Cellular metabolism

glucose metabolism

Metabolism

Living organism requires a constant supply of energy and the creation and restoration of building materials.

Metabolism - marches , in which a living organism uses and produces energy .

A summary of all the reactions occurring in the body .

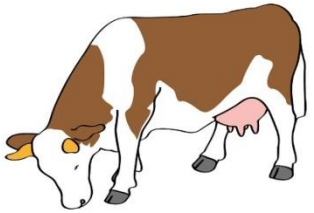
Functions of metabolism

- **maintenance of energy (catabolic - degradation)**
- **synthesis of molecules (anabolic -storage)**
- **both processes are interdependent --- Energy Affinity**

Types of organisms by metabolism

- ***by source of energy:***
- phototrophs (use of solar energy, for example green plants)
- chemotrophs (oxidation of nutrients)
- ***by source of building material:***
- autotrophs (synthesized substances from anorg. sources)
- heterotrophic (use org. substanes)

Metabolism of human



Proteins, carbohydrates, lipids

O_2

Macromolecules

Energy

CO_2

Small molecules

Organisms as open systems

- consistently receive nutrients with high enthalpy (= energy) and low entropy (= complex and ordered structure)
- nutrients are converted to waste products with low enthalpies and entropies high (= simple structure)
- Gibbs energy released during these processes is maintained in the course of biochemical processes , ensuring highly organized cell structure
- part of the energy is converted to a usable form of heat

Energy in chemical reactions

Gibbs free energy

(G Δ)

the maximum amount of useful energy that can be obtained for the reaction constant . pressure and temperature

During the conversion of nutrients into the waste substances are released Gibbs energy that keeps running biochemical processes and ensures a highly organized cellular structure. Unfortunately, you can not use all the released energy - part of it is always converted to a form unusable = heat.
Gibbs energy (G) can be defined as the maximum amount of useful energy that can be obtained in the reaction at constant pressure and temperature. For the reaction A + B → C + D , it can be

$$A+B \rightarrow C+D$$

$$\Delta G = \Delta G^0 + RT \ln \frac{[C]^c[D]^d}{[A]^a[B]^b}$$

Biochemical processes

$\Delta G^{0'}$ (pH = 7, 0 25 °C)

- **Steady-state (dynamic equilibrium) .**
- **Reaction of the successive product of one reaction is the substrate for subsequent reactions .**
- **Concentration do not meet the standard.**

With regard to the Gibbs energy in the body, we can distinguish two different types of processes :
a) Exergonic processes
b) endergonic processes

Endergonic processes (G > 0) can take place only in coupling reactions with exergonic (G < 0) . The transfer of energy from one process to another takes place by means of energy-rich molecules - most often used **ATP** (energy released in the particular process is transferred via phosphoryl groups -PO32- to other substances) .

Processes

exergonic **endergonic**

Endergonic reaction can take place only in coupling reactions with exergonic.

Energy transfer from one process to another takes place by means of energy-rich molecules.

The most commonly used is ATP.

When coupling that transfers phosphoryl groups - PO_3^{2-} other substances

Principles of coupling

Example 1:

Formation of glucosa-6-phosphate

$glucose + P_i \rightarrow glucose\text{-}6\text{-}P + H_2O$ $\quad \Delta G^{o\prime} = +13{,}8$ kJ/mol

$ATP + H_2O \rightarrow ADP + P_i$ $\quad \Delta G^{o\prime} = -30{,}5$ kJ/mol

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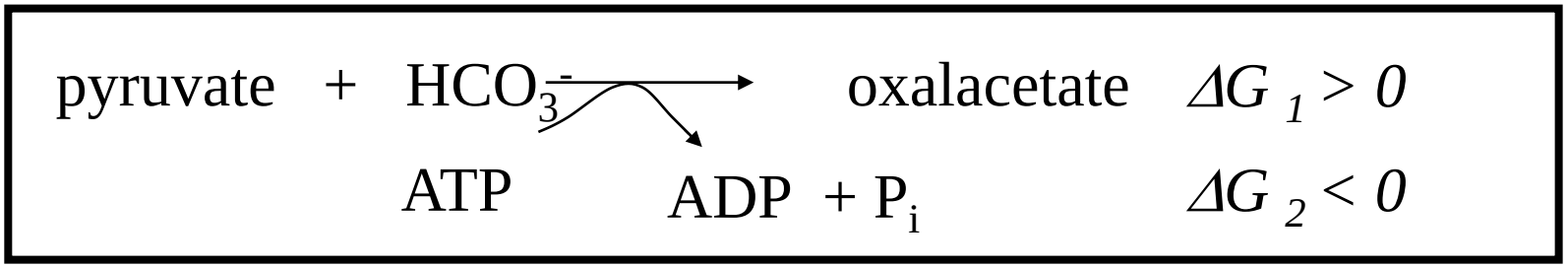
$glucose + ATP \rightarrow glucose\text{-}6\text{-}P + ADP$ $\quad \Delta G^{o\prime} = -16{,}7$ kJ/mol

$-PO_3^{2-}$ is by enzyme of kinase transported from ATP to glucose.

Example 2: Carboxylation of pyruvate



Partial reaction:

HCO_3^- + ATP → ADP + $^-$OCO-O-**PO_3^{2-}**
phosphocarbonate

$^-$OCO-O-**PO_3^{2-}** + biotin → $^-$OOC-biotine + **P_i**

$^-$OOC-biotin + pyruvate → oxalacetate

} $\Delta G < 0$

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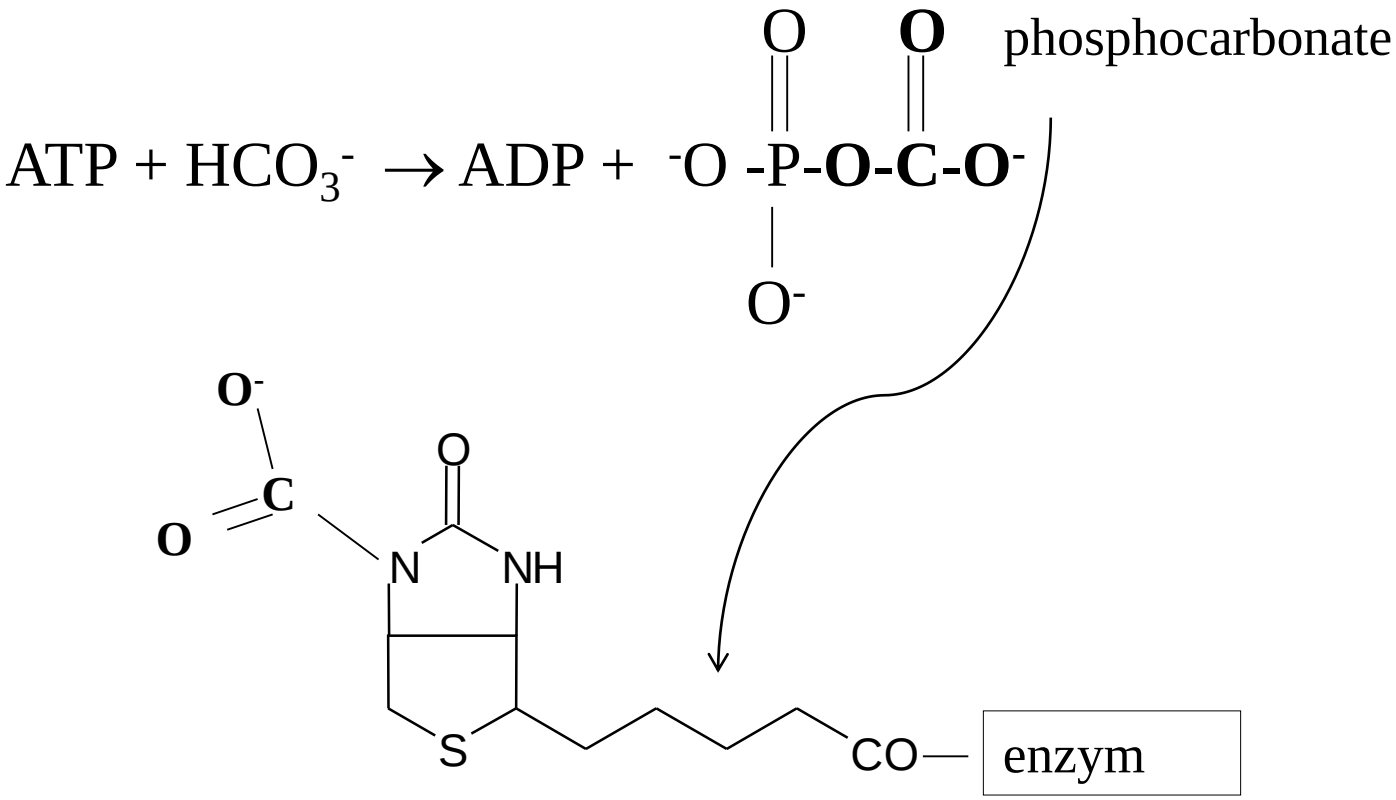
biotin + ATP + HCO_3^- → carboxybiotin + ADP + Pi

carboxybiotin + pyruvate → biotin + oxalacetate

Carboxylation of biotin



Carboxylate anion is activated by binding P_i and by biotin is transfer to pyruvate.

The term " high energy compound"

(also called " energy- rich compound "

" Macroergic Compound ")

The compound to hydrolytic cleavage of its bonds provide approximately the same or greater energy than is

$\Delta G^{0\prime}$ for ATP hydrolysis

Most often, these functional derivatives of phosphoric acid

.

The high-energy/macroergic phosphate compound

the rest contain phosphoric acid . linked most often :

- anhydride ,
- amide,
- enol esters bond.

(esters of phos. Acid are not macroergic)

Univerzal phosphate high-energic compound is ATP

Provides energy in reactions:

$ATP + H_2O \rightarrow ADP + P_i$ $\Delta G^{0'} = -30,5$ kJ/mol

$ATP + H_2O \rightarrow AMP + PP_i$ $\Delta G^{0'} = -32,0$ kJ/mol

reaction must be enzyme-catalyzed

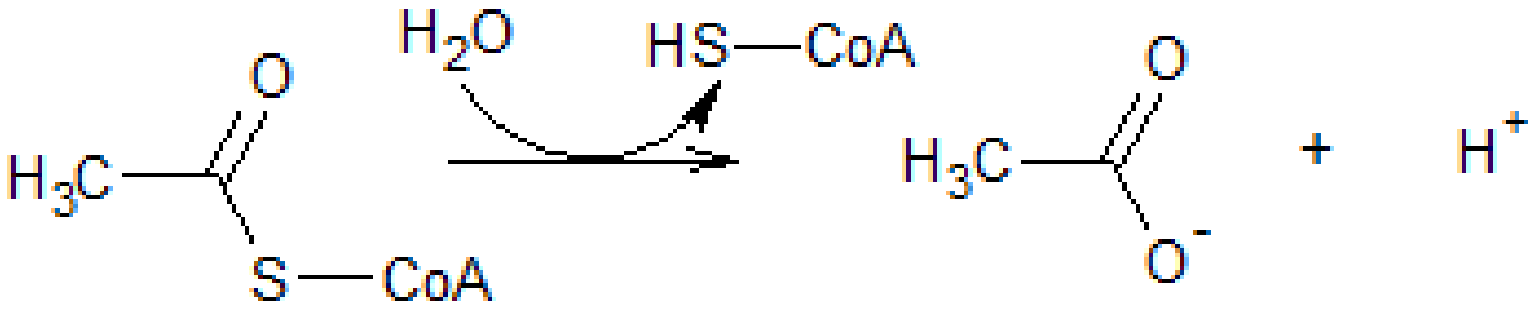
Similarly, provide energy: GTP, UTP a CTP

Another high-energy phosphate compounds

Compound	ΔG^0 (kJ/mol)	type of compounds
phosphoenolpyruvate	-62	enolester
Carbamoyl-P	-52	mixed anhydride
1,3-bisphosphoglycerate	-50	mixed anhydride
phosphokreatin	-43	amide

These substances are formed during the metabolism.
Their reactions with ADP may give ATP = substrate phosphorylation

The energy- rich compounds can also be thioesters (e.g. , an acyl group linked to coenzyme A)



$\Delta G^0 = -31{,}0$ kJ/mol

How to get high- energy compound by metabolism?

„burning nutrients “

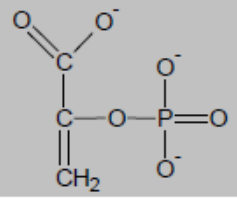
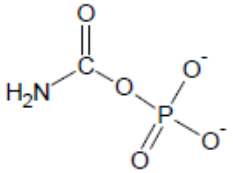
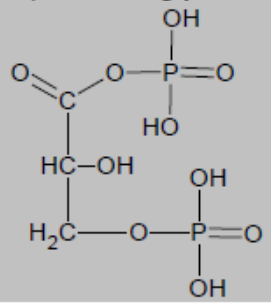
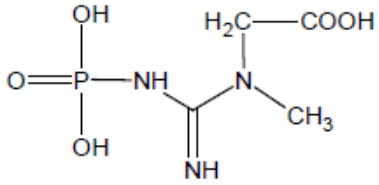
• nutrients in the diet (lipids and carbohydrates, proteins partially) contain carbon atoms with a low oxidation state

• are sequentially dehydrogenated to various intermediates, in which decarboxylation reactions cleave CO_2

• electrons and hydrogen atoms are transferred to the redox cofactors (NADH, $FADH_2$) and transported to the respiratory chain

Macroergic compounds

Tabulka 1 - Makroergní sloučeniny

Sloučenina	ΔG° [kJ/mol]	Typ sloučeniny	Děj
Fosfoenolpyruvát 	– 62	enolester	glykolýza
Karbamoylfosfát 	– 52	smíšený anhydrid	metabolismus močoviny
1,3-bisfosfoglycerát 	– 50	smíšený anhydrid	glykolýza
kreatinfosfát 	– 43	amid	metabolismus ve svalové tkáni

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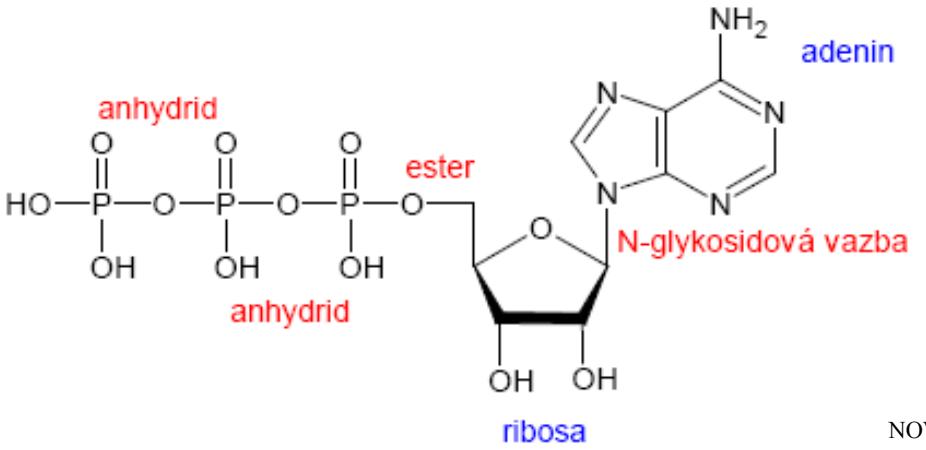

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The above- mentioned energy compounds in metabolism resulting from the combustion of nutrients.

The nutrients contained in food can be divided into lipids, carbohydrates and proteins.

These compounds contain a carbon with a low degree of oxidation . The main reactions in the combustion of nutrient oxidation, which takes the form of the dehydrogenation . Sequential dehydrogenation rise to various intermediates , releasing CO2 , electrons and hydrogen (H). Hydrogen along with electrons are transferred to the oxidation-reduction cofactors and transported to the respiratory chain.

• reoxidation of the energy released is used to generate ATP

•during degradation of the nutrients may also be formed directly high energy compound, or ATP providing subsequent **substrate phosphorylation**

Formation of ATP in cells

- **Majority of formation of ATP**

aerobic phosphorylation

= direct reaction between Pi and ADP

ADP + P_i → ATP catalyzed ATP-synthase

- Occurs in coupling in respiratory chain

- the use of energy produced by oxidation of NADH a $FADH_2$

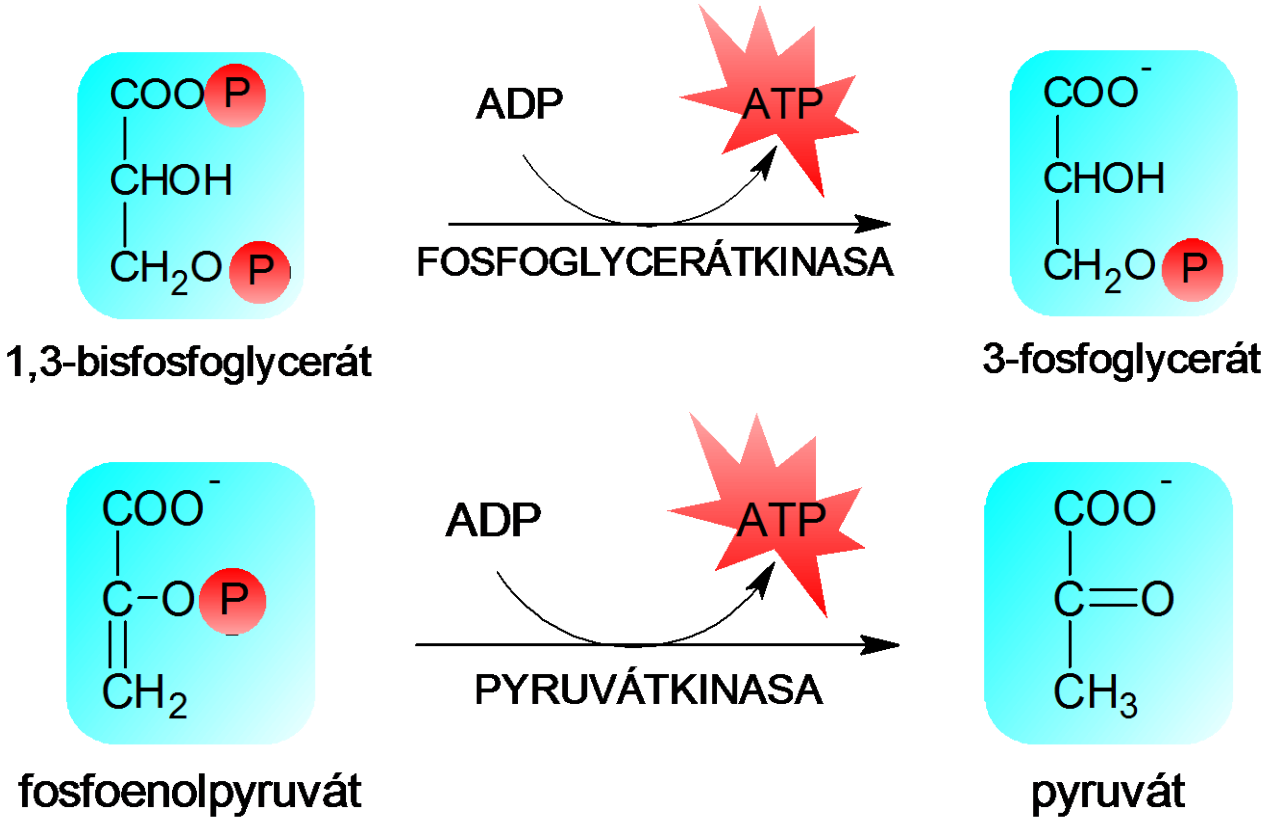
• **Other possibilities of formation of ATP**

transport $-PO_3^{2-}$ from high energetic rich compound to ADP

SUBSTRATE PHOSPHORYLATION

Examples of substrate phosphorylation

Reaction of glycolysis



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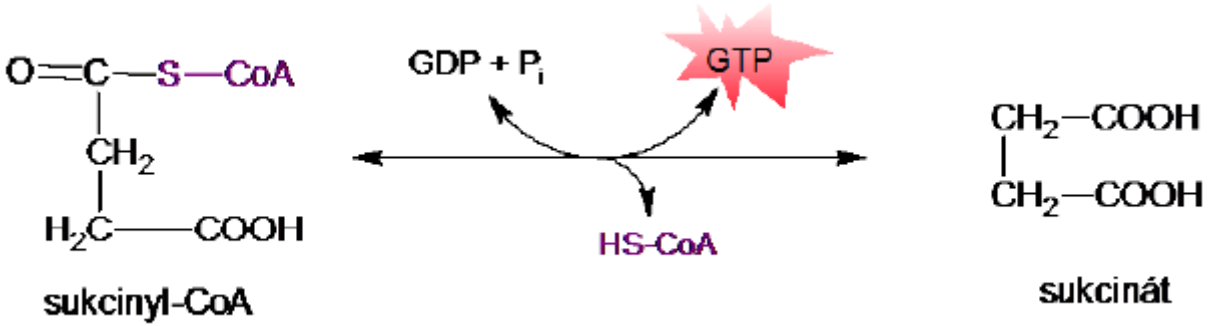
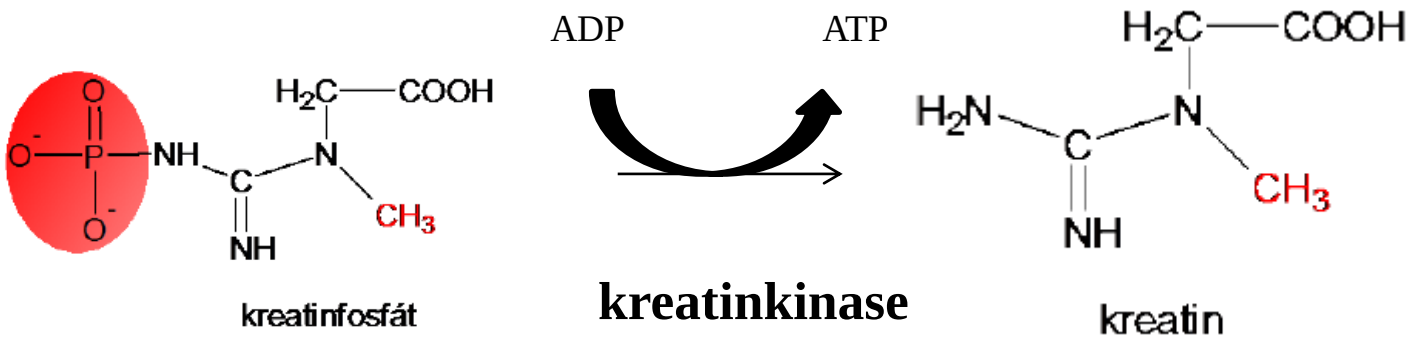
Citrate cycle



thiokinase

Muscle:



kreatinkinase

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ATP in cells

- Life time of ATP in cell cca 2 min
- They must be constantly replenished
- Instant ATP content in the body is about 100 g per day, daily is produced 60-70 kg
- Adenylatekinase keep balance between ATP, ADP and AMP

$$ATP + ADP \rightleftarrows 2\ ADP$$

- In health cell ratio [ATP]/[ADP] = 5-200

Energetic charge of cell:

$$= \frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]}$$

when drops to zero , the cell die

•Metabolism is regulated at several levels

• regulation of enzyme activity (allosteric effects product inhibition , substrate availability)

