

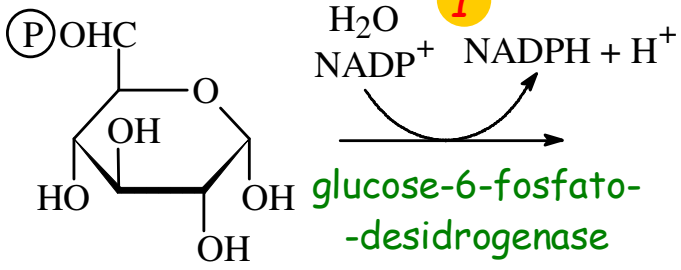
(em células adiposas, no fígado; não ocorre no músculo)



Reacções da via dos fosfatos de pentose:



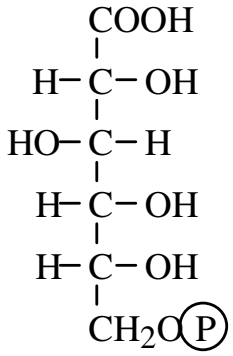
- Reacções da Fase Oxidante da Via:



glucose-6-fosfato

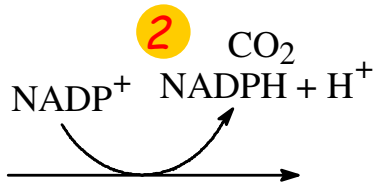
C6

glucose-6-fosfato-desidrogenase

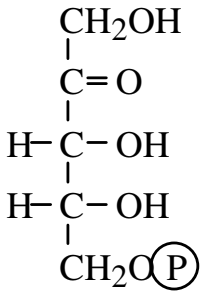