• covalent modification enzymes (phosphorylation)

• regulation of the synthesis of enzymes

• compartmentalization and organ specialization

• hormonal regulation

Different metabolic pathways are affected by the condition of the body

- condition after eating x starvation
- Rest x severe physical strain
- rest x Stress
- physiological state xdisease

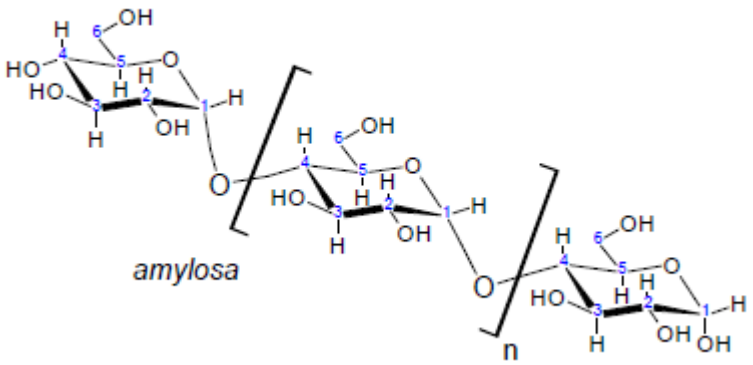
Metabolism of carbohydrates

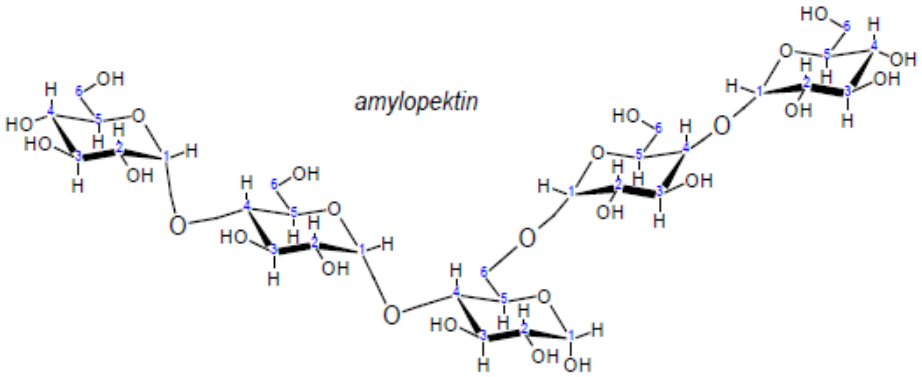
Metabolisms glucose in cells

Sources of glucose in the diet

- Glucose can be taken from food :
- a) free
- b) chemically bonded
- Free glucose gain eg . From grapes (glucose = dextrose) and other fruits (resp. Fruit juices) and honey.

- May be chemically bound glucose in polysaccharides and disaccharides .
- The main sources of glucose in food is starch. It is a polysaccharide composed glucose subunits. We distinguish its two parts :
- a) a linear amylose =
- b) branched amylopectin =





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- In addition to its structure, these two also differ in their biological effect . This difference describes variable called the **glycemic index**. The glycemic index is related to the speed at which there is an increase in blood glucose - the lower the index , thus the food is "better" (glucose is released from the more slowly , resulting in smaller demands on the pancreas and insulin production) .
- Starch sources are, for example . Potatoes (the main source of our food) , as well as bread, rice , pasta , corn and others. An important source of starch are also the pulses , which have the advantage that they contain lots of straight-chain amylose (from the glucose is cleaved more slowly), and hence have a **low glycemic index**.

- Other sources of glucose, in addition to the polysaccharides are disaccharides :
- a) sucrose
- b) Lactose
- c) maltose

- From the food can also receive free fructose .

Entry of glucose into cells

Glucose molecules are **highly polar** , they can not diffuse through hydrophobic lipid bilayer membrane (hydrogen bonds between the OH groups and water)

glucose transporters

transmembrane proteins facilitating transport of glucose into cells

- type GLUT (1-14)* or SGLT**

** glucose transporter*

*** sodium-coupled glucose transporter*

GLUT 1-GLUT 14, similarities:

~ 500 AK, 12 transmembrane helixes mechanism:

facilitated diffusion through a membrane (extends over the concentration gradient does not require energy)

Why are so many types of carriers ?

- different affinity for glucose
- They can be regulated in different ways
- They occur in various tissues

Glucose Transport

- **Na^+-Independent Facilitated Diffusion**

  Glucose Transporters (GLUT 1-14)
  With concentration gradient
  Energy Independent

- **Na^+-Monosaccharide Cotransporter:**

  Against concentration gradient
  Energy dependent
  Carrier-mediated (SGLT)
  Coupled to Na^+ transport
  Small intestine, renal tubules & choroid plexus

Glucose Transporters

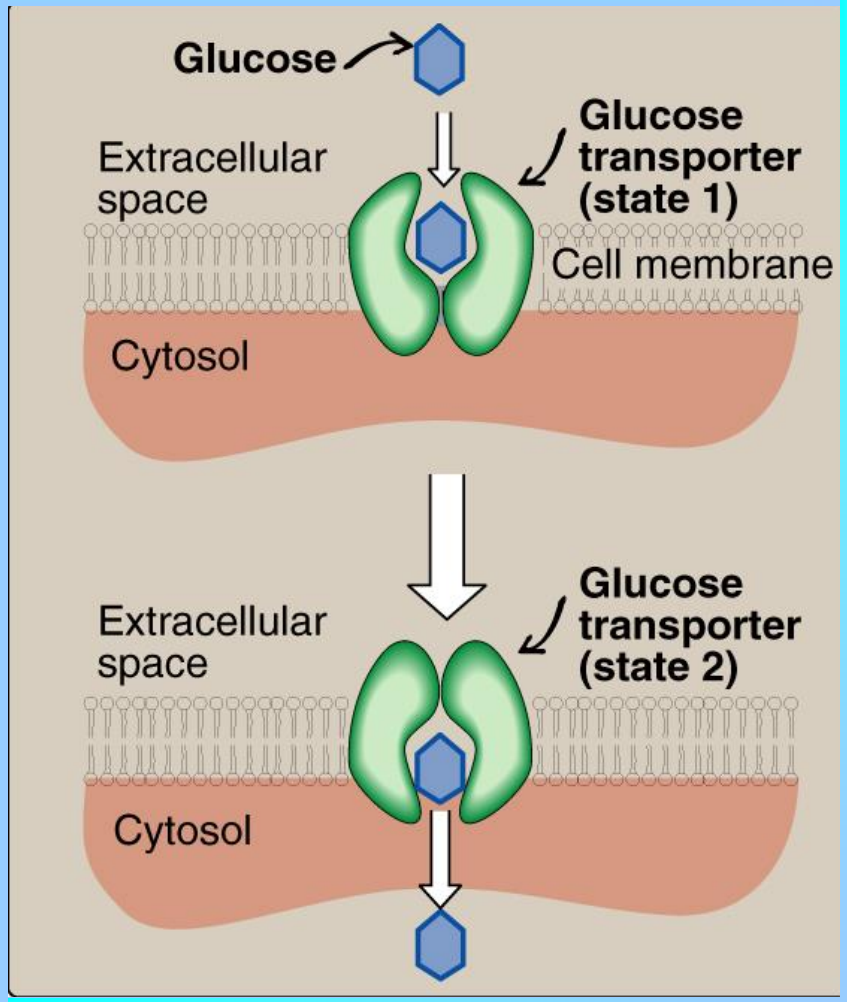
- **Tissue-specific expression pattern**

GLUT-1	**RBCs and brain**
GLUT-2	**Liver, kidney & pancreas**
GLUT-3	**Neurons**
GLUT-4	**Adipose tissue & skeletal muscle**
GLUT-5	**Small intestine & testes**
GLUT-7	**Liver (ER-membrane)**

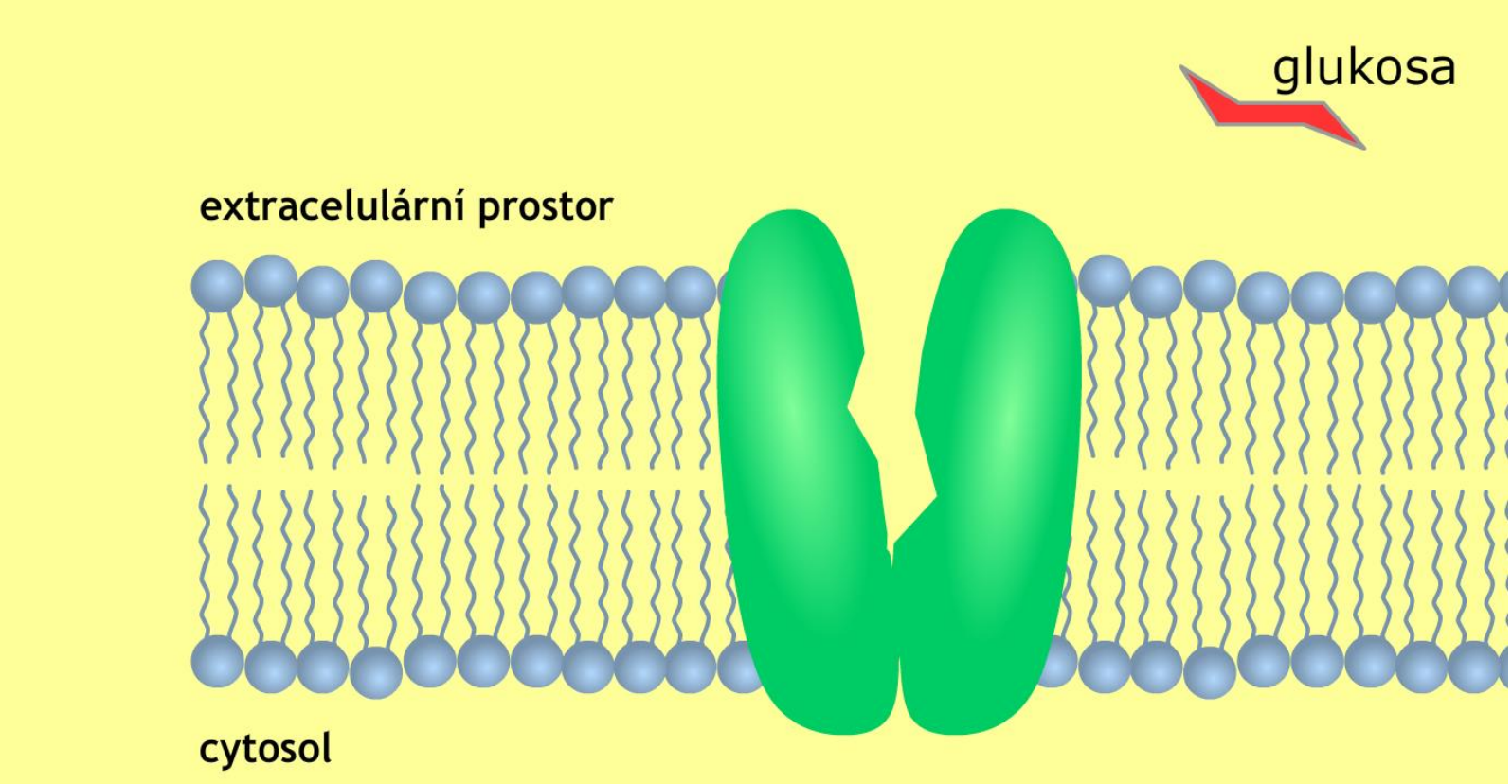
- **Functions:**

GLUT-1, 3 & 4	**Glucose uptake from blood**
GLUT-2	**Blood & cells (either direction)**
GLUT-5	**Fructose transport**

Glucose Transport: Facilitated Diffusion



Glucose transport via GLUT



Facilitated diffusion mechanism

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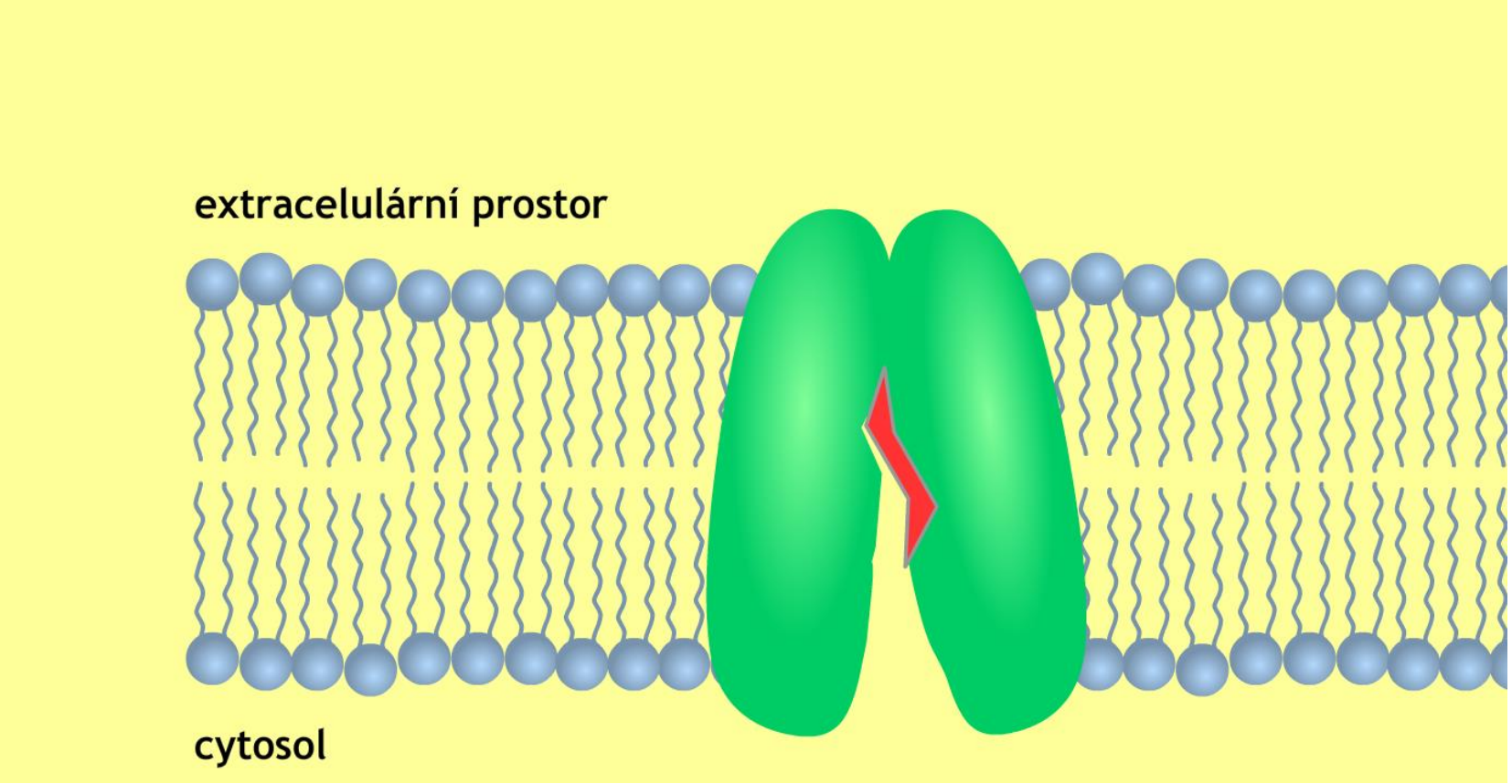

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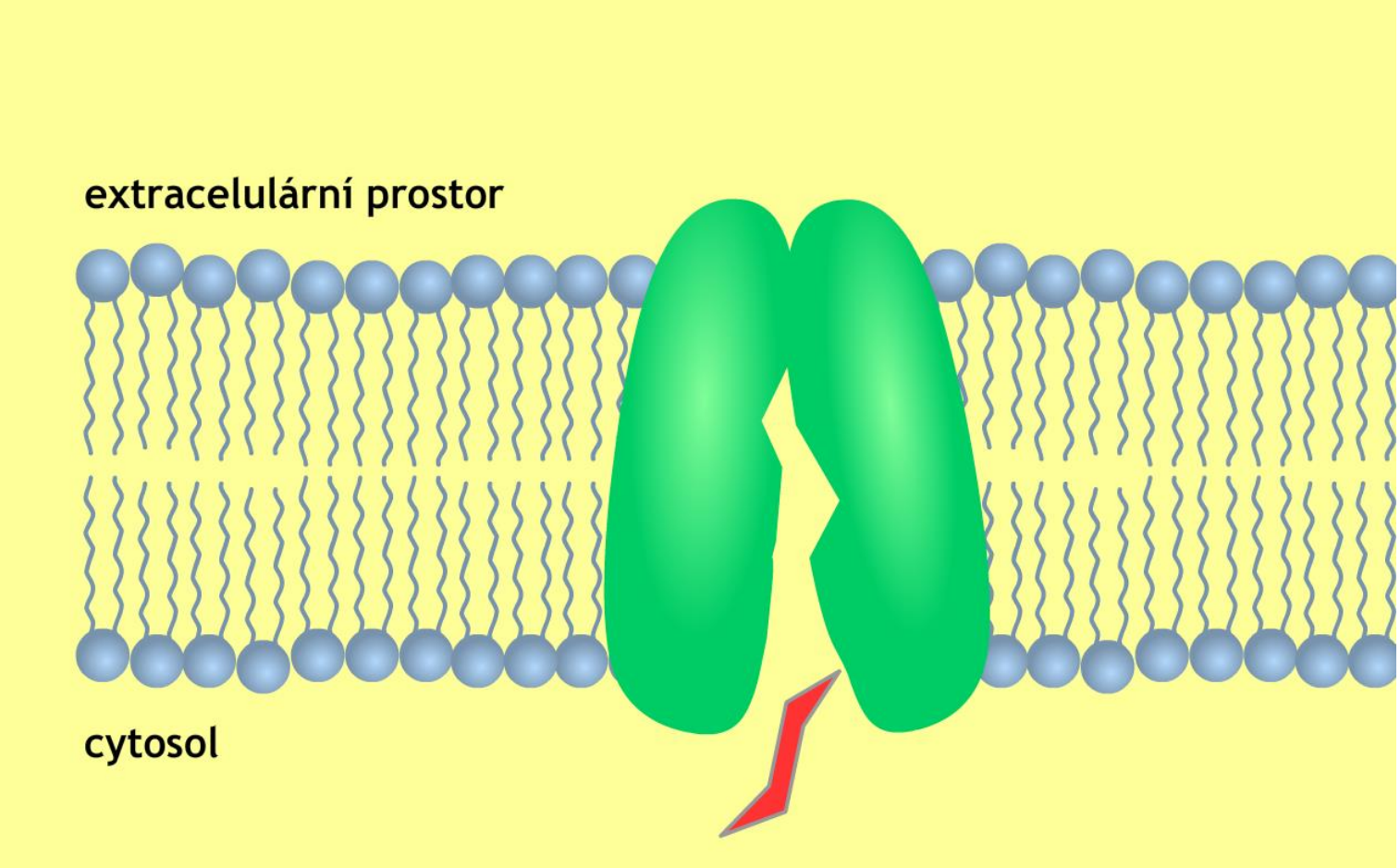

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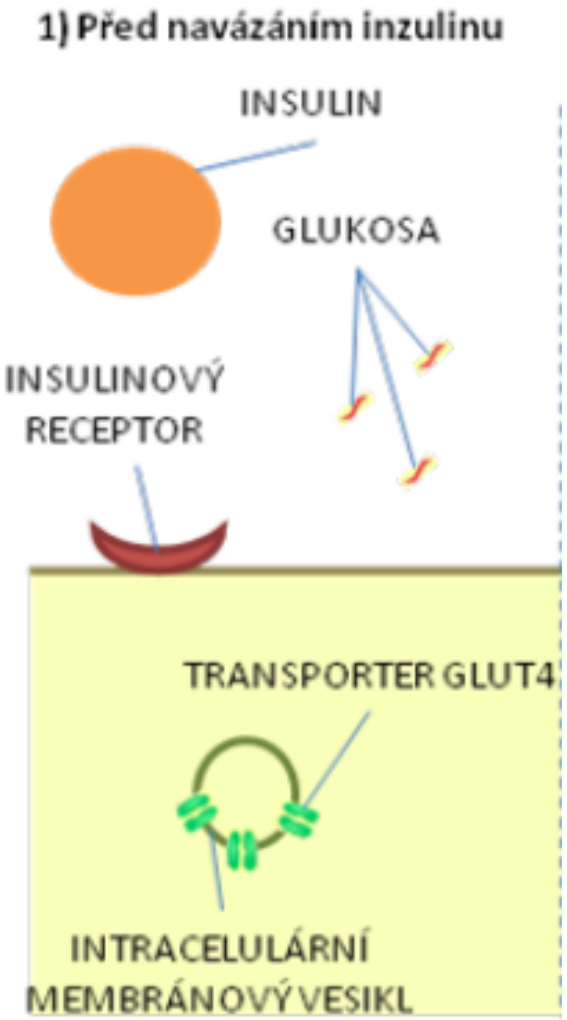
Receptor type GLUT 4 are regulated by insulin



Intracellular membrane vesicles are " sleeping " glukosovými transporters.

If insulin is not binding to the receptor , glucose can not enter into the cell .

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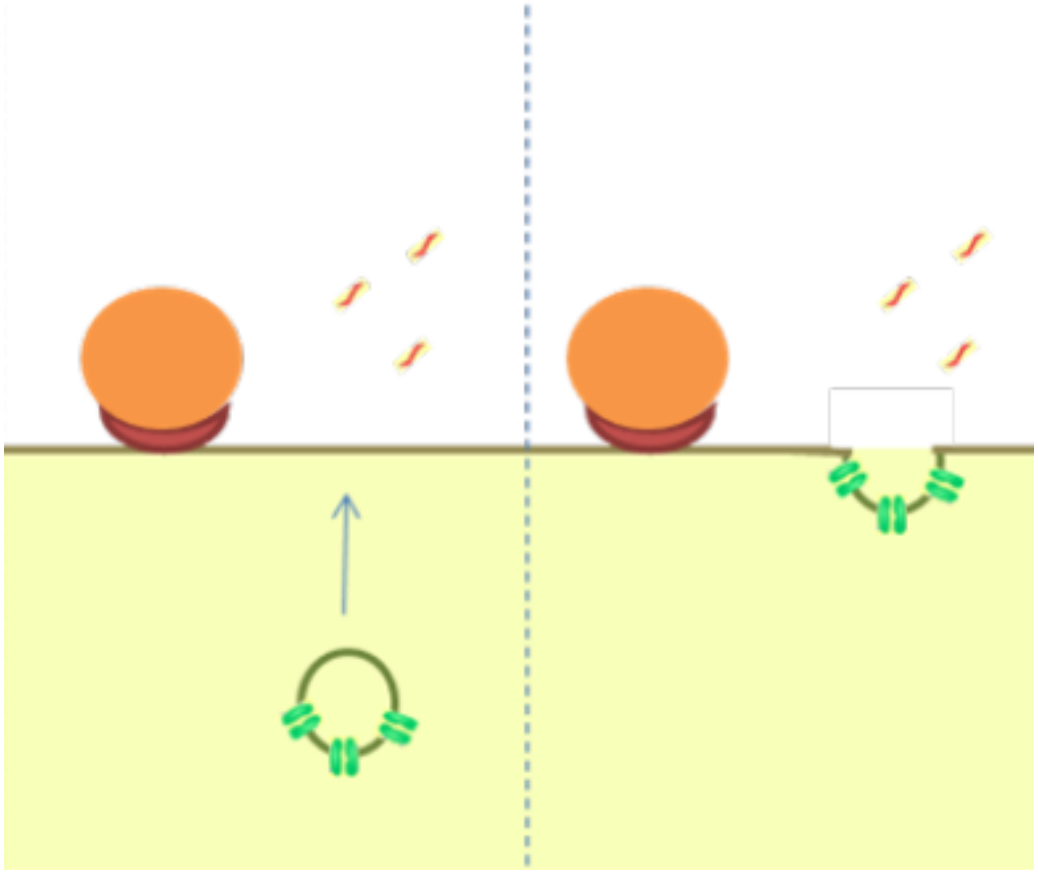
2) Binding of insulin to the receptor



After binding to the insulin receptor vesicles with membrane transporters move

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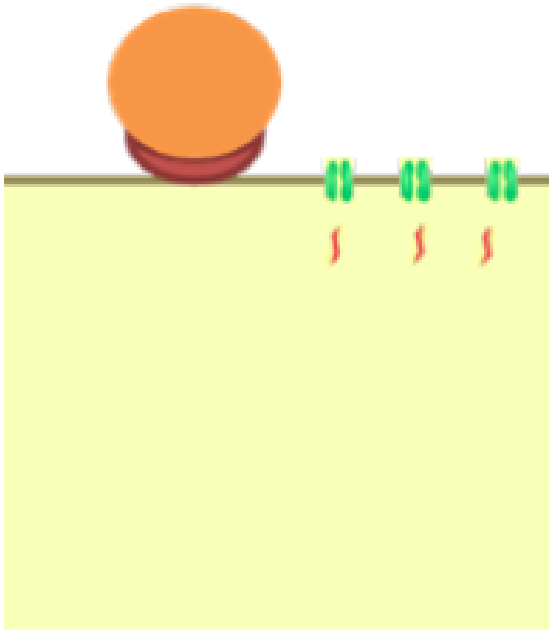
4) Glucose transport into cells



Glucose transporters penetrate into the membrane , glucose transport into the cell can begin.

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Glucose transport into the cells of the intestinal mucosa and ledviných tubules (SGLT)

Mechanism: co-transport with sodium

Secondary active transport

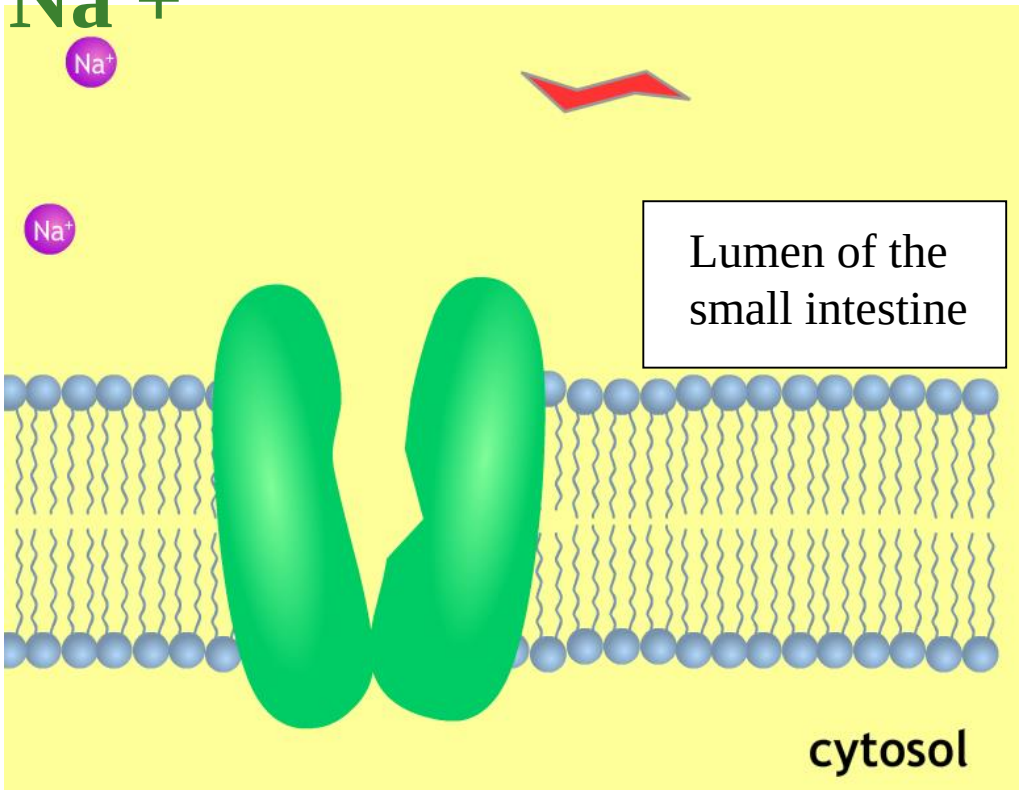
•at two specific sites linked glucose transporter and Na +

• their transport runs in parallel (without energy consumption)

• Na + is subsequently pumped from the cell ATPase (ATP consumption)

• Glucose is subsequently transported out of the cell via GLUT2

The mechanism of glucose cotransport of Na +



Mucous membrane of small intestine cells (enterocytes)

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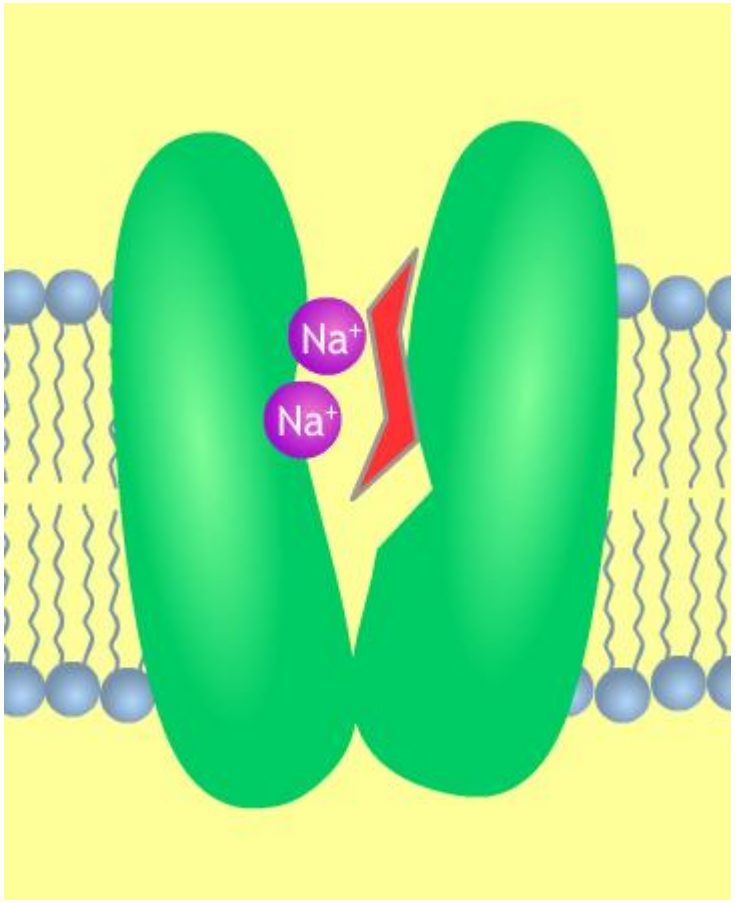

After binding of Na + and glucose transporter changes the conformation and glucose and Na + enter the cell

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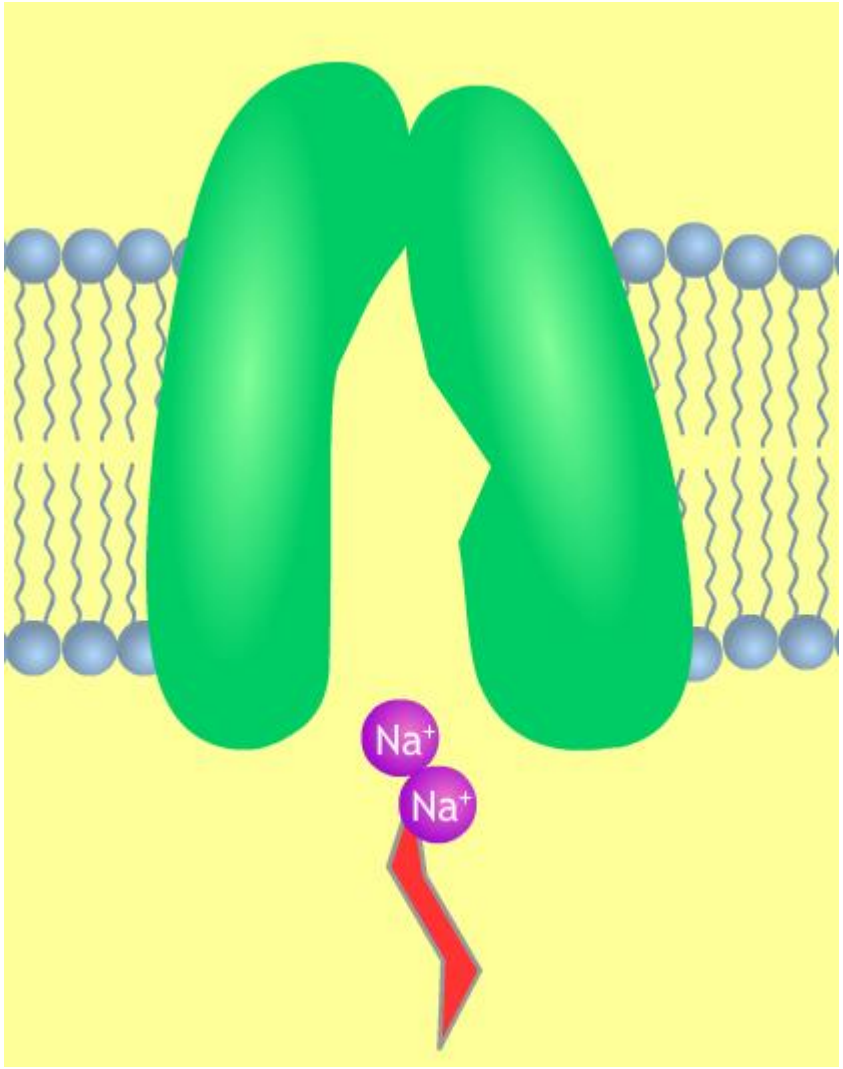
Na + and glucose are transported to the cell



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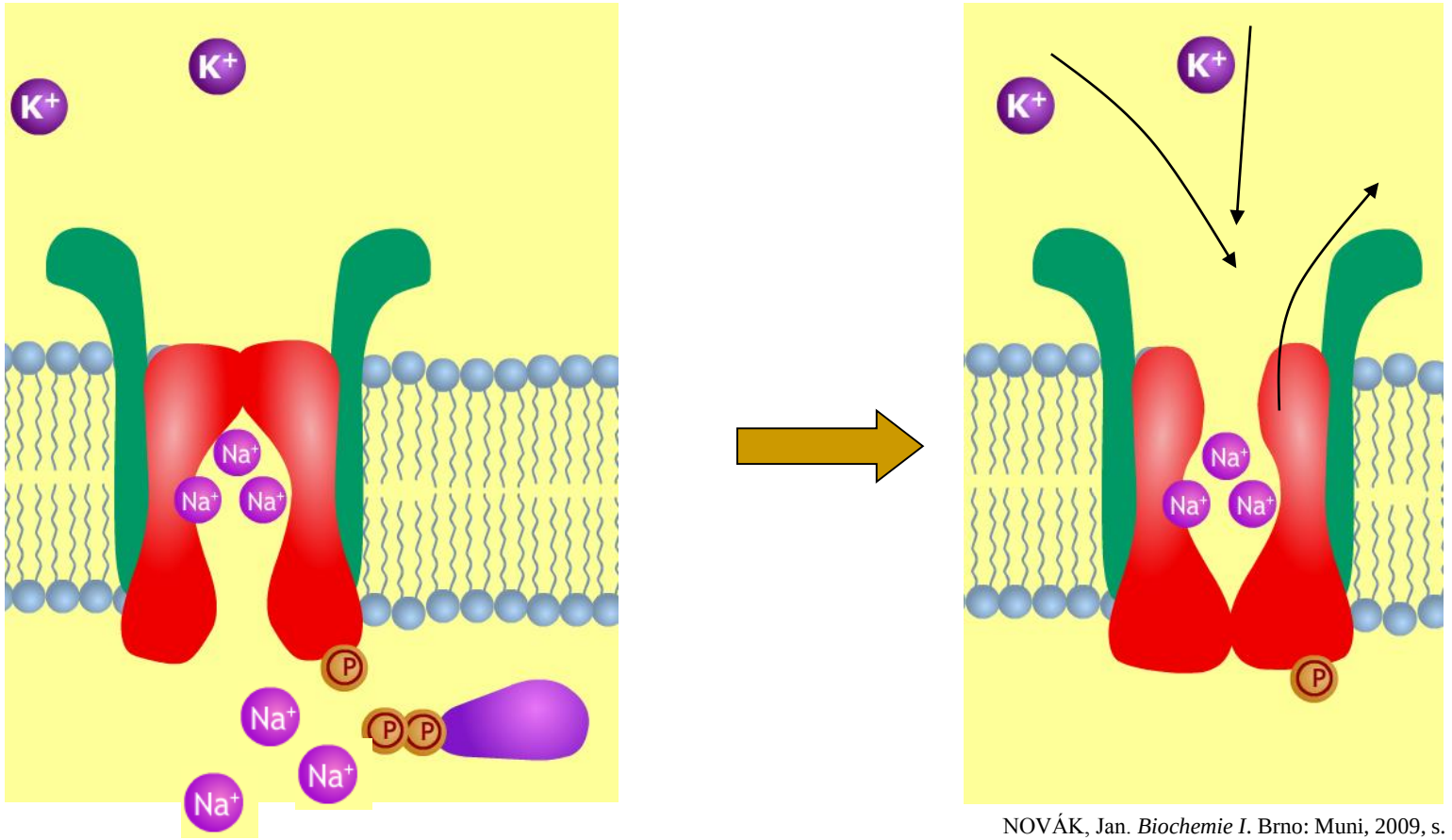
At the opposite pole of the cell (serosal side) is Na + over Na + / K + -ATPase is transported out of the cell (active transport)



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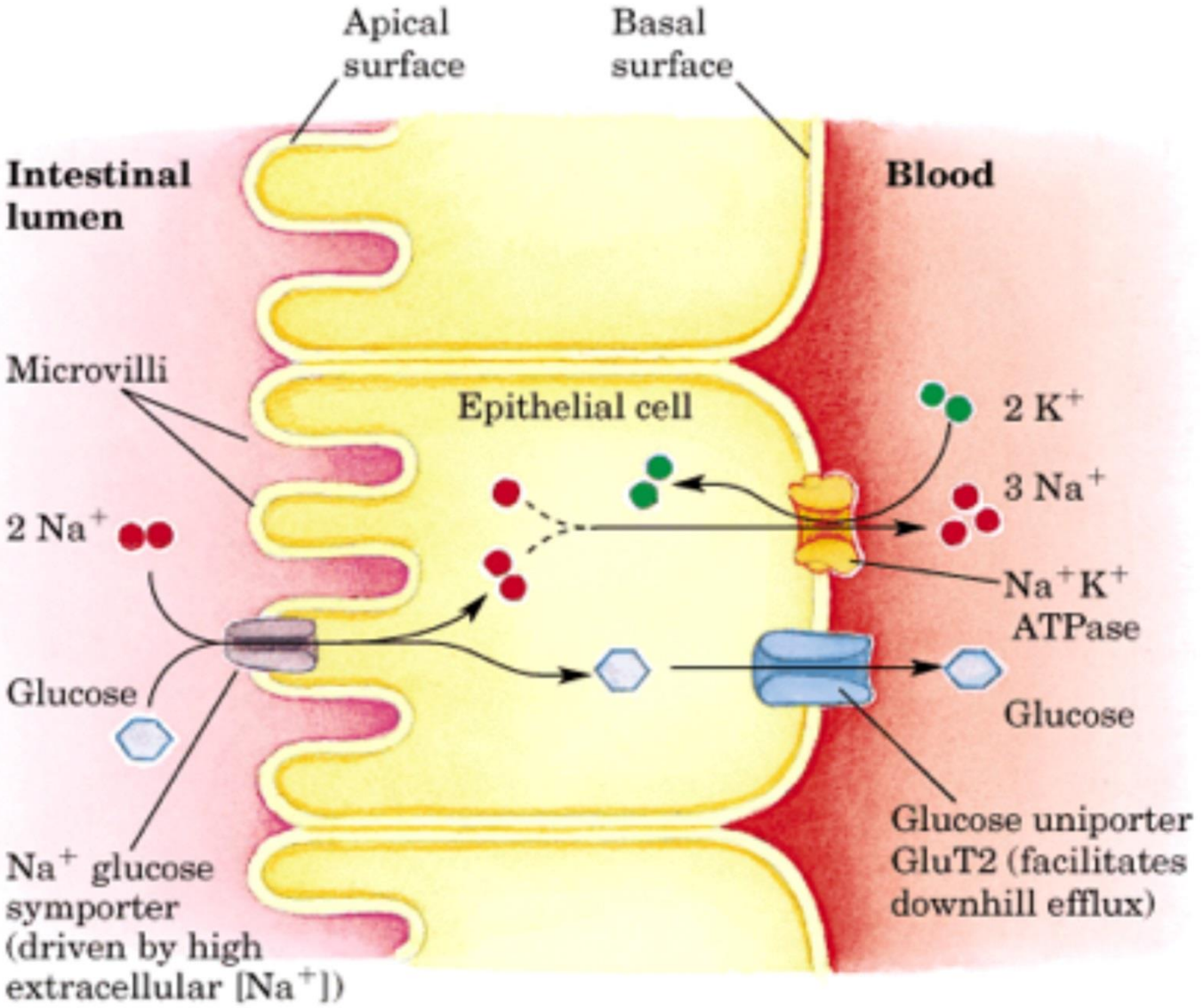
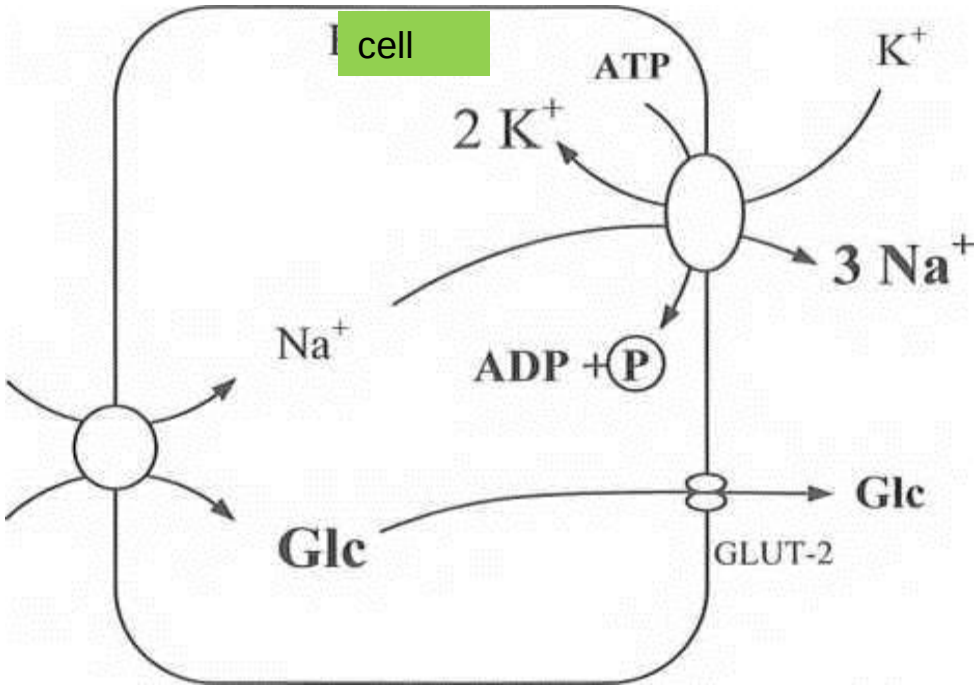
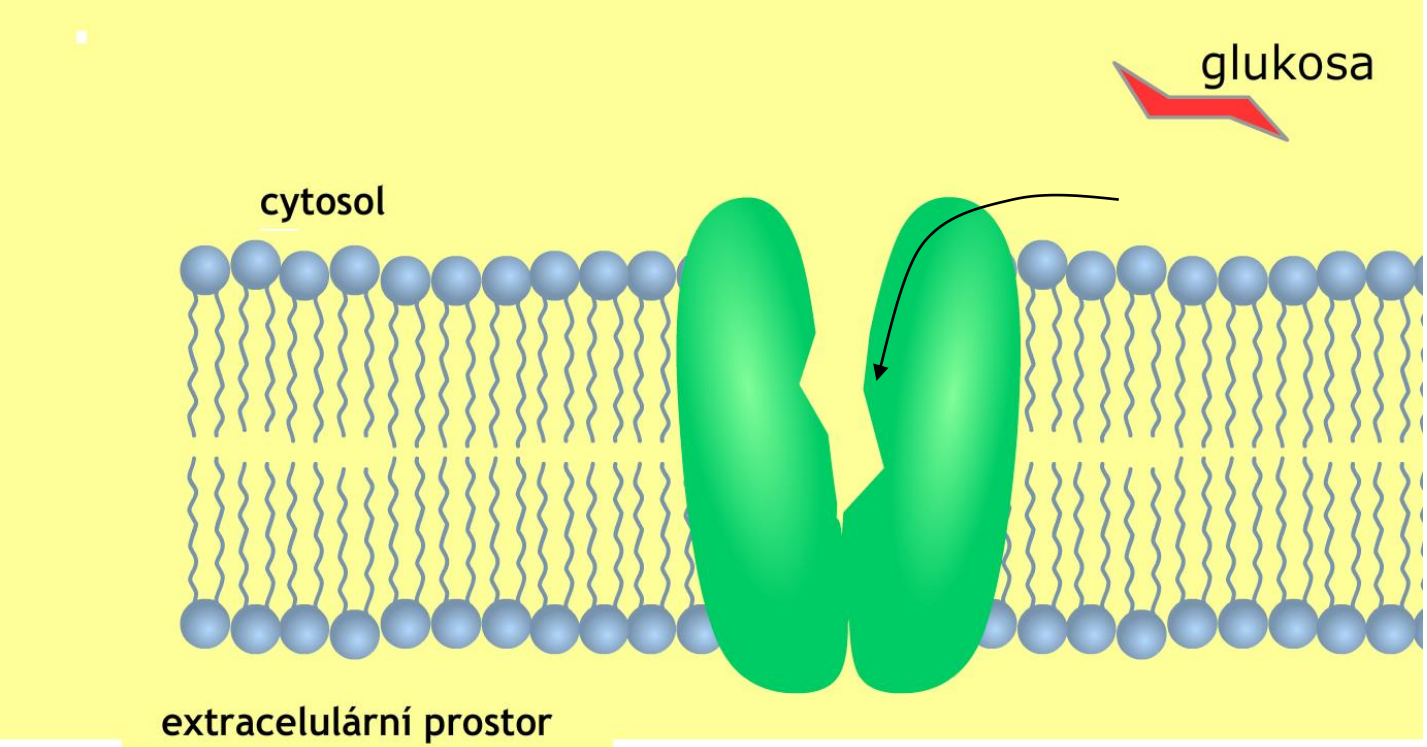