Ác. 6-fosfoglucónico

C6



6-fosfogluconato-desidrogenase



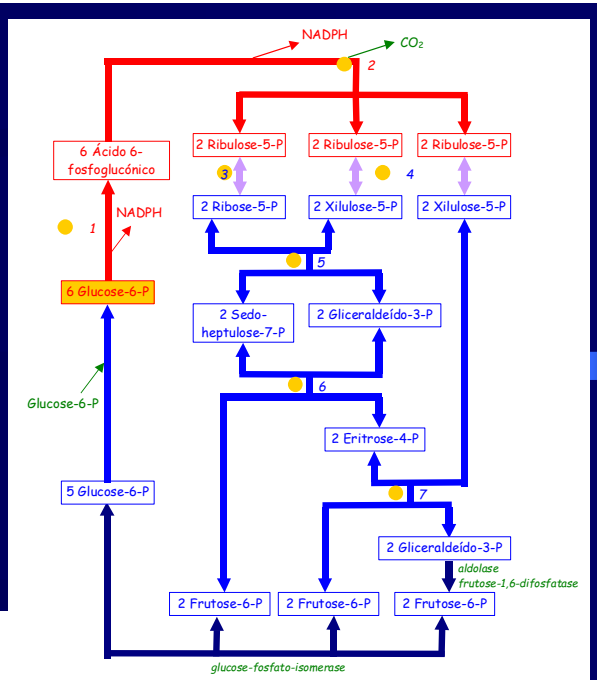
Ribulose-5-fosfato

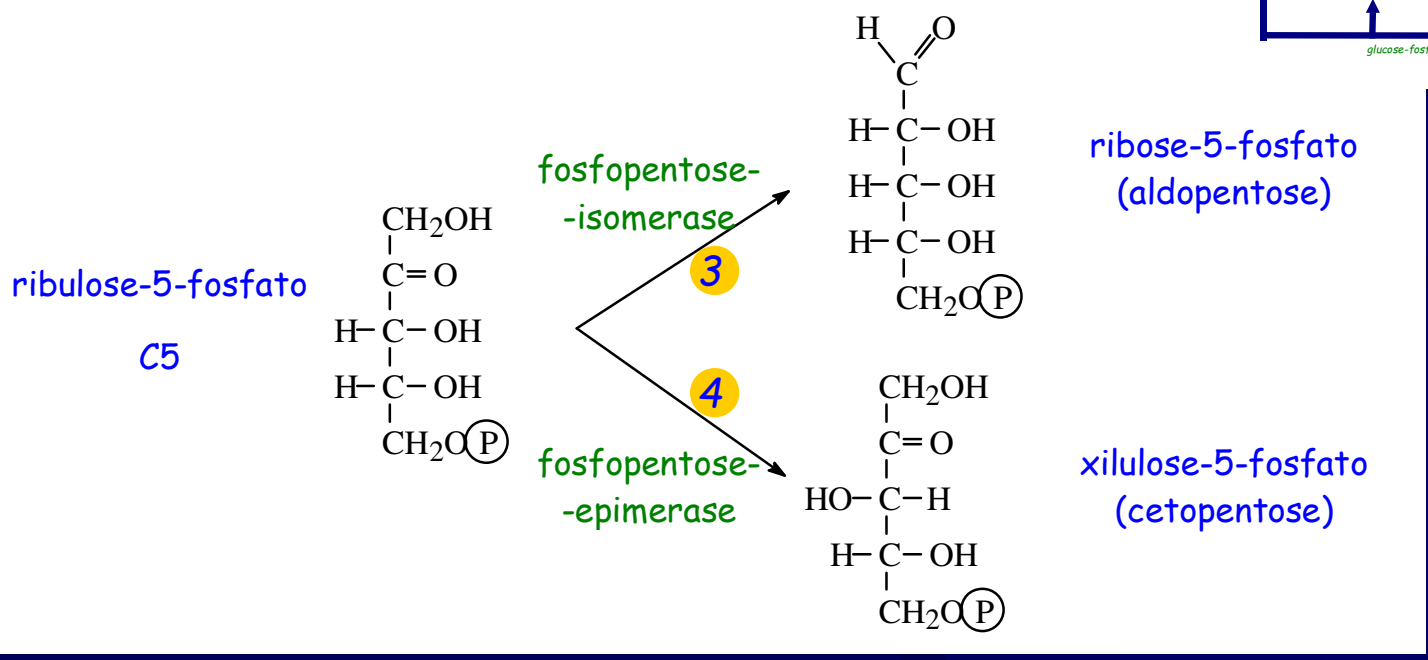
C5

Reacções da via dos fosfatos de pentose:

- Fase Não Oxidante:

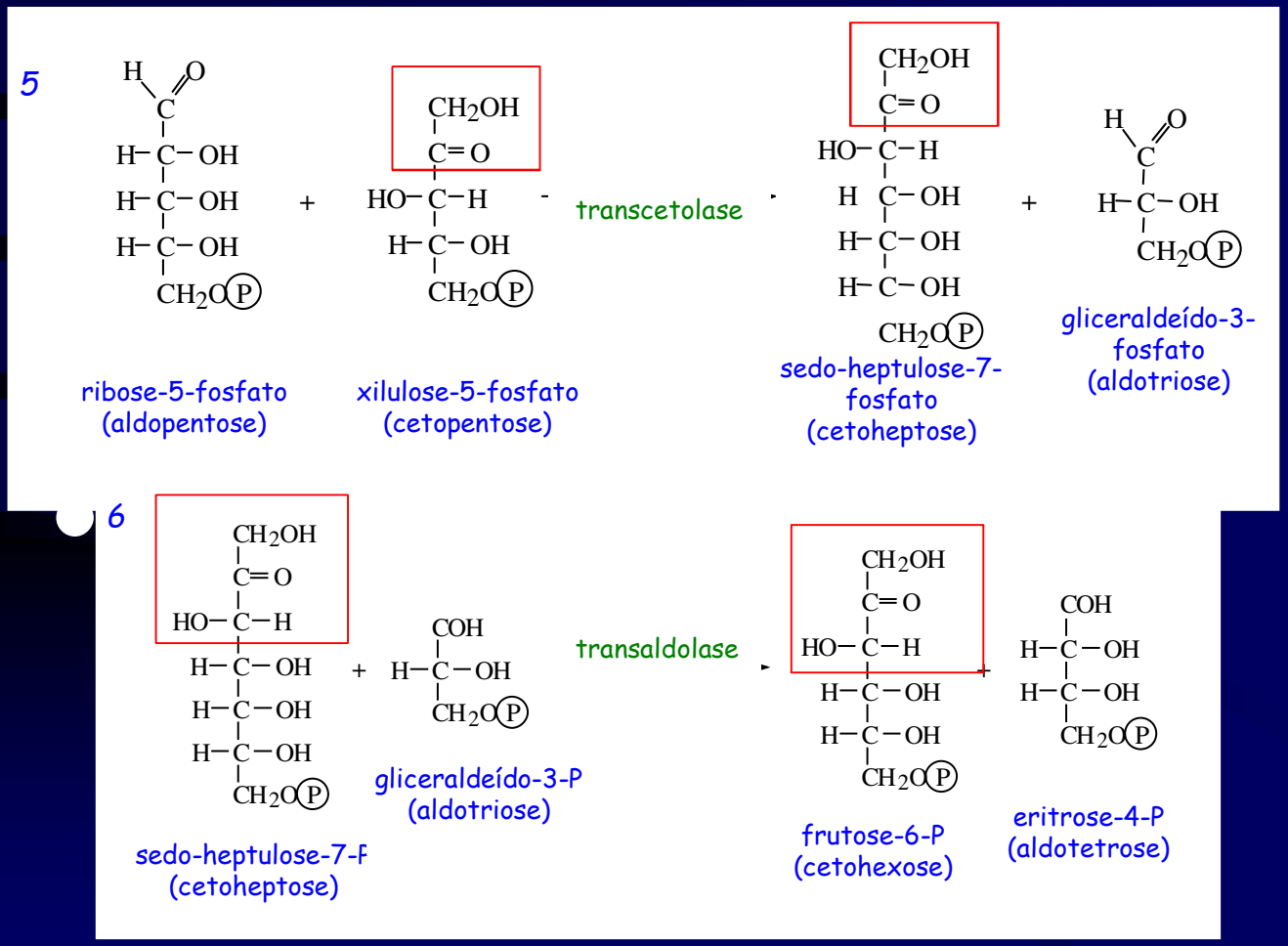
- Reacções 3 e 4 (isomerização da ribulose-5-P):





- Reacções 5, 6 e 7 (catalizadas por transaldolases e transcetolases)

ocorre *interconversão* dos açucares em C3, C4, C5, C6 e C7

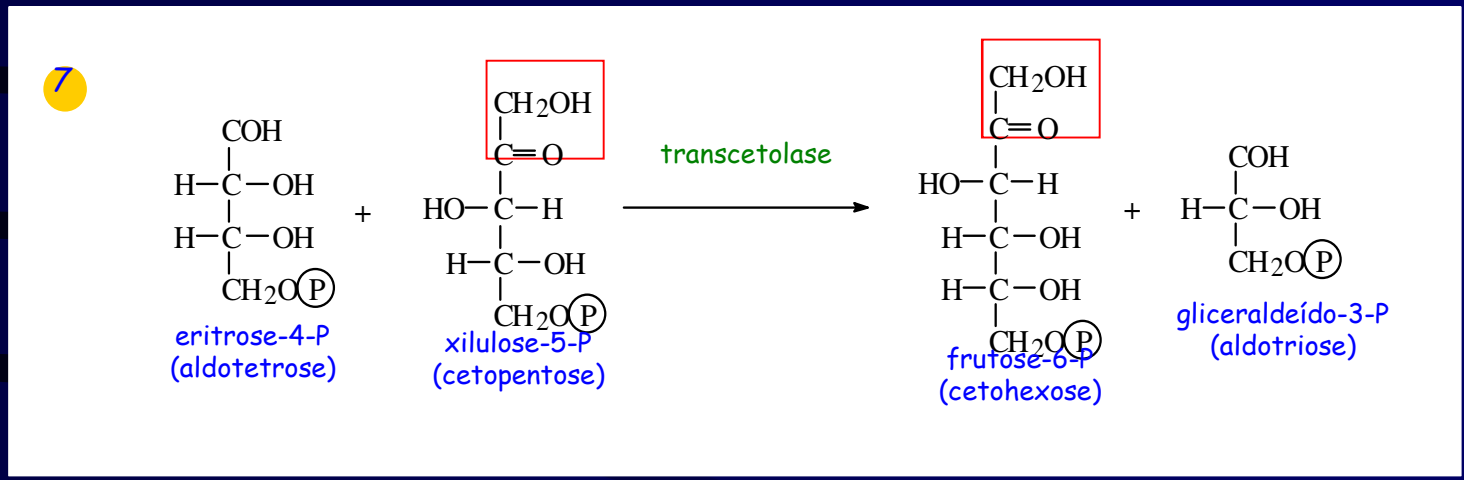


transcetolase: transfere uma unidade de 2 C (quebra a ligação entre C2 e C3 de uma cetose) (dador é cetose)

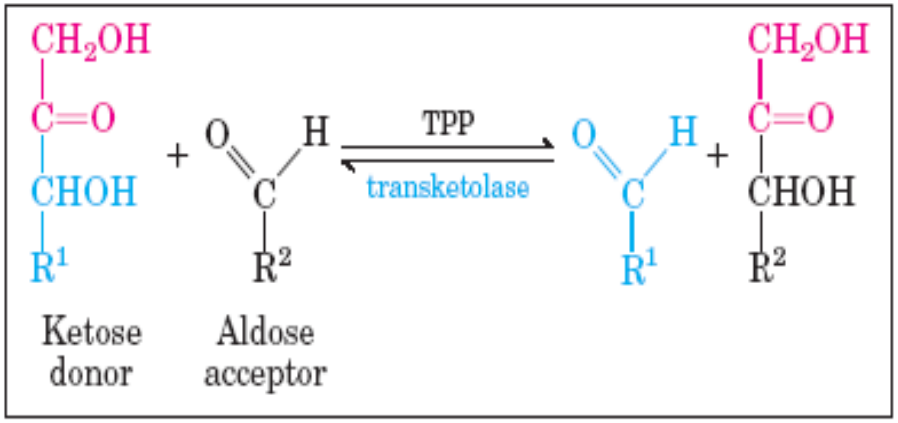
transaldolase: transfere uma unidade de 3 C (quebra a ligação entre C3 e C4 de uma cetose) (dador é aldose)

- Reacções 5, 6 e 7 (catalizadas por transaldolases e transcetolases)

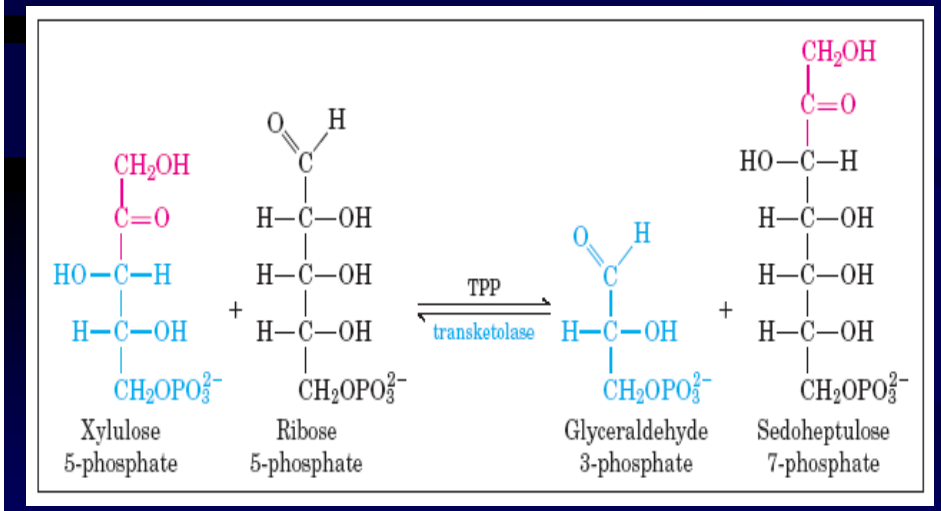
ocorre *interconversão* dos açucares em C3, C4, C5, C6 e C7