Apical surface
Basal surface
Intestinal lumen
Blood
Microvilli
Epithelial cell
2 K+
3 Na+
2 Na+
Na+K+ ATPase
Glucose
Glucose
Na+ glucose symporter (driven by high extracellular [Na+])
Glucose uniporter GluT2 (facilitates downhill efflux)


cell
ATP
K+
2 K+
3 Na+
Na+
ADP + P
Glc
Glc
GLUT-2

Glucose is the serosal side of the enterocyte transported out of the cell via GLUT -2 (passive transport)



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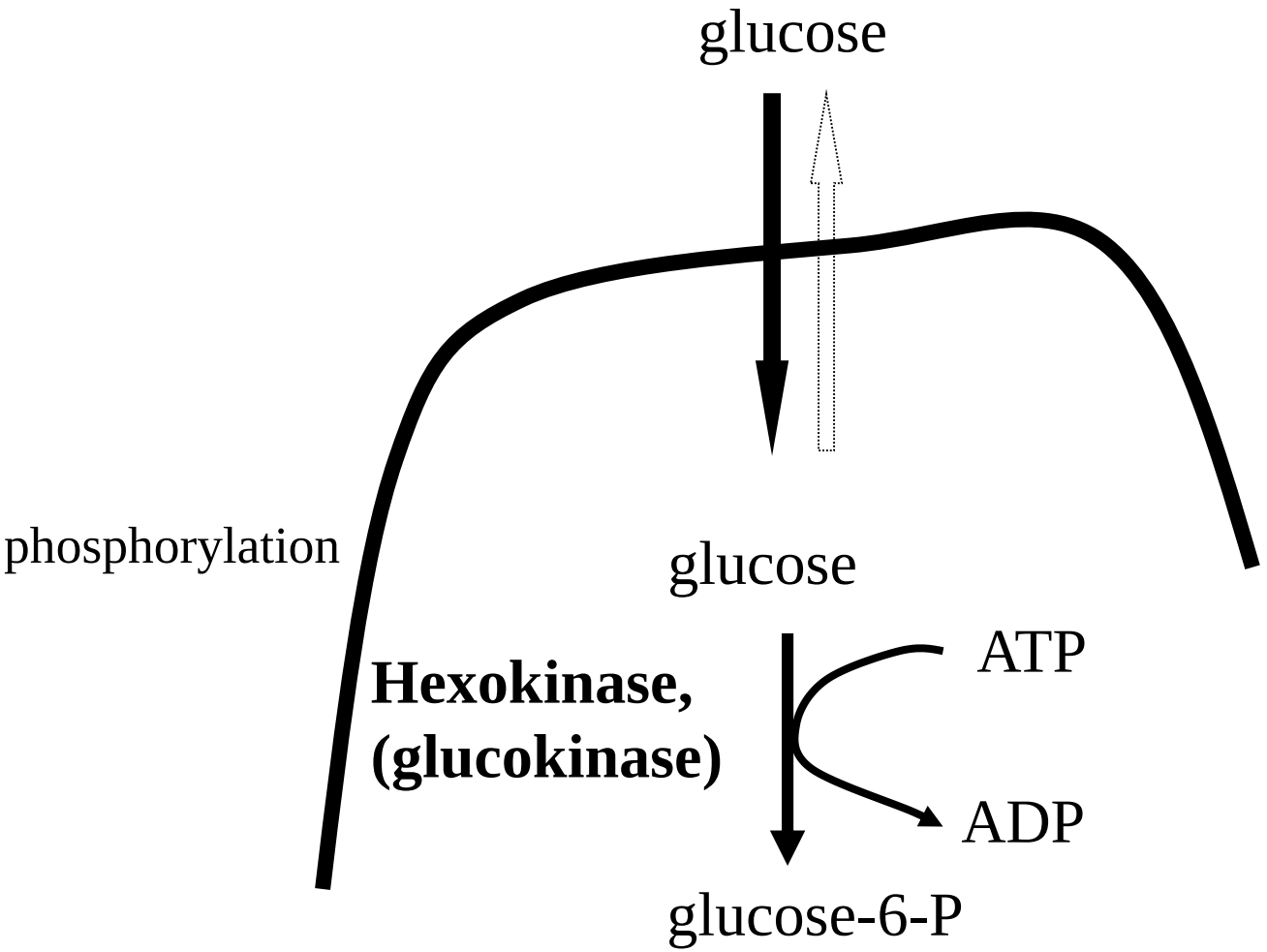
Glucose metabolism in cells

Formation of glucose-6- phosphate after the entry of glucose into the cell :

glucose + ATP ⟶ glucose-6-P + ADP

Enzymes **hexokinase or glucokinase**

Significance of the formation of glucose-6 -P for further glucose metabolism



Catalyzes the reverse reaction of glucose-6 - P phosphatase

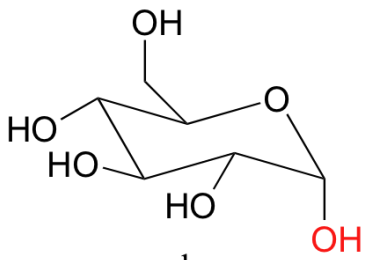
It is only in the liver (and kidneys)

Consequences:

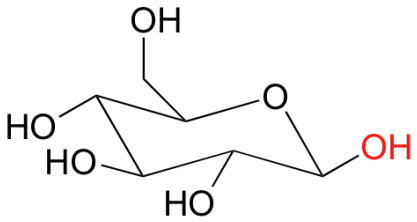
•The conversion of glucose to Glc -6-P in the cell allows additional supply of glucose along a concentration gradient

• Once phosphorylated glucose can not have a cell out (trap glucose)

• Only the liver (and kidneys) can be converted Glc -6-P back to glucose and send that back to the blood



α-glucose



β-glucose

SODERBERG, Tim. *ChemWiki: The Dynamic Chemistry E-textbook*. Dostupné z: http://chemwiki.ucdavis.edu/Organic_Chemistry/Organic_Chemistry_With_a_Biological_Emphasis/Chapter__3%3A_Conformations_and_Stereochemistry/Section_3.2%3A_Conformations_of_cyclic_organic_molecules

Glucose concentration and the rate of phosphorylation of glucokinase and hexokinase

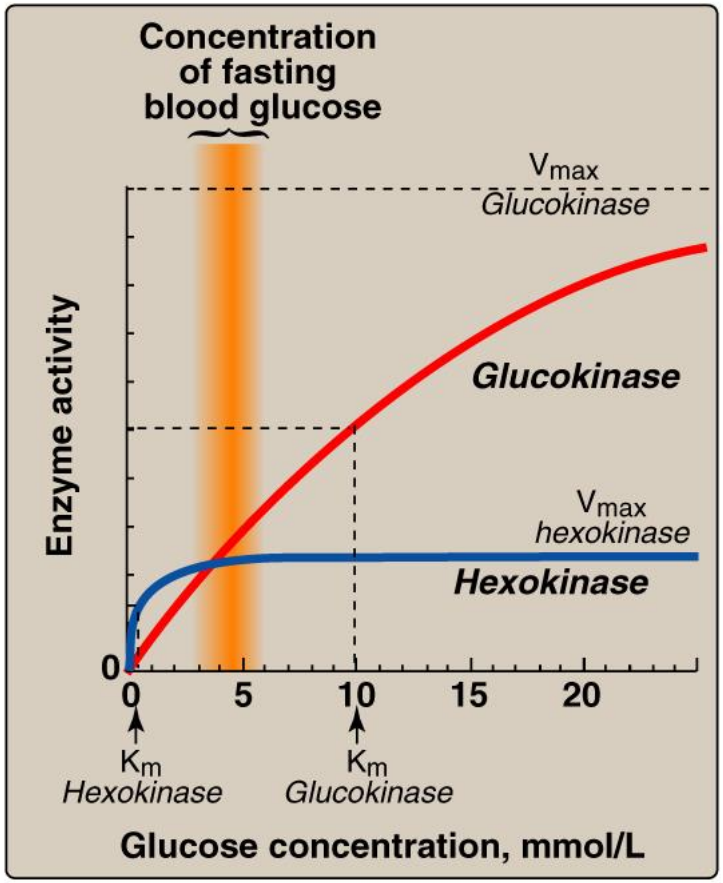


Figure 8.13
Effect of glucose concentration on the rate of phosphorylation catalyzed by *hexokinase* and *glucokinase*.

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glucokinase X HEXOKINASe

Characteristic	Hexokinase	glucokinase
location	Most tissues	Hepatocytes Islet cells (pancreas)
specify	broad (hexoses)	narrow (glucose)
inhibition	Glc-6-P	no inhibition
Affinity to Glc	high	low
inductive	no	insulin
K_M (mmol/l)	0,1	10

- phosphorylation of glucokinase in the liver occurs at a sufficient supply of glucose in the liver (after meal)
- at lower concentrations it hexokinase

- phosphorylation in cells other than hepatic (hexokinase) takes place only if the glucose to be metabolized

Role glukokinasy v pankreatu

- glucokinase in the pancreatic cells β- sensor in blood glucose
- At elevated blood glucose levels , glucose enters into cells of the pancreas (GLUT2) and is phosphorylated by glucokinase
- Another glucose metabolism mediated insulin release from cells β

Transformation of Glc-6P in cell and significance

pathway	significance
glycolysis	Energy, transformation of acetylCoA to FA
Synthesis of glycogen	Storage of Glc
Pentose cycle	Source of pentose, source of NADPH
Synthesis of Glc derivates	Synthesis of glycoprotein's and proteoglycans

GLYCOLYSIS

- Glycolysis occurs in almost every living cell.
- It occurs in cytosol.
- It was the first metabolic sequence to be studied.
- Most of the work done in 1930s by the German biochemist G. Embden Meyerhof Warburg.
  - That is why it is also called Embden-Meyerhof pathway.
- It is a Greek word.
  - Glykos → sweet
  - Lysis → loosing
  - Glycolysis → loosing or splitting of glucose

Glycolysis

Glykos (sugar) lysis (cleavage)

- Meaning : energy yield , production of other substances , including metabolism, galactose and fructose
- In virtually all cells
- Location: cytoplasm
- Reversible enzyme- catalyzed reactions
- Three reactions are irreversible

aerobic glycolysis

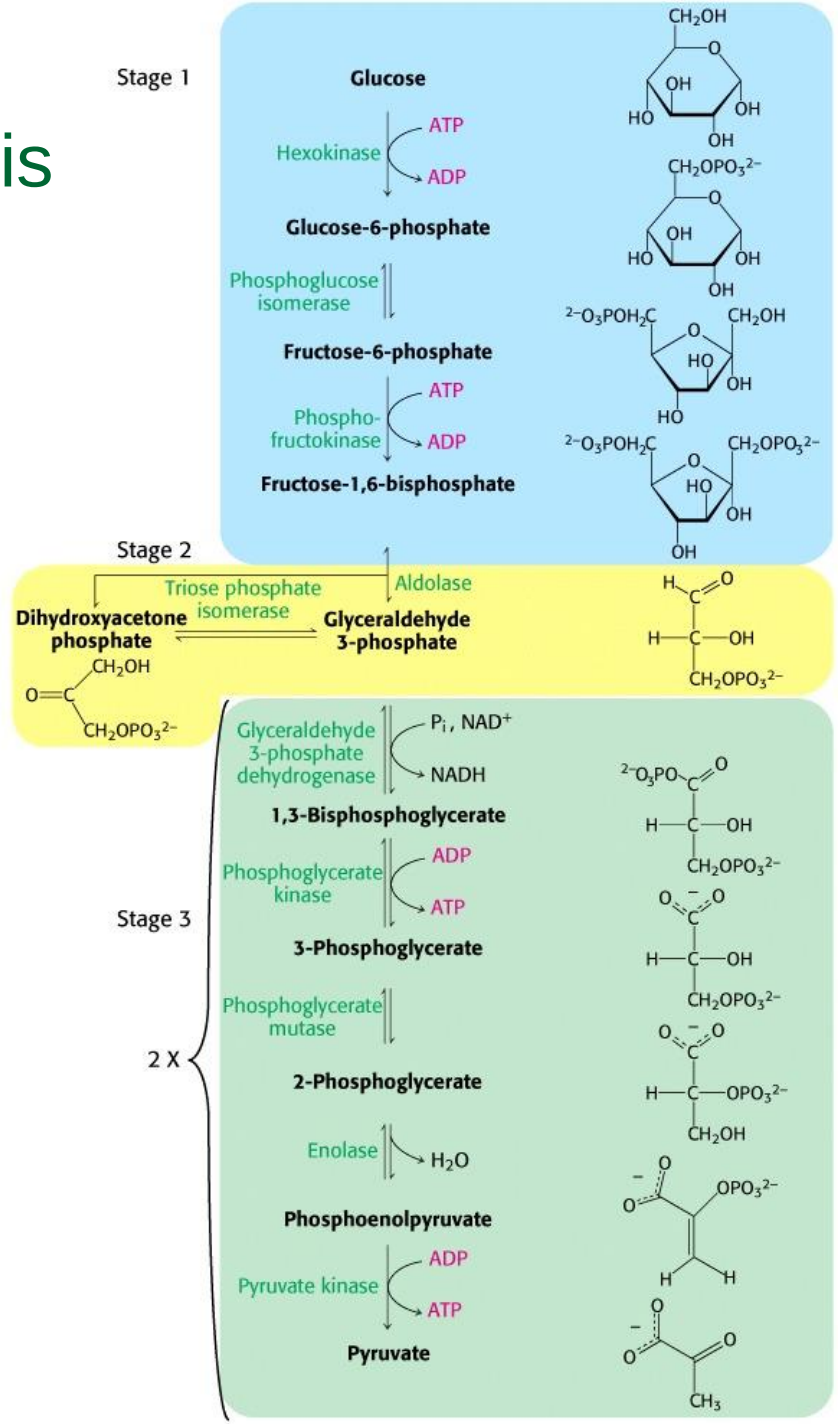
oxygen , pyruvate is converted to <u>acetyl-CoA</u>