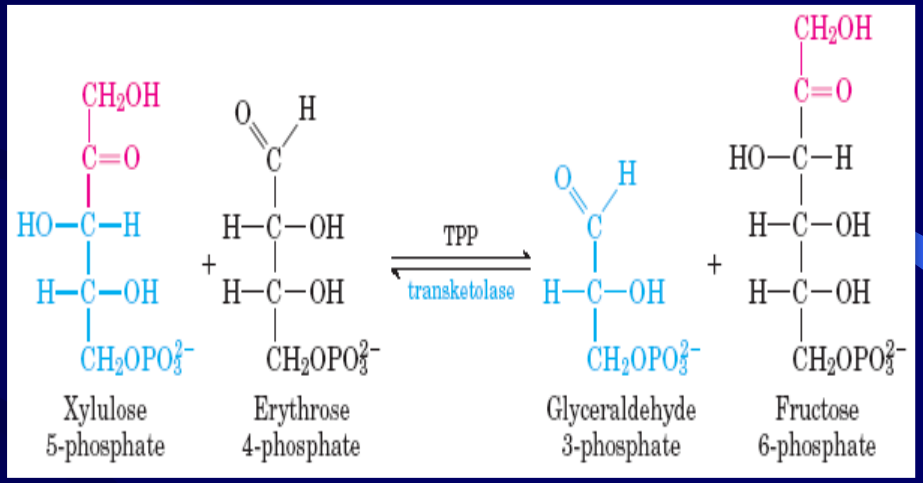


Transketolase

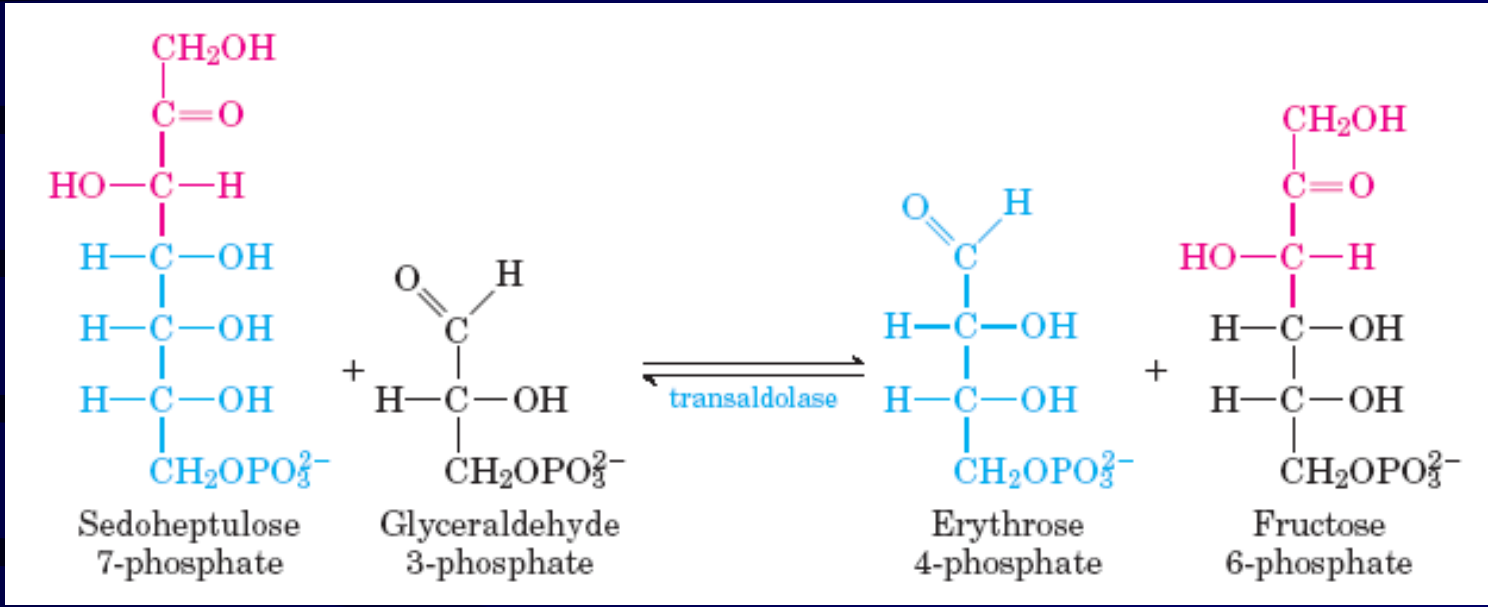


- **Transketolase** catalyzes the transfer of a two-carbon fragment from a ketose donor to an aldose acceptor.
- **TPP (thiamine pyrophosphate-vit. B1)** is needed as cofactor





Transaldolase



- **transaldolase** similar to the aldolase reaction of glycolysis: a three-carbon fragment is removed from sedoheptulose 7-phosphate and condensed with glyceraldehyde 3-phosphate, fructose 6-phosphate and erythrose 4-phosphate.

Reacção global:

6 glucose-6-P + 12 $NADP^+$ $\rightarrow$ 5 glucose-6-P + 6 CO_2 + 12 NADPH + 12 H^+ + Pi

- **Metabolização da glucose-6-P:**
  Transformação da glucose em pentoses sem utilização da glicólise

- **Formação de NADPH (fase oxidante):**
  Utilizado como poder redutor:
  - síntese de ácidos gordos e de outros lípidos
  - mantém sistema antioxidante activo

- **Formação de ribose-5-P (fase oxidante e fase não-oxidante em sentido inverso):**
  Percursor da síntese de nucleótidos
  (relacionado com a sintese proteica)

Oxidative PPP Vs. Nonoxidative PPP

- Oxidative PPP is **irreversible**; nonoxidative PPP is reversible – converting hexose phosphates to pentose phosphates (**reductive pentose phosphate pathway of** plants – photosynthetic assimilation of CO_2)
- All in cytosol- glycolysis, gluconeogenesis, and PPP are connected (the cell's relative needs for pentose phosphates, NADPH, and ATP)
- **Wernicke-Korsakoff Syndrome** (severe memory loss, mental confusion, and partial paralysis) **Is exacerbated by a Defect in Transketolase (1/10 affinity for TPP)**. The syndrome is more common among alcoholics (chronic alcohol consumption interferes with the intestinal absorption of some vitamins, including thiamine).

Regulação da via das pentoses na célula (intracelular):

Condições metabólicas em que ocorre a via das pentoses:

- Nível de $NADP^+$:

O organismo requer muito mais NADPH do que Ribose-5-P → -Dá-se o ciclo completo (fase oxidante com produção de NADPH, seguida da fase não oxidante), com regeneração de glucose-6-P.

-Em alternativa a frutose-5-P e gliceraldeído-3-P vão para a glicólise com produção de ATP

Se o organismo requer muito mais Ribose-5-P do que NADPH: → Dá-se a fase não oxidante da via, no sentido inverso.

Se o organismo requer Ribose-5-P e NADPH: → Dá-se a fase oxidante da via, apenas.