anaerobic glycolysis

in the absence of oxygen , pyruvate is converted into <u>lactate</u>

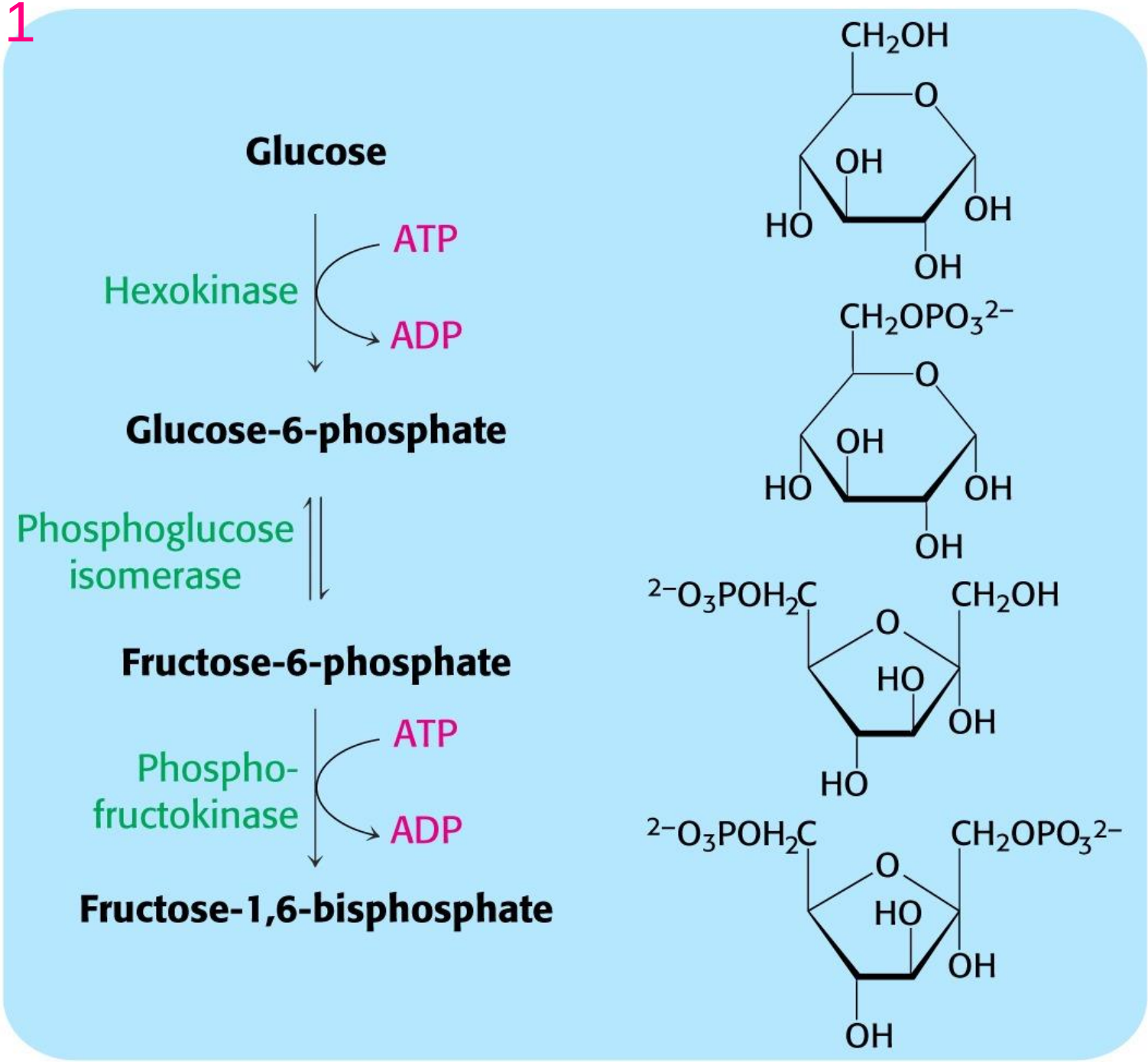
Stages of Glycolysis

Three stages

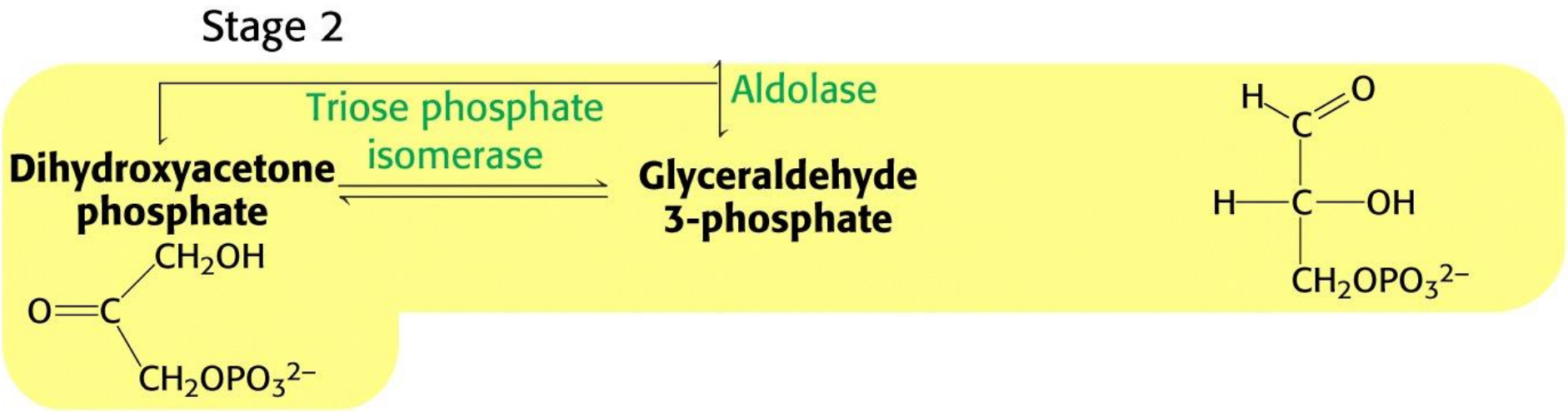


Glycolysis: 1

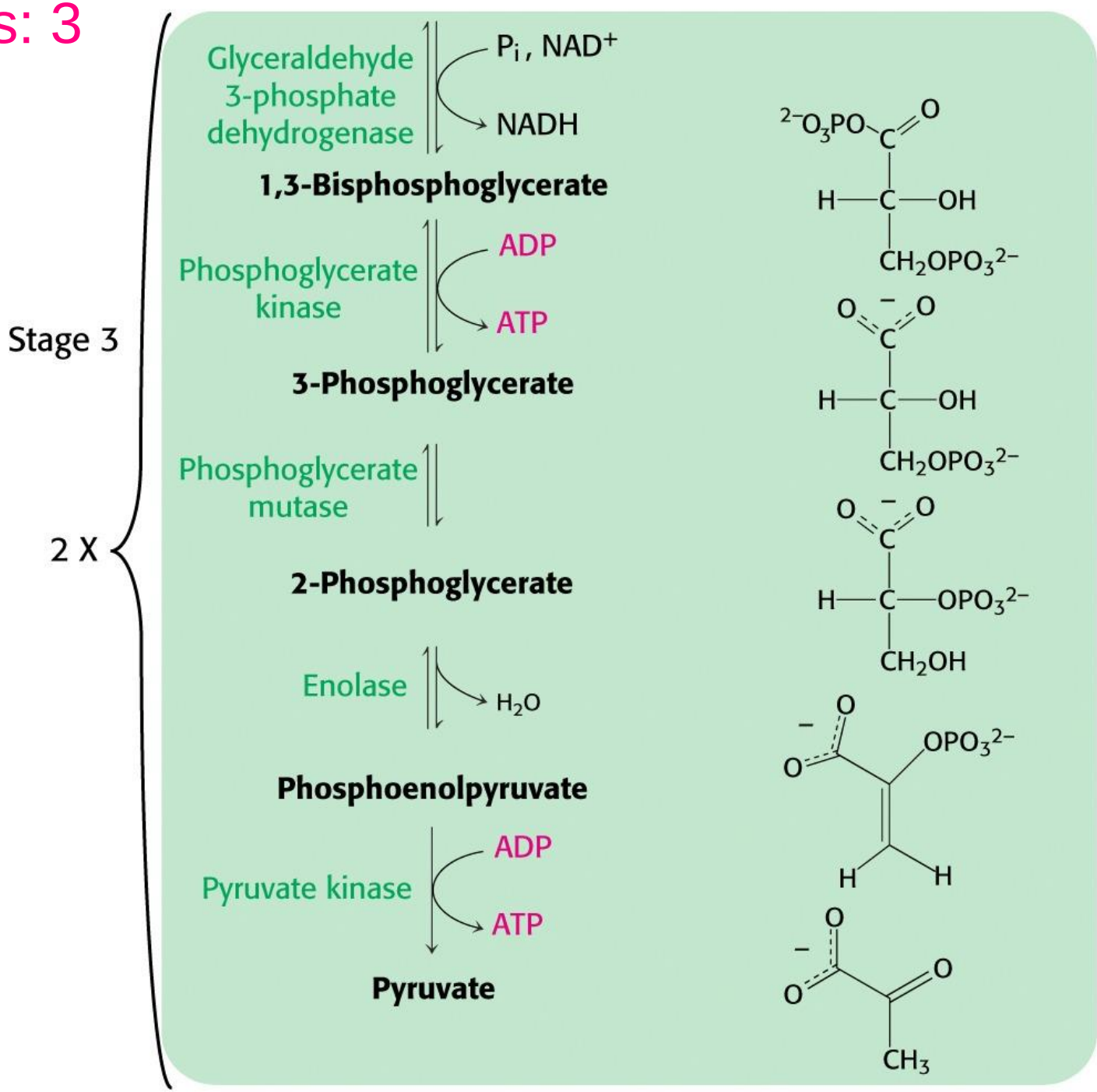
Stage 1



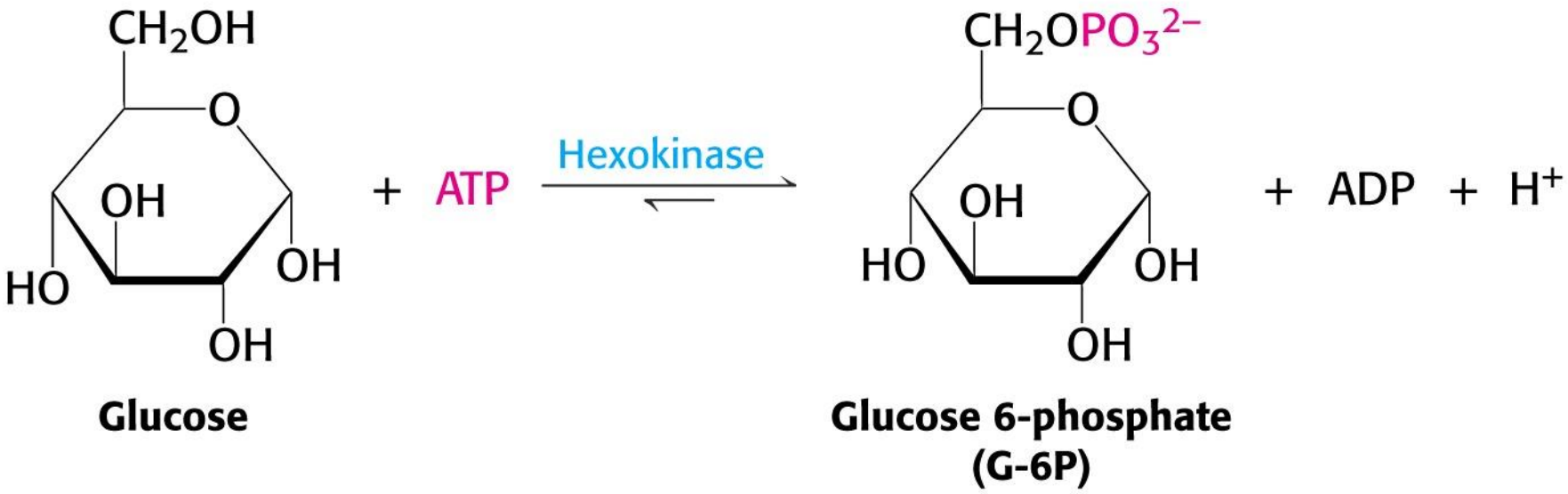
Glycolysis: 2



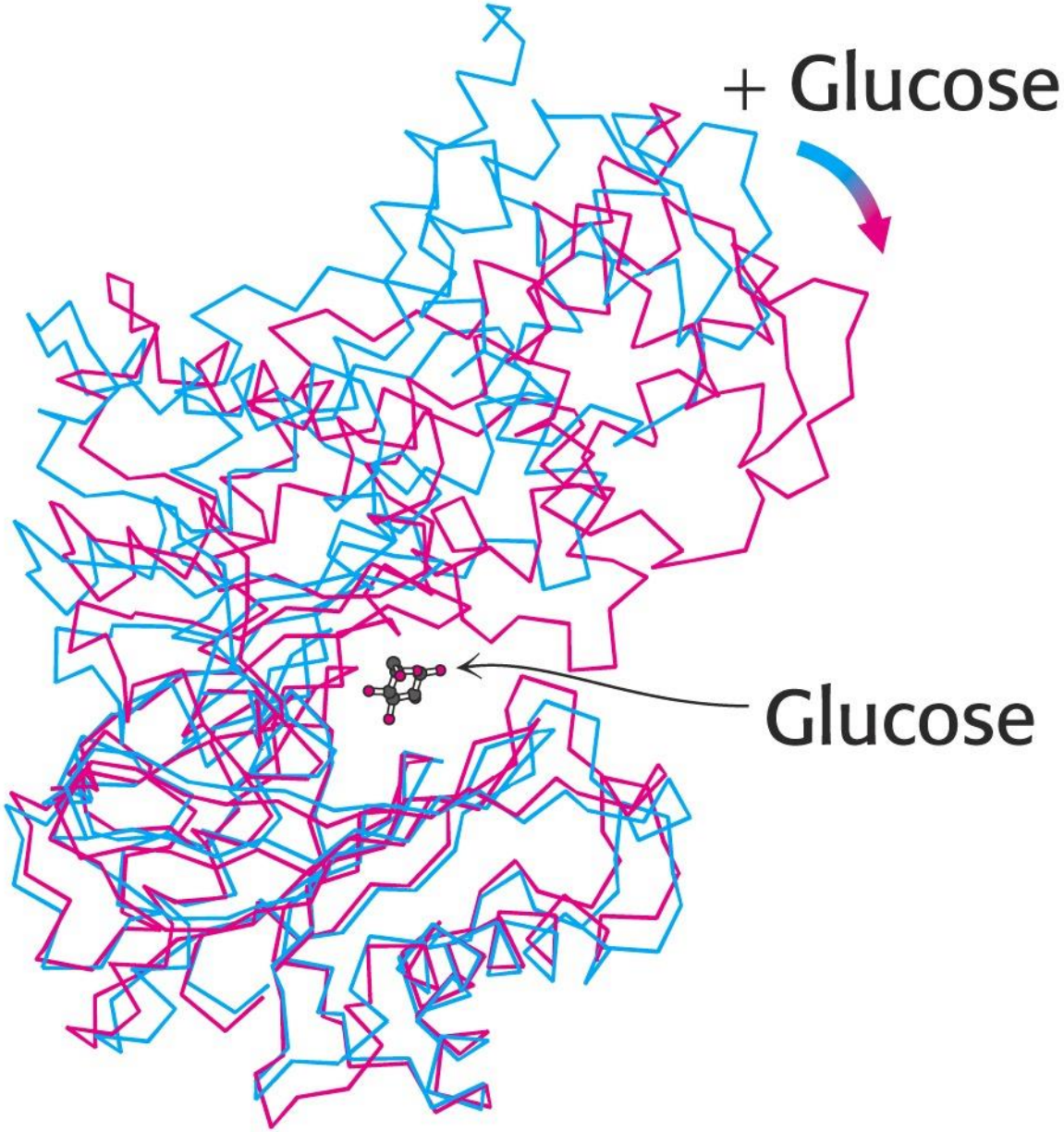
Glycolysis: 3



Glucose phosphorylation: step 1



Induced fit in hexokinase



Conformation changes on binding glucose,

the two lobes of the enzyme come together and surround the substrate

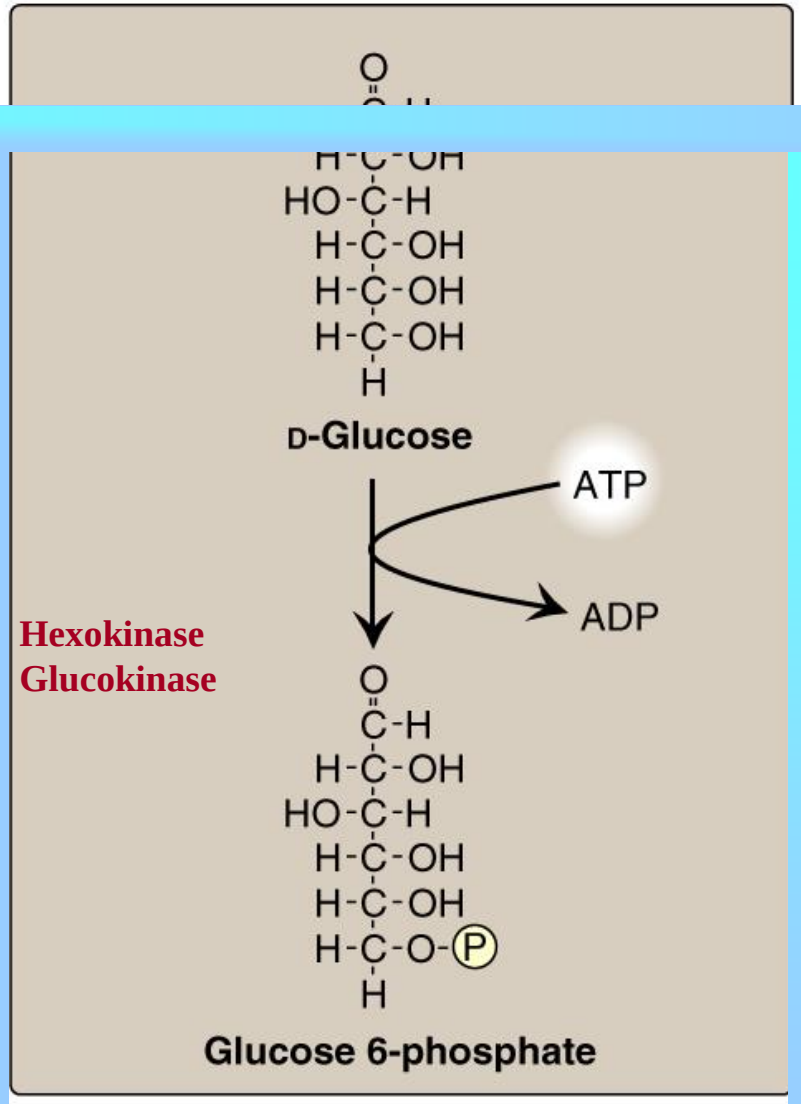


Figure 8.12

Energy investment phase: phosphorylation of glucose.

	Hexokinase	Glucokinase
Site	Most tissues	Hepatocytes Islet cells (pancreas)
Kinetics	Low Km (0.1mmol/l) Low Vmax	High Km (1mmol/l) High Vmax
Regulation	G-6-phosphate	F-6-phosphate Insulin: Induction
Function	Low glucose conc.	High glucose conc. Glucose sensor

glucokinase X HEXOKINASe

Characteristic	Hexokinase	glucokinase
location	Most tissues	Hepatocytes Islet cells (pancreas)
specify	broad (hexoses)	narrow (glucose)
inhibition	Glc-6-P	no inhibition
Affinity to Glc	high	low
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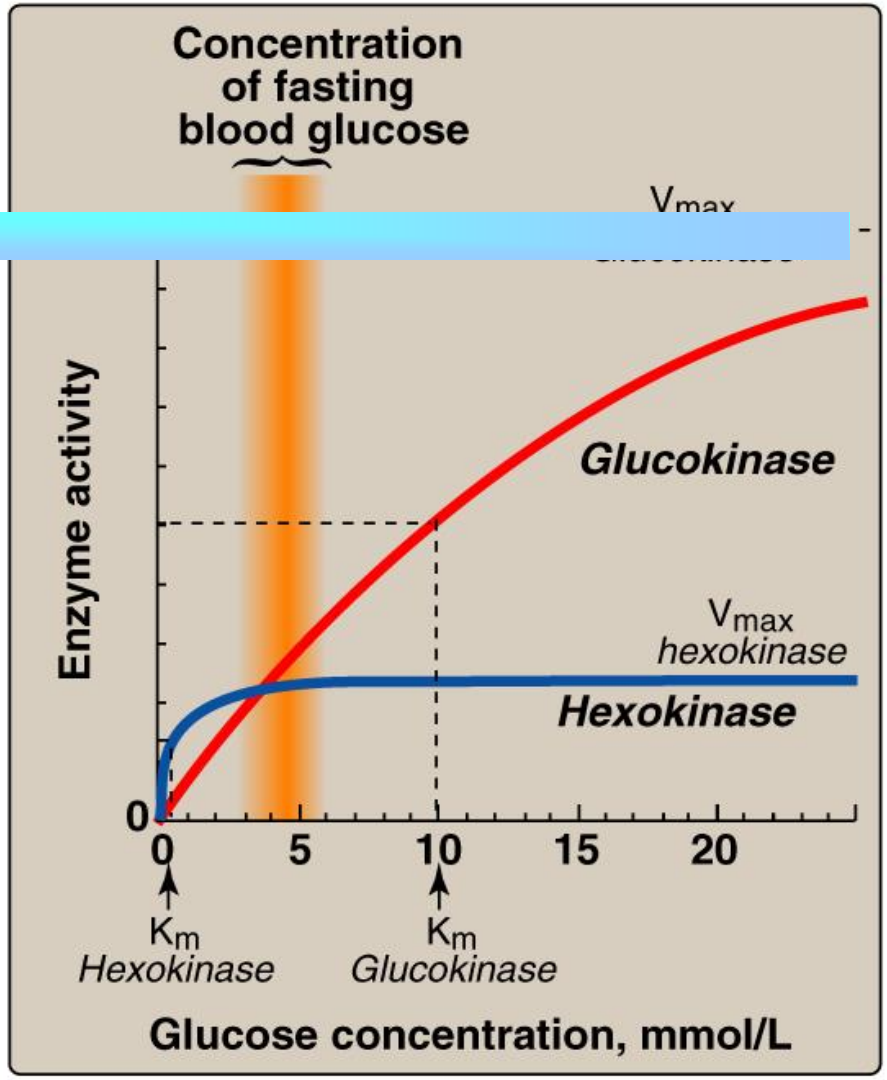


Figure 8.13
Effect of glucose concentration on the rate of phosphorylation catalyzed by *hexokinase* and *glucokinase*.

Difference between hexokinase and glucokinase

- Hexokinase:
  - Its function is to make sure there is enough glc for the tissues, even in the presence of low blood glc concentrations, by phosphorylating all the glc concentration gradient between the blood and the intracellular environment.
- Glucokinase:
  - Its function is to remove glc from the blood following a meal.
- **Hexokinase, phosphofructokinase and pyruvate kinase are 3 regulatory enzymes of glycolysis.**

Hexokinase vs glucokinase

- Liver has an additional enzyme, glucokinase, that phosphorylate only glc

1. Glucokinase has a high Km, because it phosphorylates glc only when its concentration is high. This occurs during the brief period after consumption of a carbohydrate rich meal, when high glc are delivered by portal vein.

2. Glucokinase has a high Vmax, allowing the liver to remove effectively this flood of glc from the portal blood. So this prevents extreme hyperglycemia after meals.

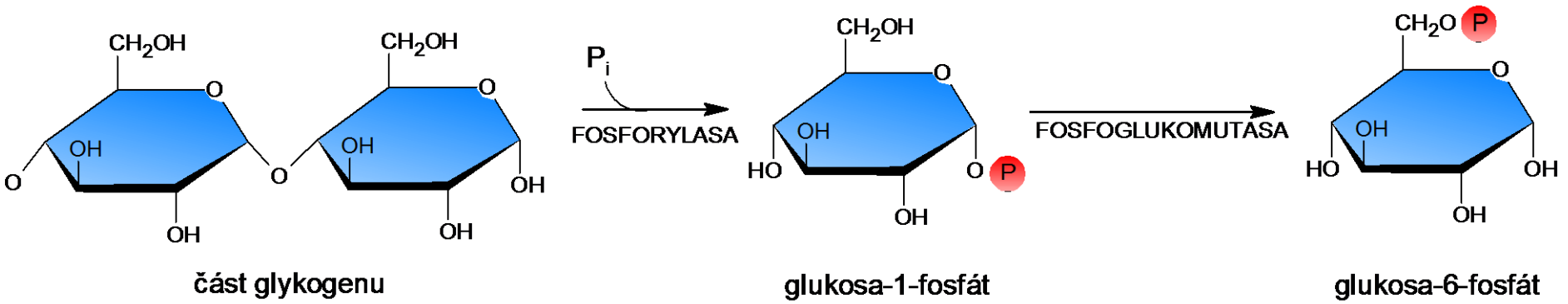
SUMMARY

	Hexokinase	Glucokinase
Tissue dist	all	liver
Km	low	high
Vmax	low	high
Substrate	D-glc and other	D-glc only
Inhibition by G-6-P	Yes	no

More about HK

- Hexokinase, like adenylate kinase and all other kinases, requires Mg (or Mn) for activity.
- Hexokinase is also one of the induced-fit model enzymes.
  - It has two lobes that move towards each other when Glc is bound!
- Substrate-induced cleft closing is a general feature of kinases.
- Other kinases (Pyruvate kinase, phosphoglycerate kinase and PFK) also contain clefts between lobes that close when substrate is bound.

Formation of Glc-6-P: cleavage of glycogen (without ATP)



část glykogenu

glukosa-1-fosfát

glukosa-6-fosfát

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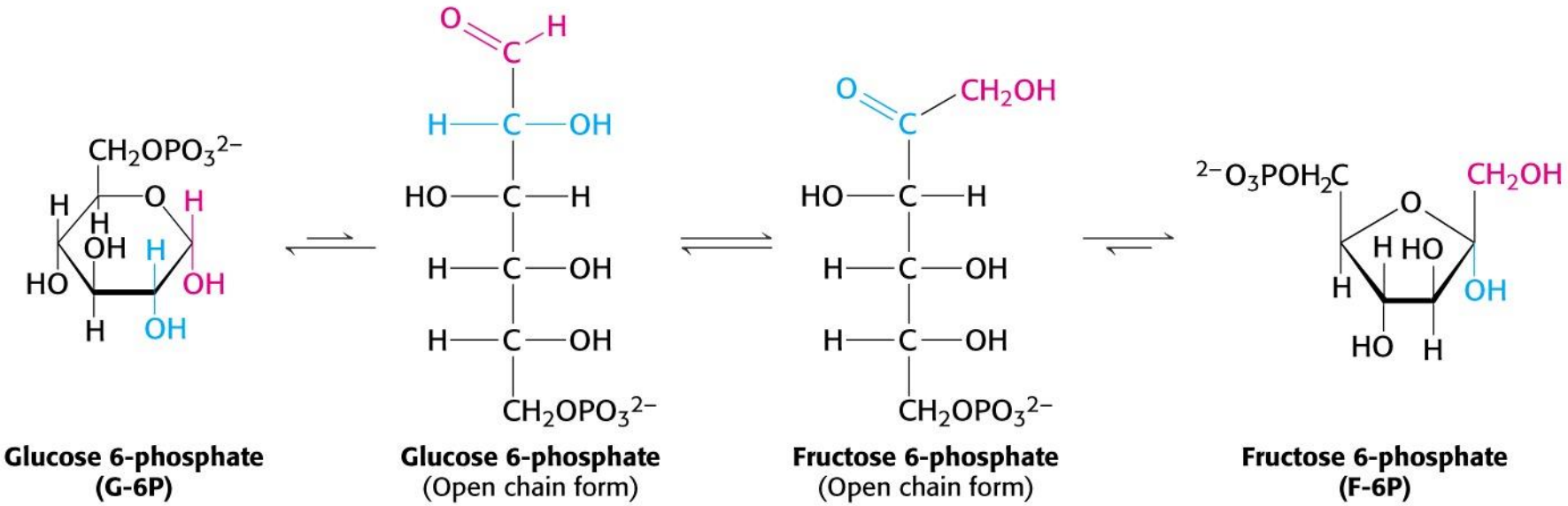
Formation of fructose-6-phosphate: step 2

by phosphoglucose isomerase



Conversion of an aldose to a ketose

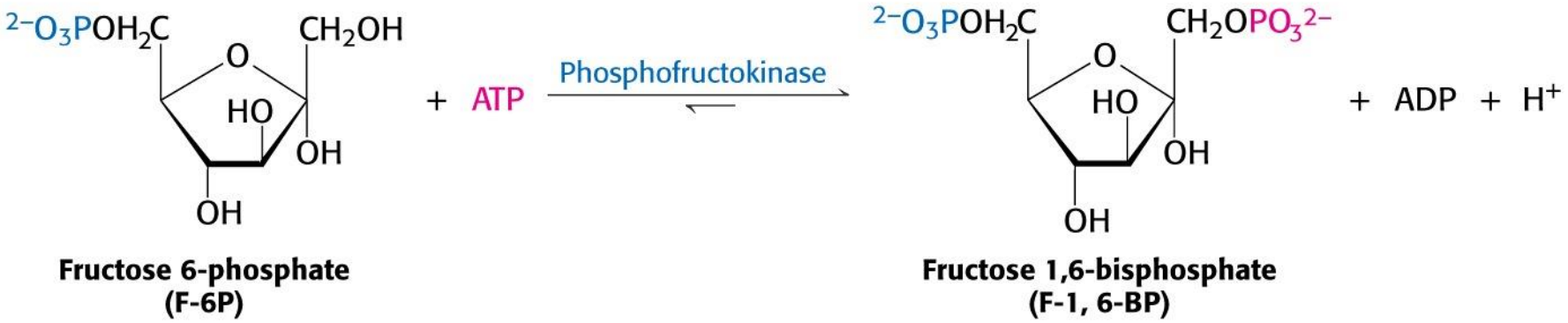
2. ISOMERIZATION OF G-6-P

- The isomerization of G-6-P (an aldose sugar) to F-6-P (a ketose sugar) is catalyzed by phosphoglucose isomerase. The reaction is readily reversible, is NOT a rate limiting or regulated step.

3. PHOSPHORYLATION OF F-6-P

- Irreversible phosphorylation reaction catalyzed by PFK (phosphofructokinase) is the most important control point of glycolysis.
- Within the cell, the PFK reaction is the rate-limiting step in the glycolytic breakdown of glc. It is controlled by the concentrations of the substrates ATP and F-6-P

by phosphofructokinase (PFK): an allosteric enzyme
that regulates the phace of glycolysis.



Fructose 6-phosphate (F-6P)

Fructose 1,6-bisphosphate (F-1, 6-BP)

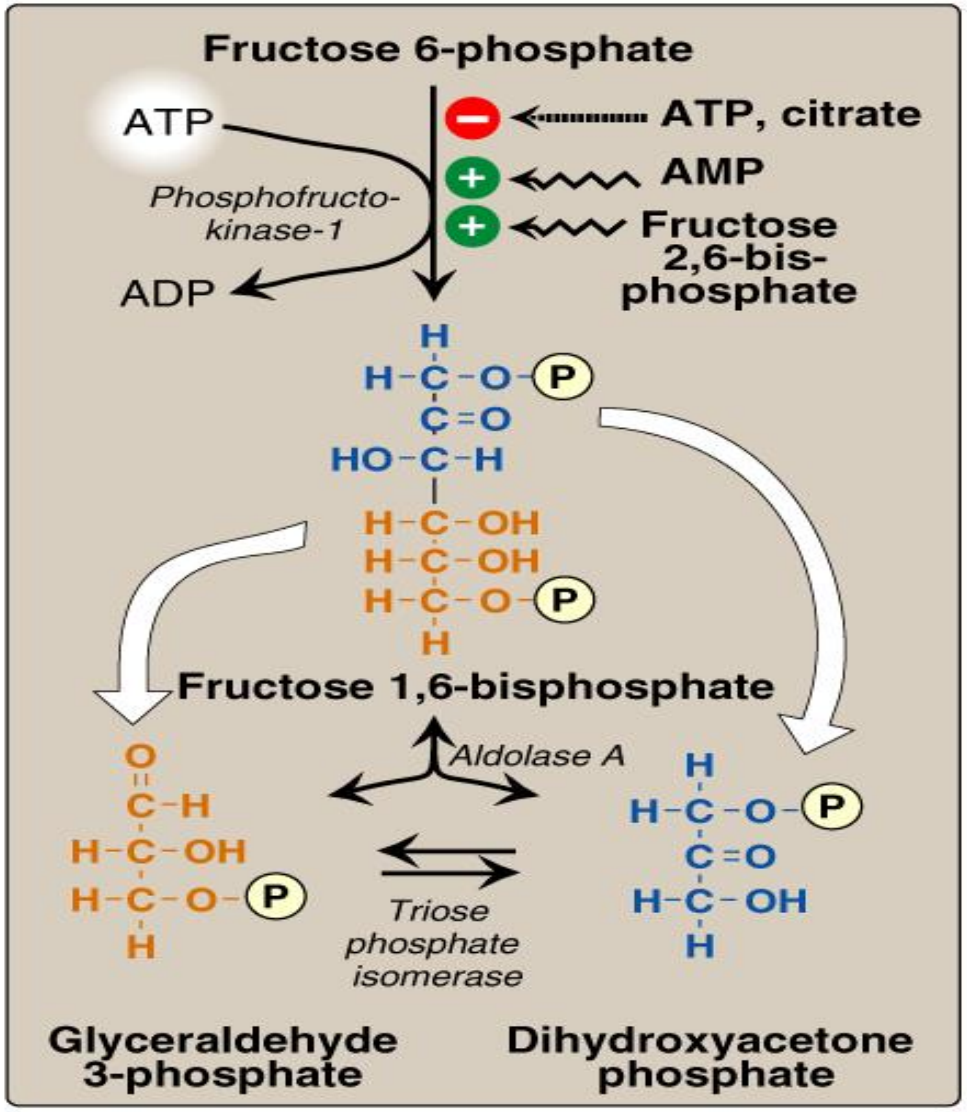
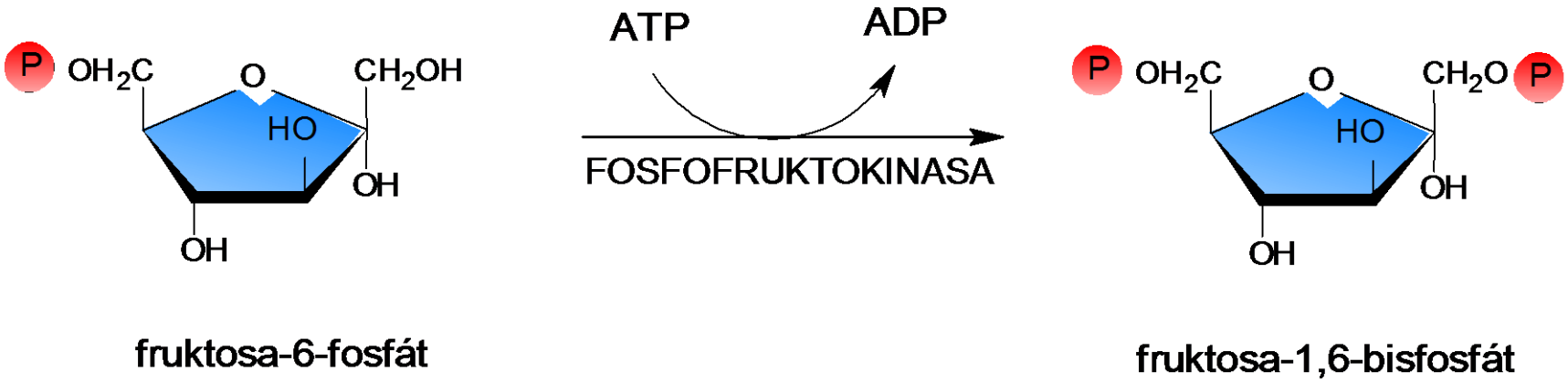


Figure 8.16

Energy investment phase (continued): Conversion of fructose 6-phosphate to triose phosphates.

3. Formation of fructose 1,6-bisphosphate



fruktosa-6-fosfát

fruktosa-1,6-bisfosfát

The rate of reaction is determinative of the rate of glycolysis whole

NOVÁK, Jan. *Biochemie I*. Brno: Muni, 2009, s. 82.

Principles of regulation phosphofructokinase

allosteric inhibition of ATP and citrate

allosteric activation of AMP , ADP and hepatic fructose 2,6 - bisphosphate - *

(* Fru - 2,6- Bispo arises under hormonal control)

What are allosteric enzymes ?

Properties of enzymes which limit the rate of reaction

- slowest enzyme pathway
- operating at Vmax (the only way to increase the speed of response is to add more enzyme - no more substrate). The rate of reaction is independent of [S].
- the reaction is irreversible (the reaction takes place in the opposite direction , it is necessary the action of a different enzyme). Other enzymes tracks may be reversible.

The regulatory effect of fructose -2,6- biP in glycolysis and gluconeogenesis in the liver

2,6-biP is **allosteric** efector

Phosphofructokinase
(glycolysis)
Activation

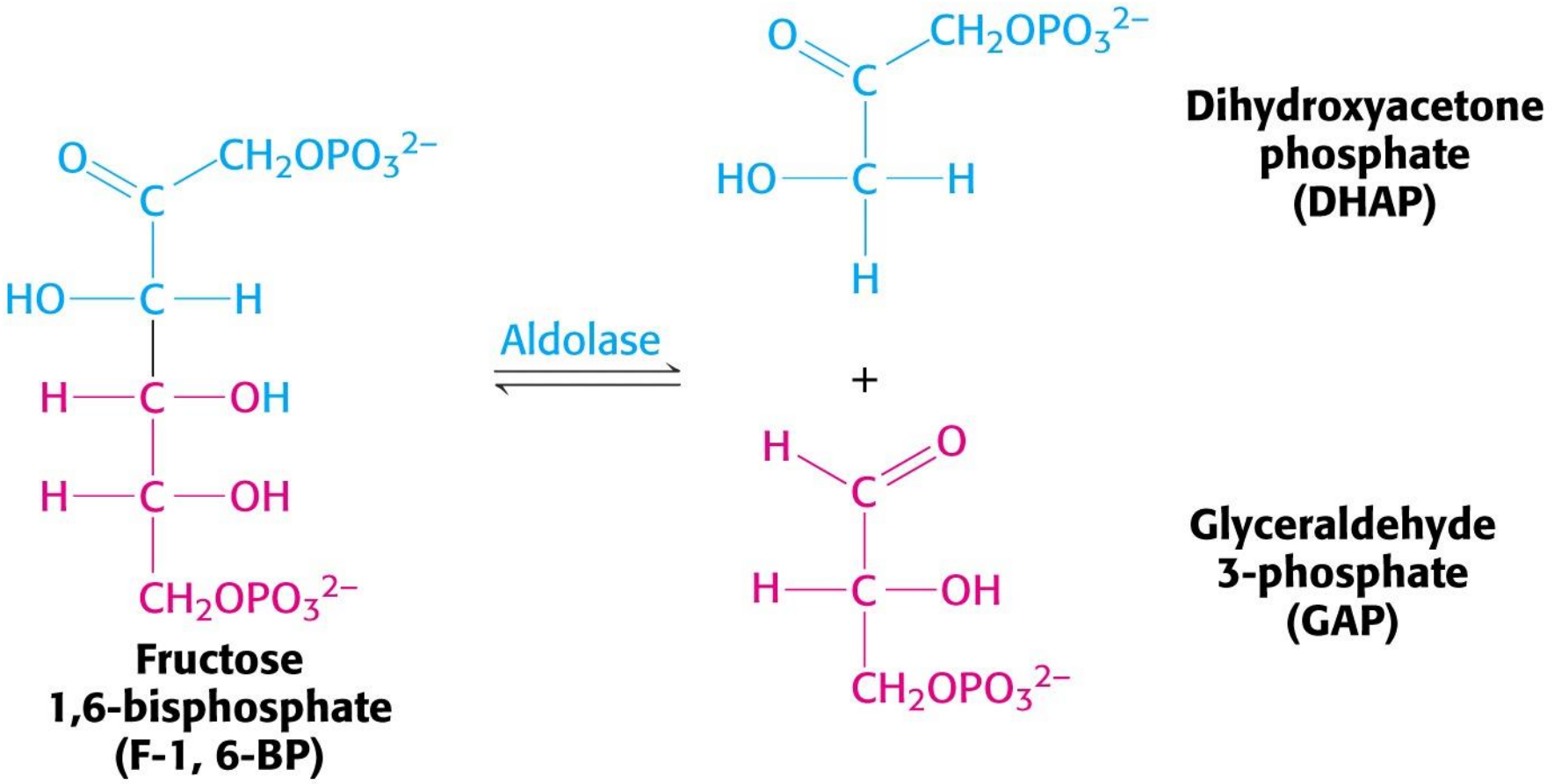
fructosa 1,6-bisphosphatase
(gluconeogenesis)
Inhibition

Formation of fructose-2,6-biP

↑ Stimulation of fructosa-6P

↓ inhibition glucagon

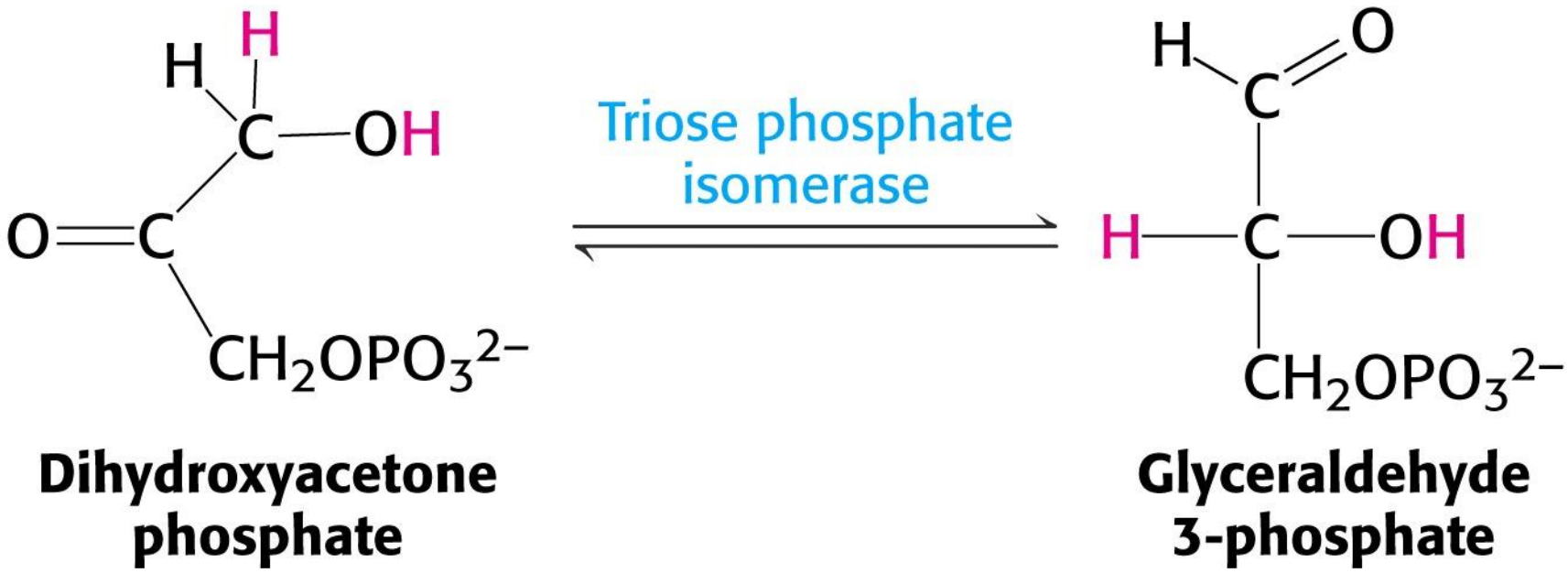
Cleavage of six-carbon sugar: step 4



5. ISOMERIZATION OF DIHYDROXYACETONE-P

- Triose phosphate isomerase (TIM) catalyzes the reversible interconversion of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate.
- X-ray crystallographic and other studies showed that Glu 165 plays the role of a general acid-base catalyst.
- TIM has 8 parallel beta and 8 alpha helices ($\alpha\beta$ barrel). This structure is also found in
  - Aldolase
  - Enolase
  - Pyruvate kinase

Salvage of three-carbon fragment: step 5



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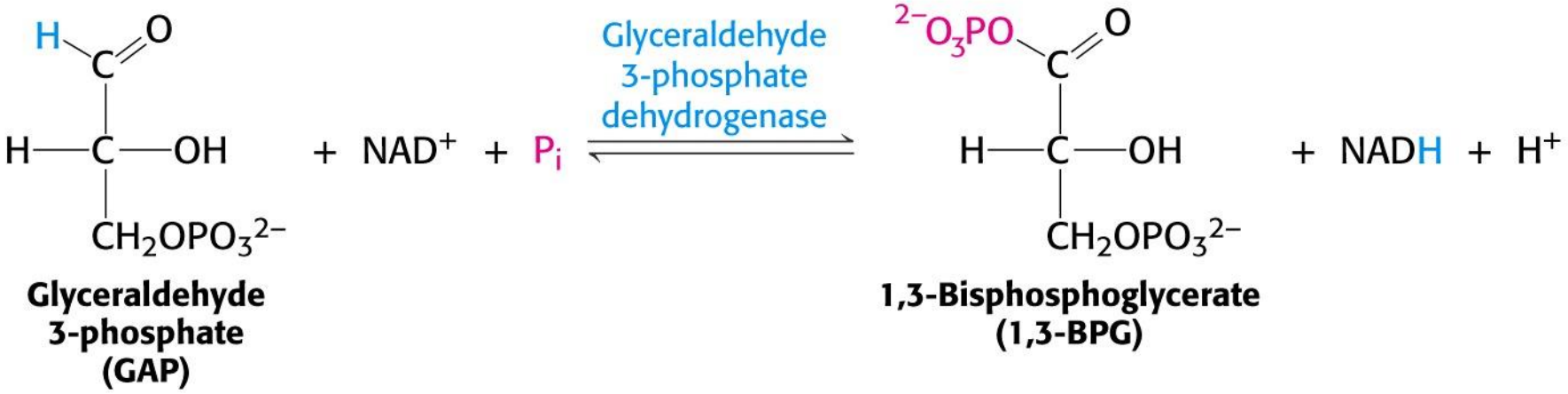
- TIM has 8 parallel beta and 8 alpha helices ($\alpha\beta$ barrel). This structure is also found in
  - Aldolase
  - Enolase
  - Pyruvate kinase

6. OXIDATION OF GLYCERALDEHYDE 3-P.

- Because there is only a limited amount of NAD+ in the cell, the NADH formed in this reaction must be reoxidized back to NAD+ for glycolysis to continue. Two major mechanisms for oxidizing NADH are
  - The NADH-linked conversion of pyruvate to lactate
  - Oxidation by the respiratory chain.
- The high energy P group at carbon of 1,3-bisPG conserves much of the free energy produced by the oxidation of Glycerate-3-P