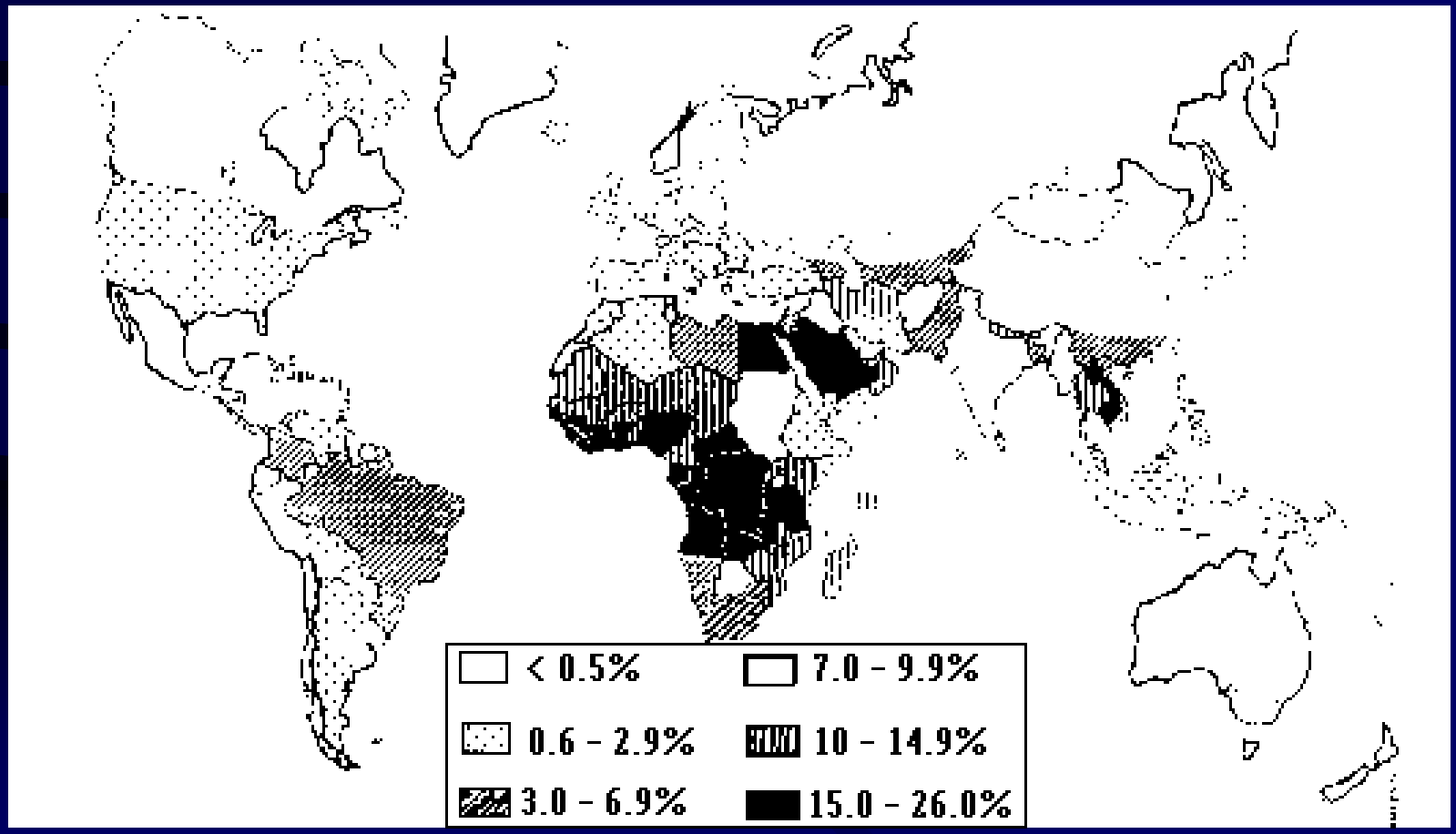
Regulation

- Glucose-6-P dehydrogenase
  - First step
  - Rate limiting
- Allosteric Regulation
  - Feedback inhibited by NADPH
- Inducible enzyme
  - Induced by insulin

G6PDH Deficiency and Hemolytic Anemia

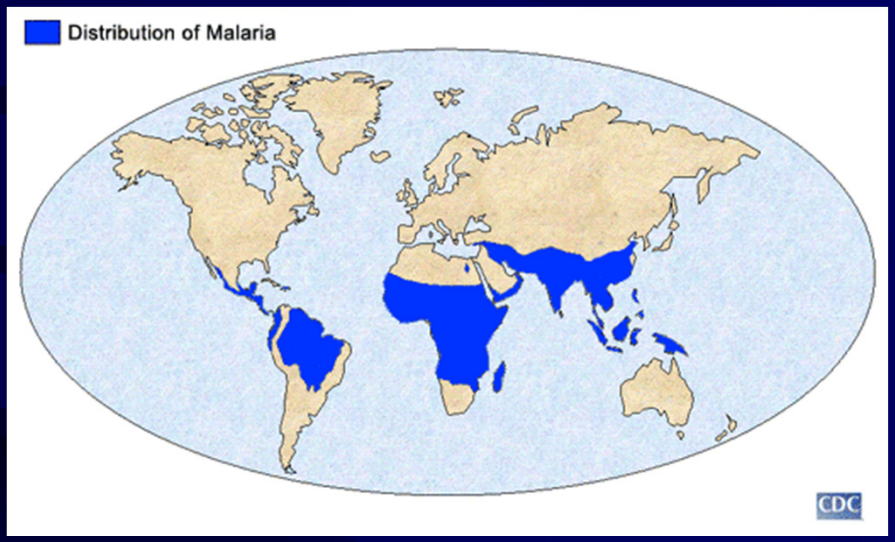
- Most common genetic enzymopathy
  - 400 hundred variants of G6PDH deficiency
  - Mediterranean, Asian, African descent
    - 400 million people affected worldwide
    - 50% of Kurdish men
    - 10-14% of African-American men with G6PD deficiency

Worldwide distribution of G6PD deficiency: 1995



G6PD Deficiency

- Distribution of G6PD deficiency coincides prevalence of malaria

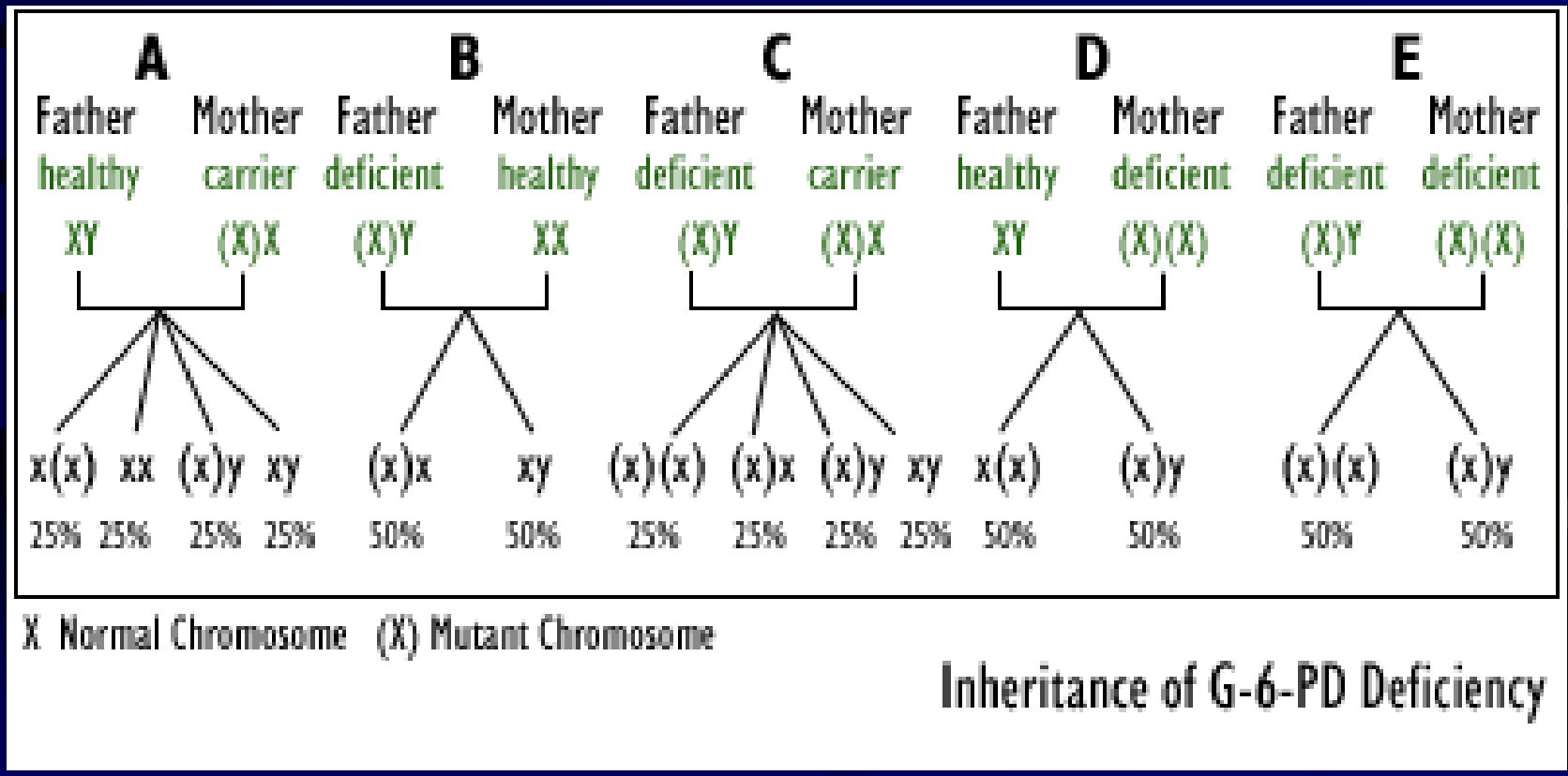


- G6PD deficiency may impart some degree of malaria resistance
  - Also sickle cell anemia

Genetics

- Recessive sex-linked mutation
  - X-chromosome
  - Rare in females (two X-chromosomes)
- Homozygous mutation:
  - high hemolysis and anemia
- Heterozygous mutation:
  - Normally asymptomatic
    - unless exposed to drugs (primaquine, anti-malarial drug) or compounds (fava bean) that produce superoxide or hydrogen peroxide

Inheritance of G6PD Deficiency



G6PD Deficiency

- Exposure to anti-malarial drugs (Primaquine) results in increased cellular production of superoxide and hydrogen peroxide (Primaquine sensitivity)
- Other chemicals known to increase oxidant stress
  - Sulfonamides (antibiotic)
  - Asprin and NSAIDs
  - Quinadine and quinine
  - Napthlane (mothballs)
  - Fava beans (vicine & isouramil)

Fava Bean

- Grown worldwide
  - Important in Middle East
  - High in protein
  - Frost resistant perennial
- Genetically modified fava bean being developed
  - Low in vicine and isouramil
- Favism

Case Study

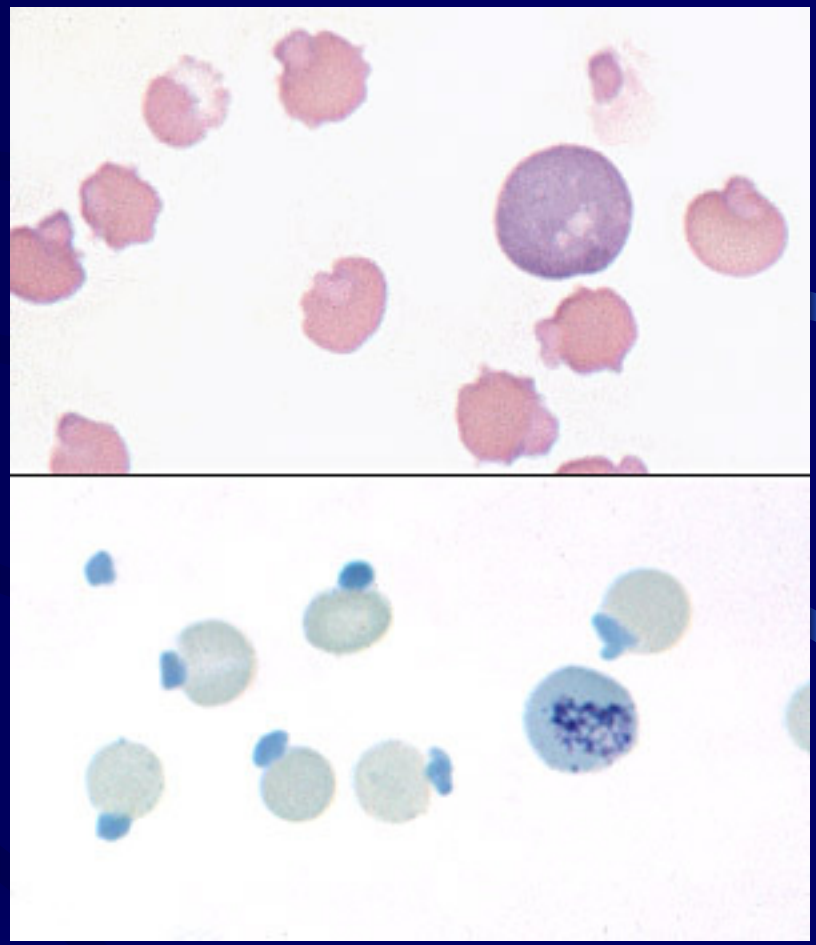
- 21 yo male medical student with malaria
- Treated with primaquine
- Four days later:
  - Black colored urine
  - Low RBC count
  - Elevated reticulocyte count
  - RBC with Heinz bodies
  - Low hemoglobin
  - Elevated serum bilirubin
- Pt recovered in a few days