Formation of 1,3-Bisphosphoglycerate: step 6

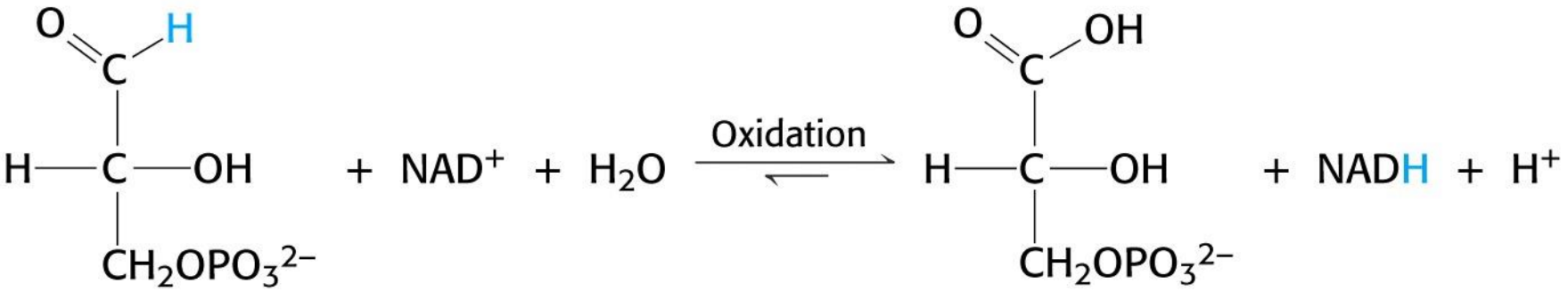
Done in two steps

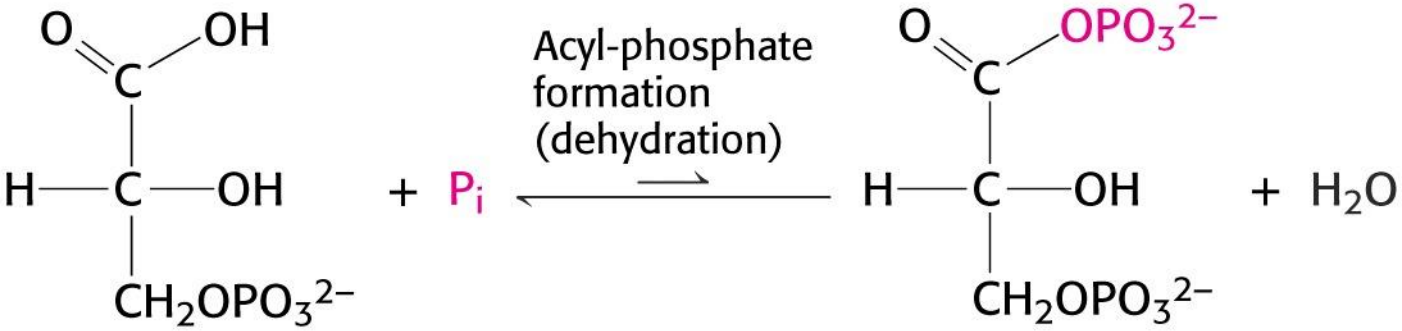


Two-process reaction

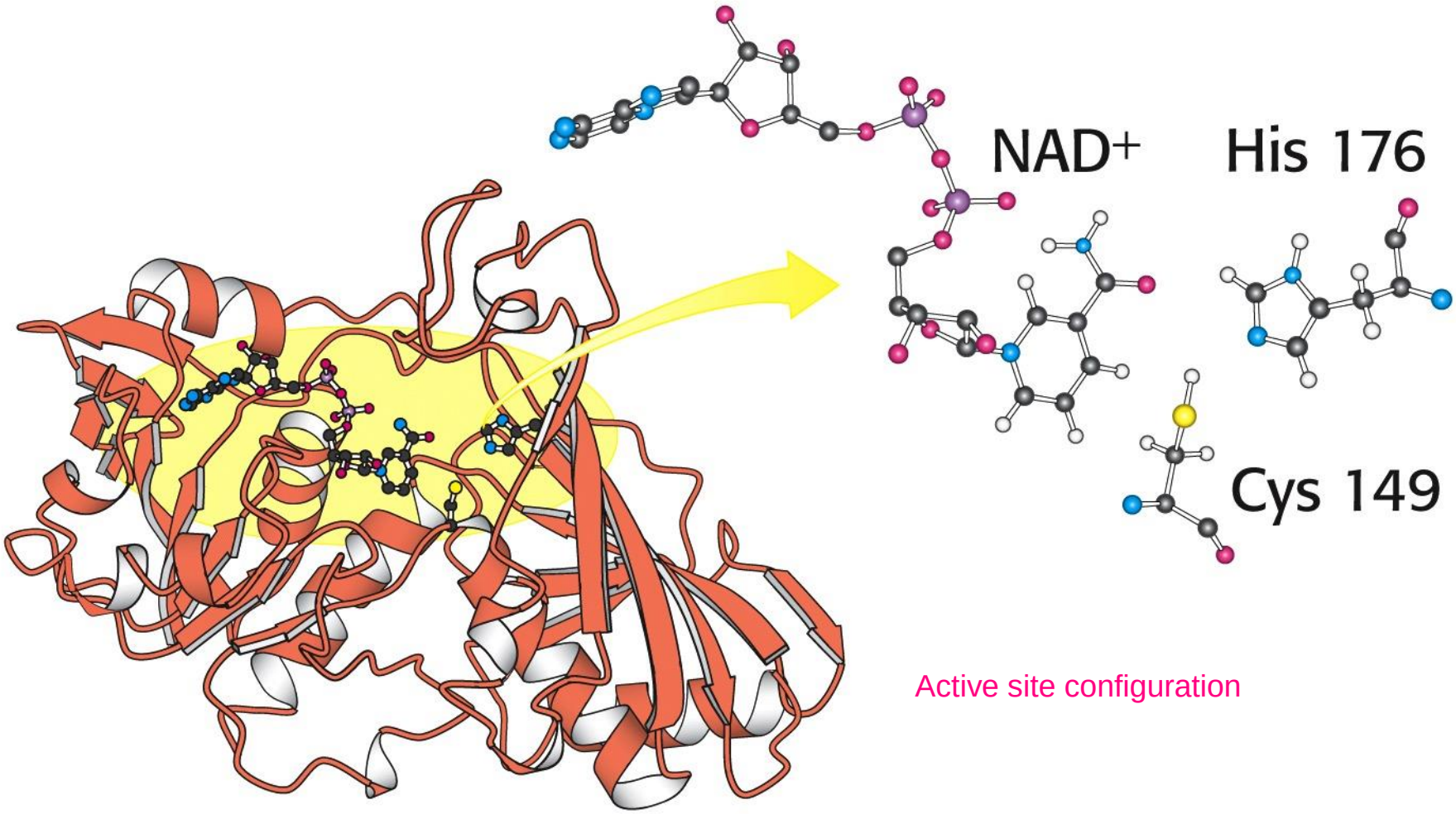
Aldehyde

Acid





Glyceraldehyde 3-phosphate dehydrogenase



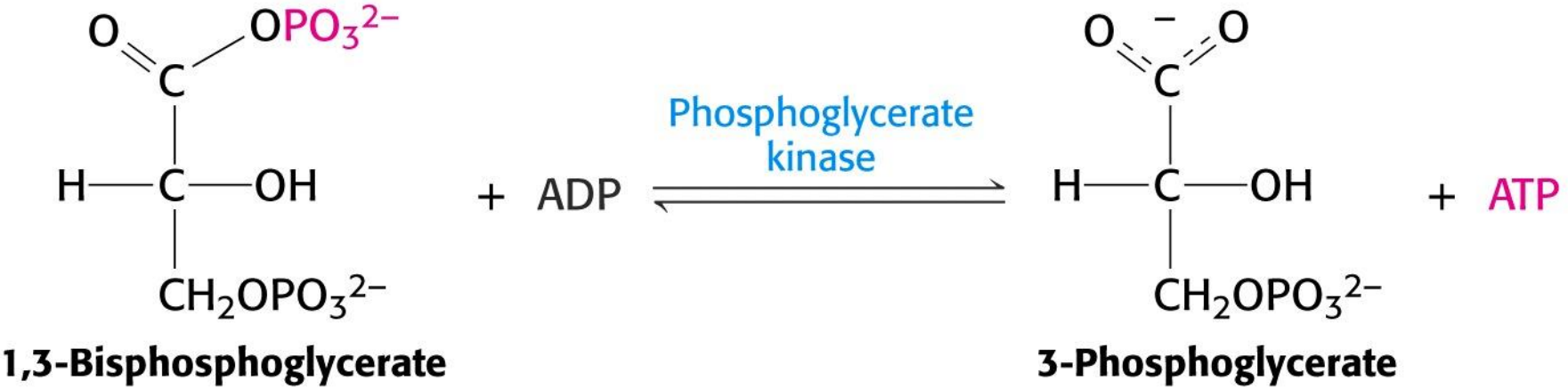
Active site configuration

7: FORMATION OF ATP FROM 1,3-BisPGLYCERATE AND ADP

- This step is a substrate-level phosphorylation in which the production of a high-energy P is coupled to the conversion of substrate to product, instead of resulting from oxidative phosphorylation. The energy trapped in this new high-energy P will be used to make ATP in the next reaction of glycolysis. The formation of ATP by P group transfer from a substrate such as 1,3-bisphosphoglycerate is referred to as a substrate-level phosphorylation. Unlike most other kinases, this reaction is reversible.
- 2 mols 1,3biPGlycerate → 2ATP replaces the 2ATP consumed earlier with the formation of G-6-P and fructose 1,6bisP.

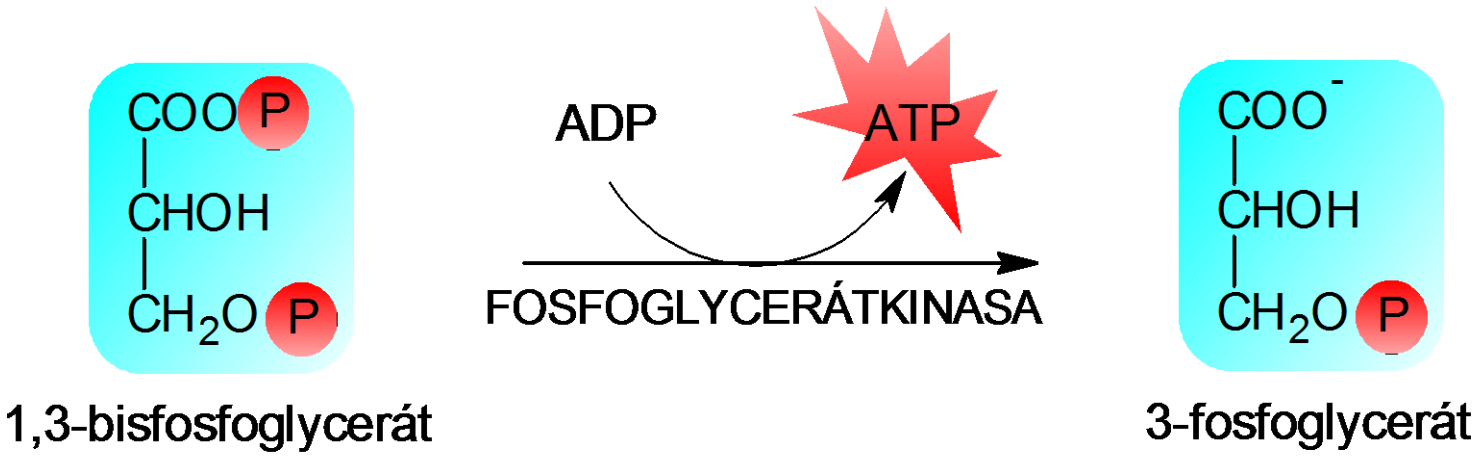
Formation of ATP from 1,3-Bisphosphoglycerate: step 7

High phosphoryl-
transfer potential



1,3-Bisphosphoglycerate **3-Phosphoglycerate**

7. Formation of 3-phosphoglycerate and ATP



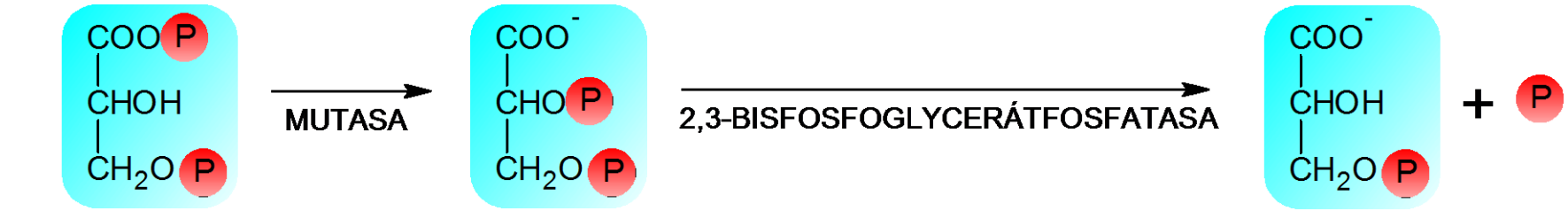
Formation of ATP on the principle of substrate phosphorylation :

1.3 BPG is a high energy compound (mixed anhydride) ,

Energy released during the transmission $PO_3 2-$ is utilized to synthesize ATP

NOVÁK, Jan. *Biochemie I*. Brno: Muni, 2009, s. 84.

The formation of 2,3- bisfosfoglycerátu - side road in erythrocytes



1,3-bisfosfoglycerát

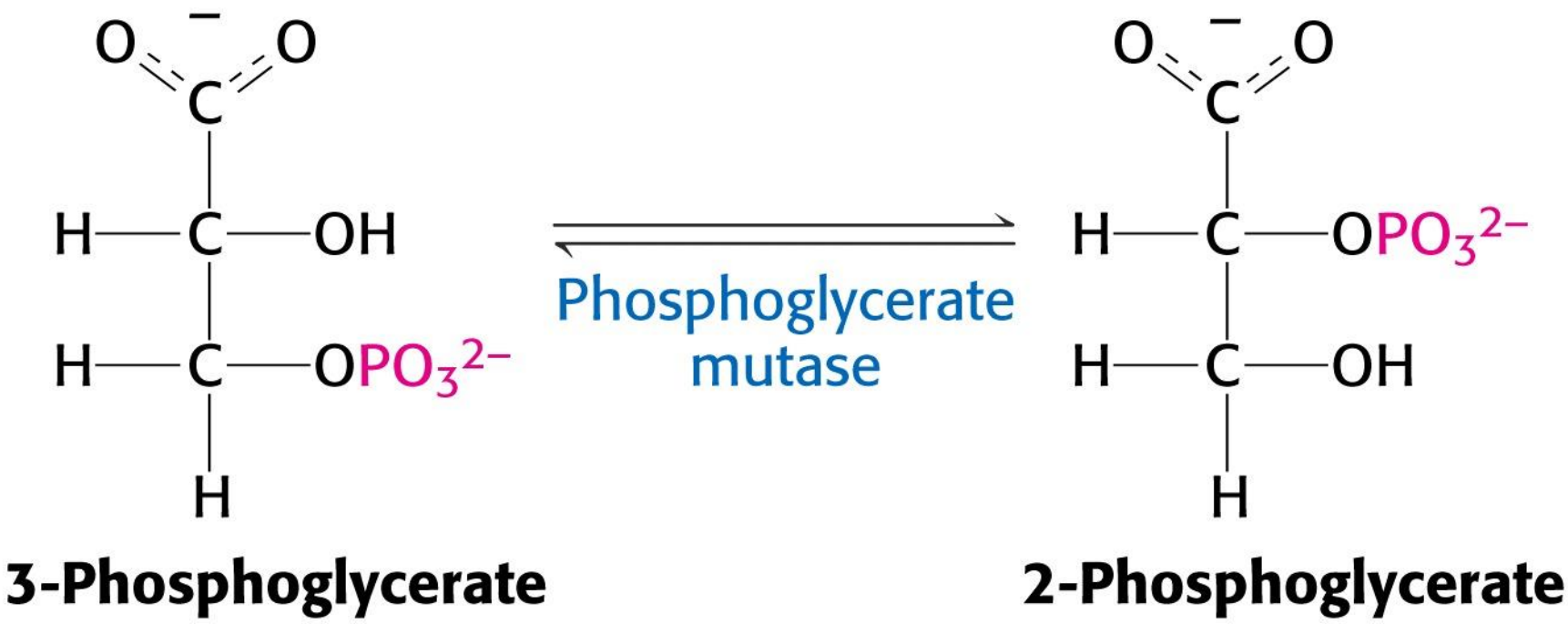
2,3-bisfosfoglycerát (2,3-BPG)

3-fosfoglycerát

Binding to Hb

NOVÁK, Jan. *Biochemie I*. Brno: Muni, 2009, s. 84.

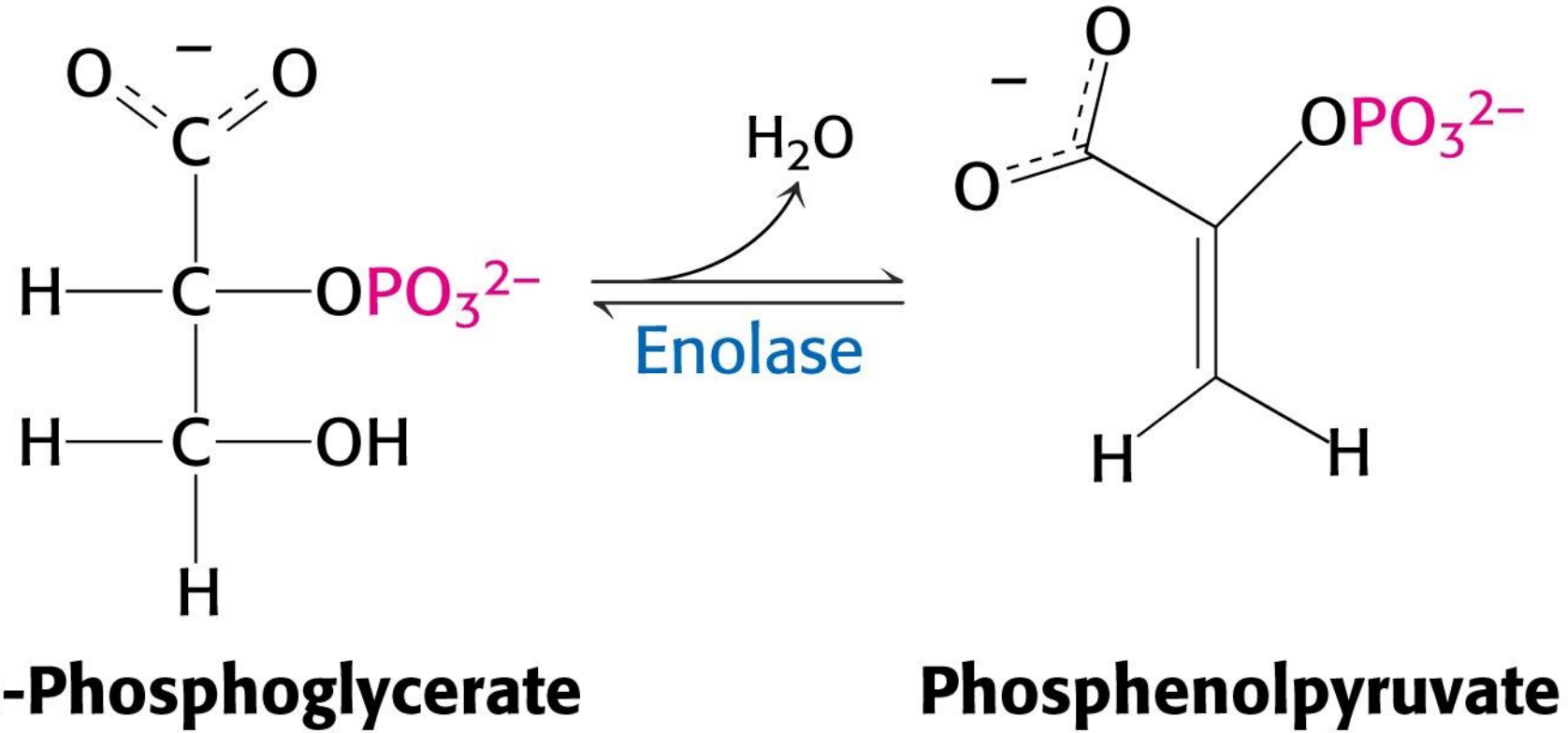
Rearrangement: step 8



3-Phosphoglycerate **2-Phosphoglycerate**

An enol phosphate is formed: step 9

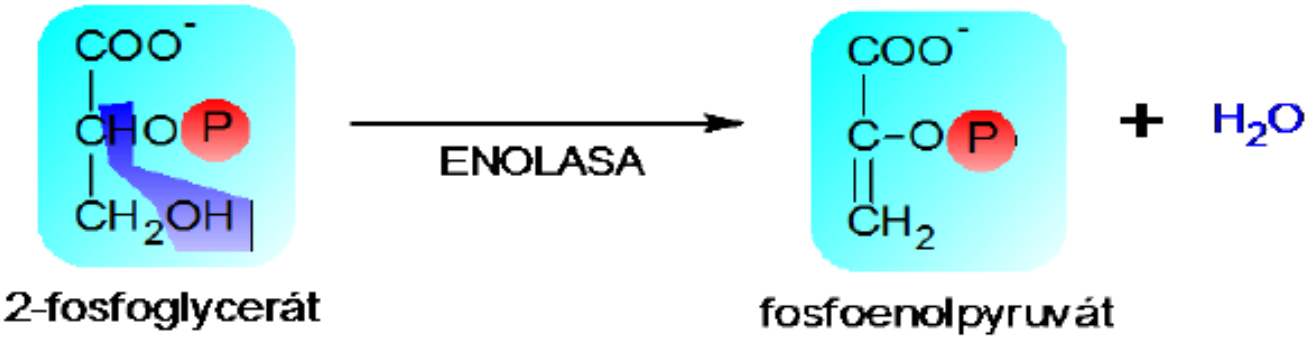
Dehydration elevates the transfer potential of the phosphoryl group, which traps the molecule in an unstable enol form