Symptoms

- Black colored urine
  - Hemolysis may result in urinary excretion of hemoglobin
- Low RBC count & low hemoglobin
  - Result of high rate of hemolysis
- Elevated bilirubin
  - Catabolism of heme

RBCs with Heinz Bodies

- Precipitation of hemoglobin due to disulfide bond formation between Hb molecues
- Upper photo shows distorted RBCs with large Heinz bodies
- Bottom photo shows RBC stained with methylene blue



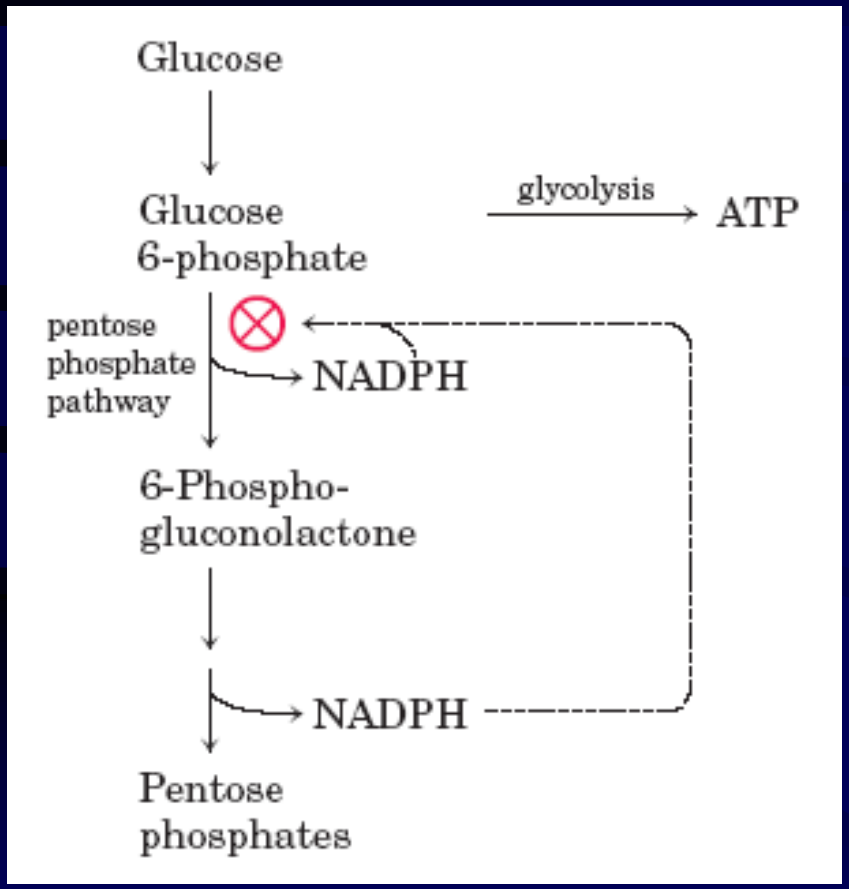
Elevated Reticulocytes

- A RBC containing granules or filaments representing an immature stage in cell development
- Normally constitutes 1% of circulating RBCs
- Reticulocytosis
  - Elevation of reticulocytes
  - Indicative of active erthropoiesis in red bone marrow

Defective G6PDH

- Results in enzyme with unstable structure
  - Patient with 10% of normal activity
  - Enough to generate NADPH under normal condition
- Newly made RBCs have normal 6PDH activity
  - Patients recover quickly (8 days)

Glucose 6-Phosphate Is Partitioned between Glycolysis and the Pentose Phosphate Pathway



- When a cell is rapidly converting NADPH to NADP in biosynthetic reductions, the level of NADP rises, allosterically stimulating G6PD and thereby increasing the flux of glucose 6-phosphate through the pentose phosphate pathway.
- When NADPH is forming faster than it is being used for biosynthesis and glutathione reduction , [NADPH] rises and inhibits the first enzyme in the pentose phosphate pathway. As a result, more glucose 6-phosphate is available for glycolysis.