2-Phosphoglycerate **Phosphenolpyruvate**

Enol: molecule with hydroxyl group next to double bond

9. Formation of fosfoenolpyruvate

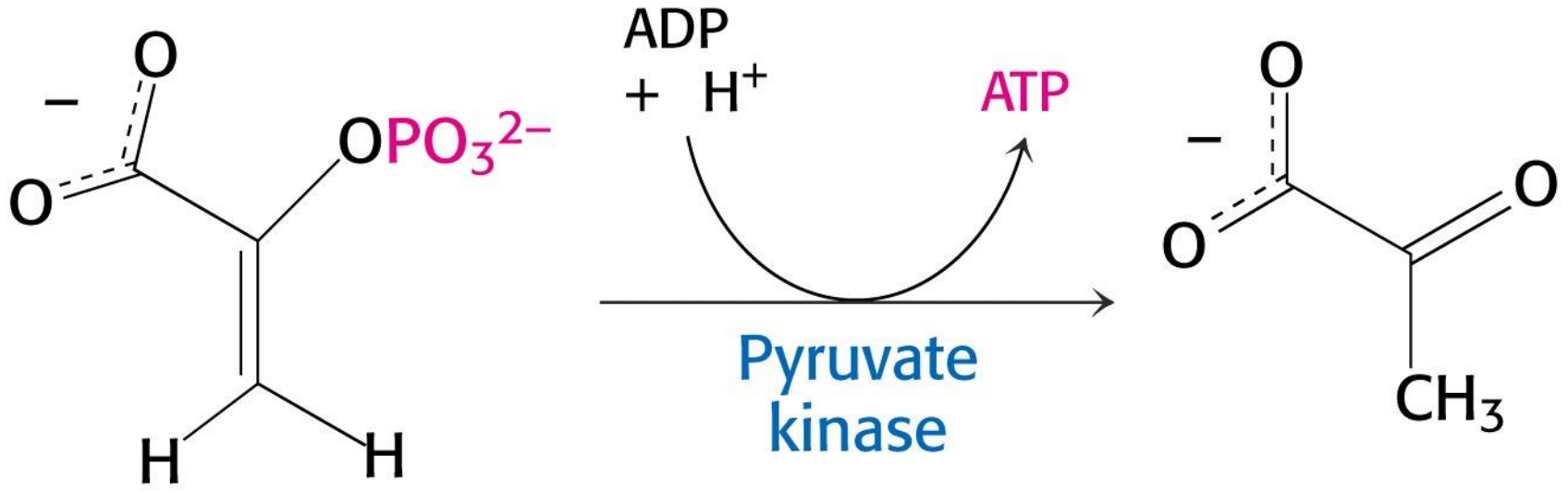


enolase

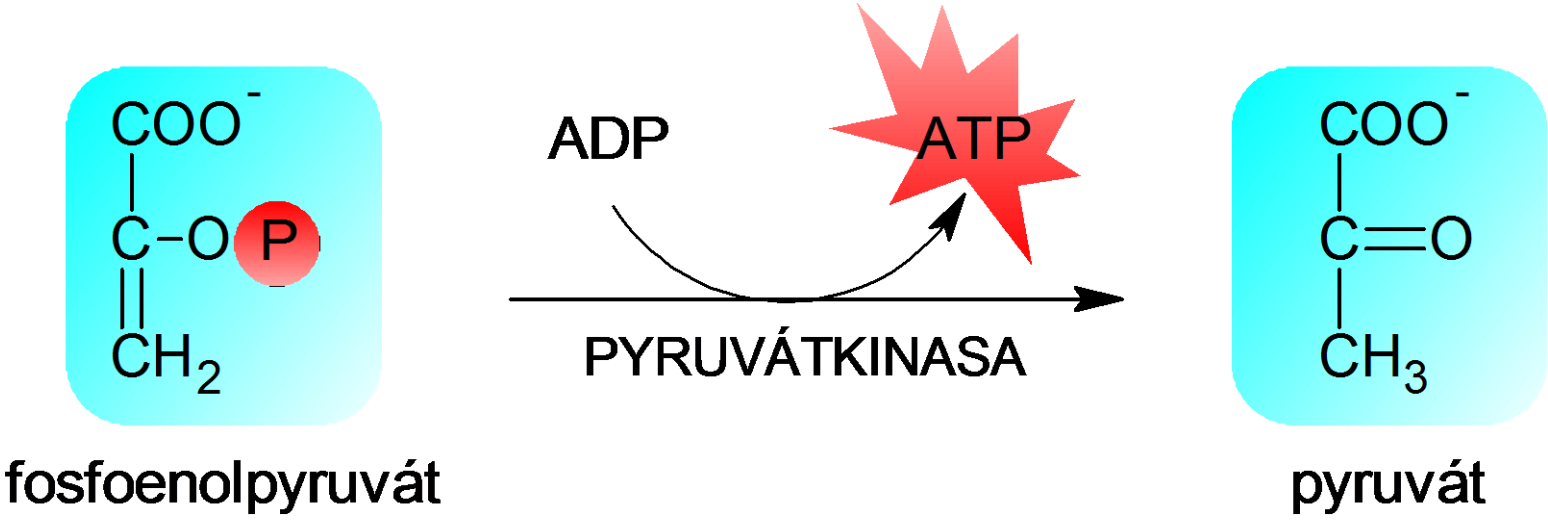
(inhibition F- - arrest of glycolysis by taking a blood sample)

NOVÁK, Jan. *Biochemie I*. Brno: Muni, 2009, s. 84.

Formation of Pyruvate & ATP: step 10



9. Formation of pyruvate



pyruvate kinase

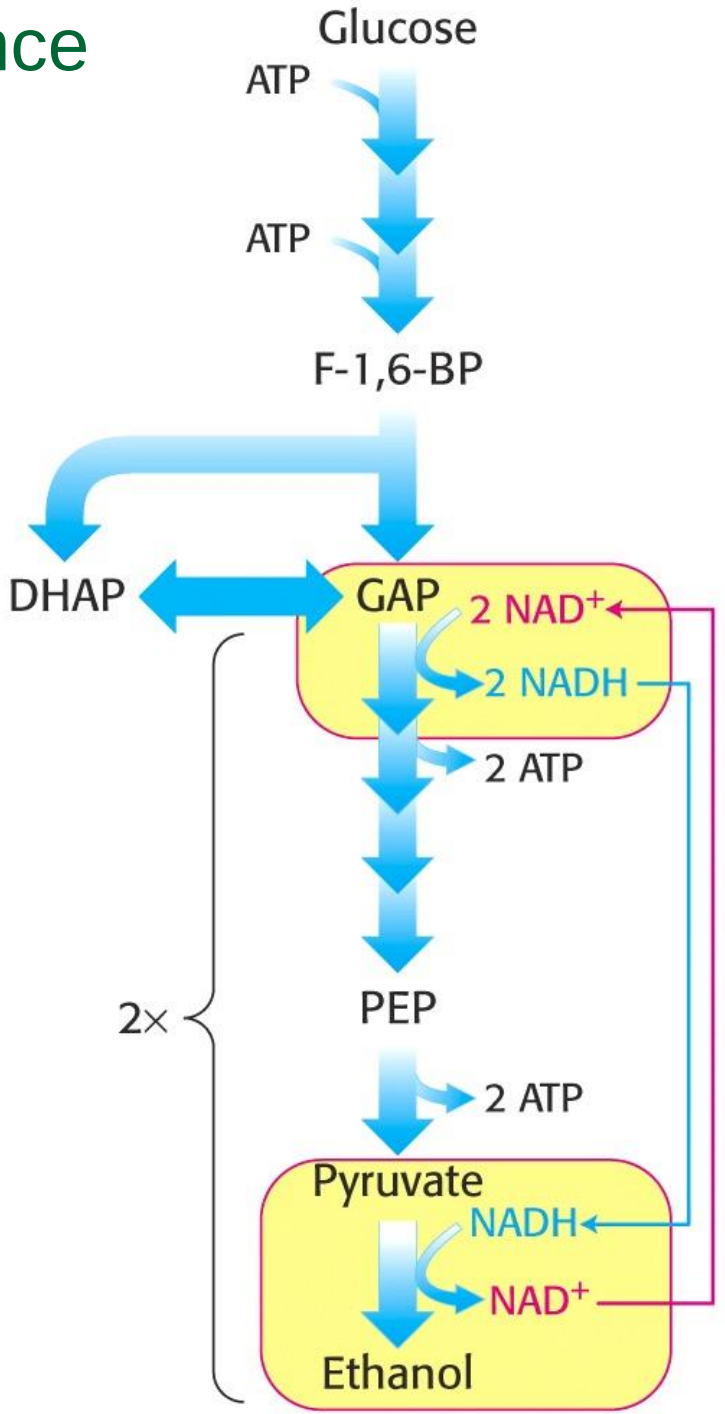
activation of fructose -1,6- BISP

hormonally regulated glucagon (inactivation)

(substrate phosphorylation)

NOVÁK, Jan. *Biochemie I*. Brno: Muni, 2009, s. 85.

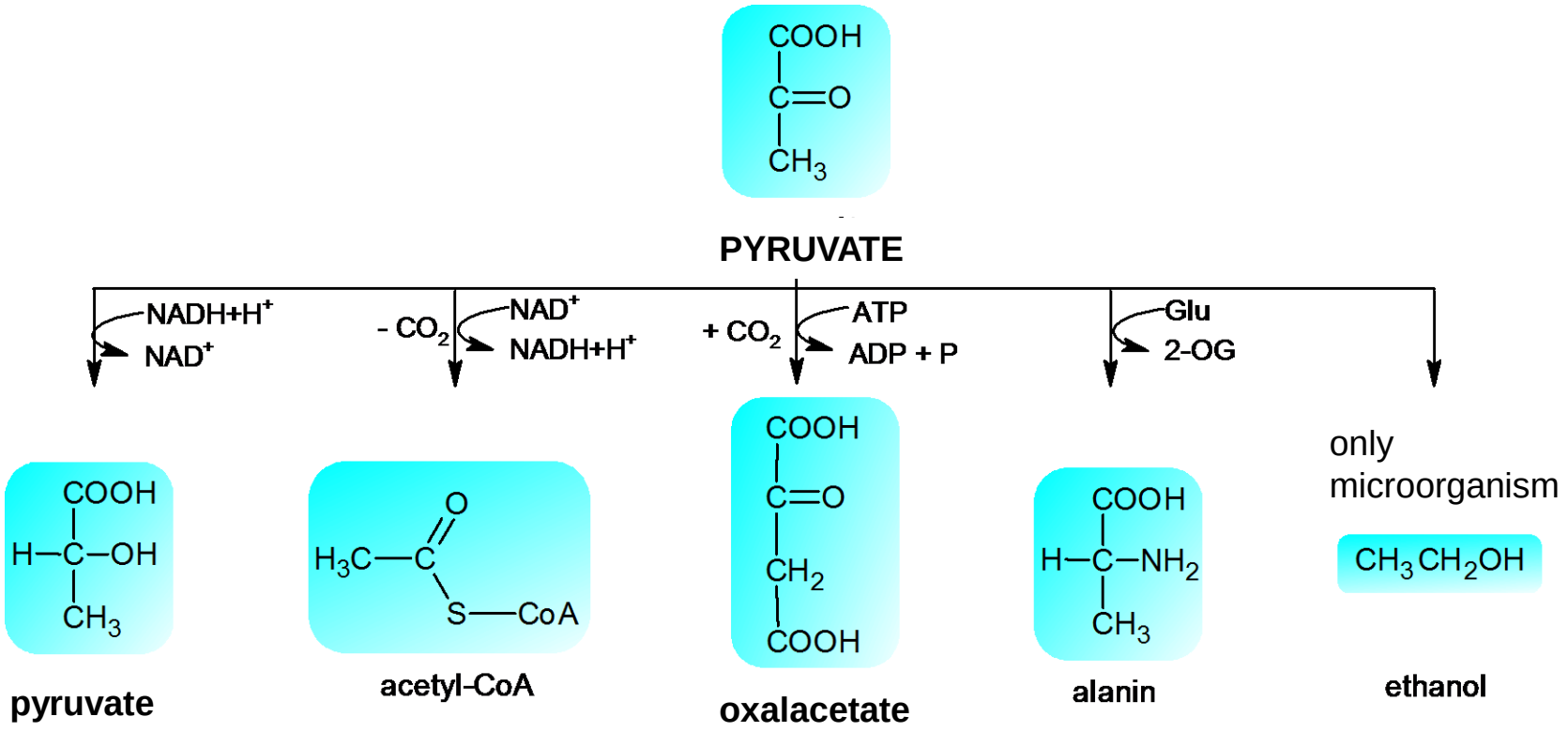
Maintaining Redox Balance



NAD^+ must be regenerated for glycolysis to proceed

Glycolysis is similar in all cells, the fate of pyruvate is variable

Conversion of pyruvate



TEST

NOVÁK, Jan. *Biochemie I*. Brno: Muni, 2009, s. 85.

Conversion of pyruvate

1) During **anaerobic glycolysis**, pyruvate is reduced to lactate

2) During **aerobic glycolysis** (under air) followed by oxidative decarboxylation of pyruvate , which is then converted to **acetyl-CoA** .

3) carboxylation of pyruvate (one of **anaplerotic reaction of the citric acid cycle**) for energy arises oxaloacetate , which can be used in various orbits (conversion to aspartate , CC).

4) transamination of pyruvate obtained **Alanine** - reaction involved glutamate (Glu) and produces the 2-oxoglutarate (2- OG) .

5) Another possibility is the degradation of pyruvate alcohol microorganisms. In this reaction, **ethanol** is formed .

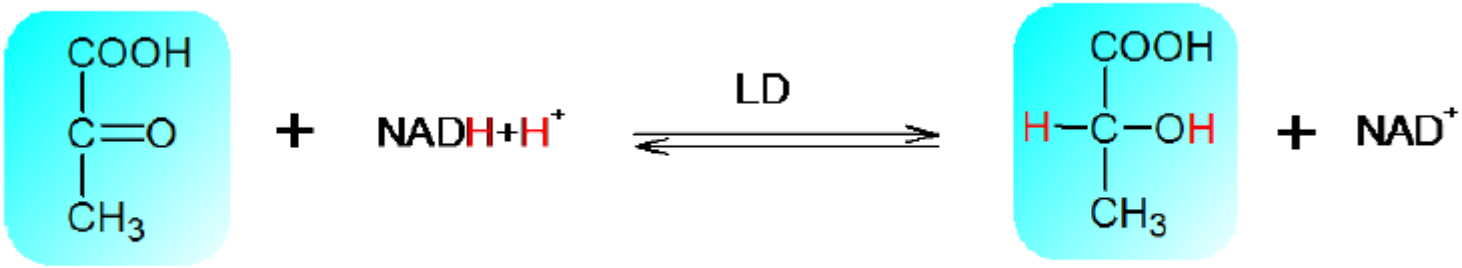
Fermentation

- An anaerobic process beyond glycolysis.
- In our body it is used to make NAD^+ when there is not enough oxygen.
- NAD^+ must be regenerated from NADH or glycolysis will stop.
- We'll look at two types of fermentation:
- Lactate and Ethanol.

Lactate fermentation

- Lactate
- Produced by muscles when the body can't supply enough oxygen.
  - pyruvate → lactate (NADH + H^+ → NAD^+)
- Anaerobic conversion of pyruvate to lactate permits regeneration of NAD^+.
- Body can then make more ATP - at a cost.
- Creates an oxygen debt.
- Body must take in extra O_2 to oxidize lactate.

The formation of lactate - anaerobic



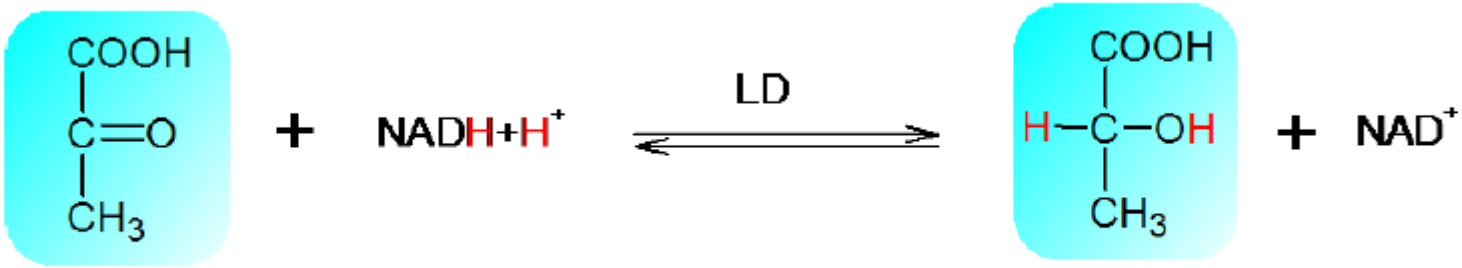
Pyruvate **lactate**

meaning :
Regeneration of NAD + consumed in the formation of 2,3- BisP - glycerate

if not enough NAD + , not more glucose molecules to enter glycolysis

NOVÁK, Jan. *Biochemie I*. Brno: Muni, 2009, s. 86.

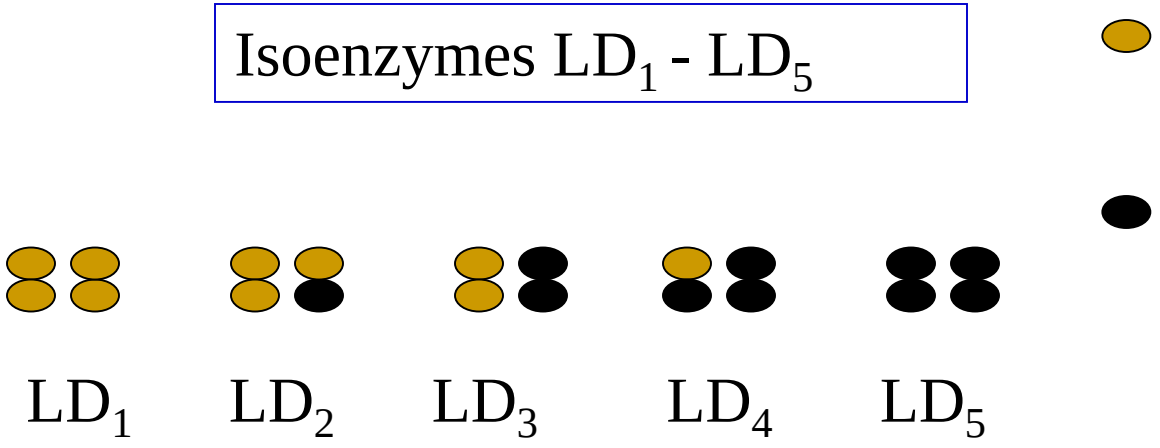
Lactate dehydrogenase (LD)



Pyruvate **lactate**

Katalyzuje reakci v obou směrech

Isoenzymes LD_1 - LD_5



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The formation of lactate

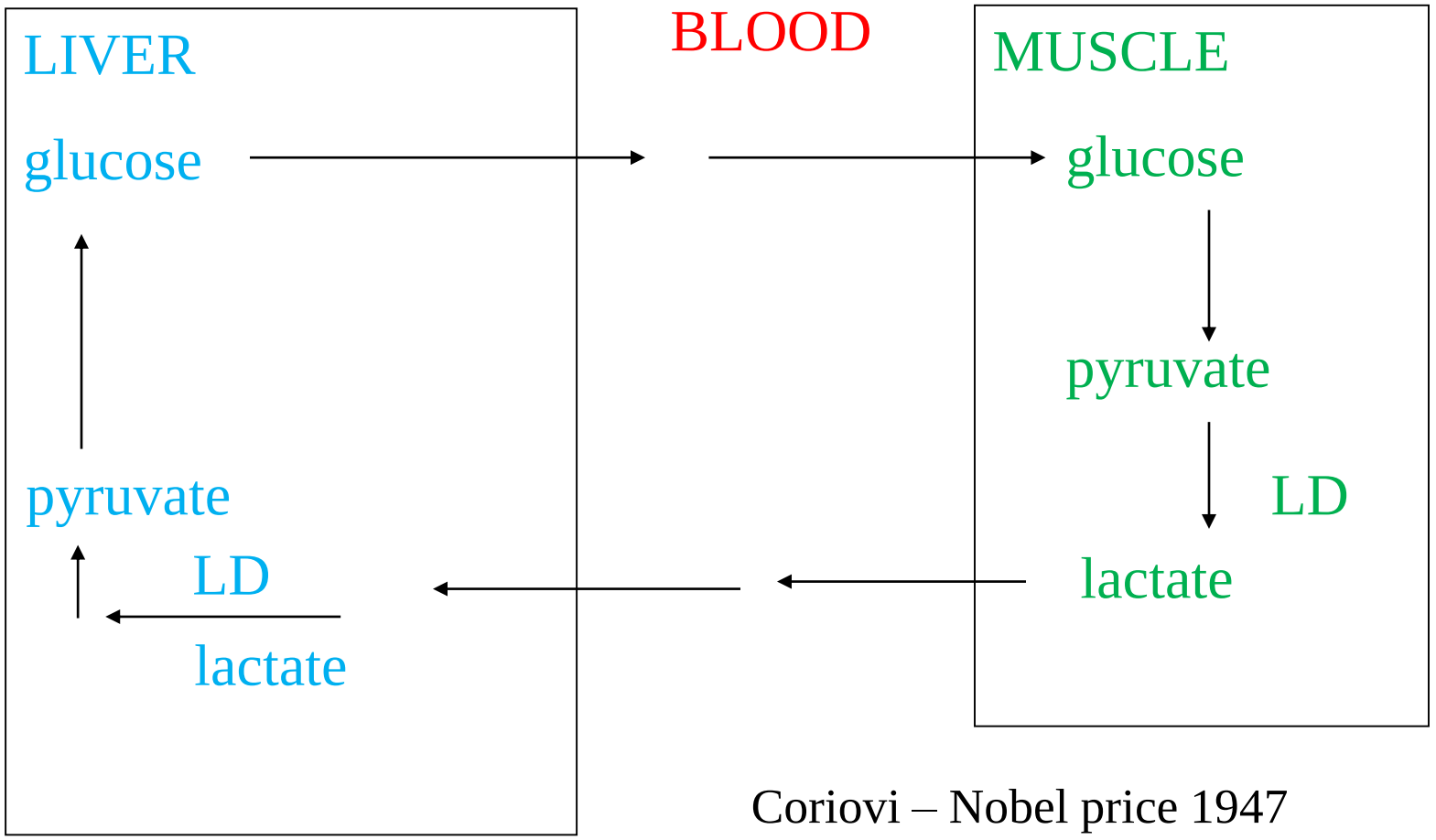
average of 1.3 mol / day (70 kg man)

- in the short term intensively working muscle 14 %
- in erythrocytes (have mitochondria) 25%
- Skin 25 %
- Brain 14 %
- in intestinal cells 8 %

The concentration of lactate in the blood : 1 mmol / l

changes during intensive muscle work (up to 30 mmol / l)

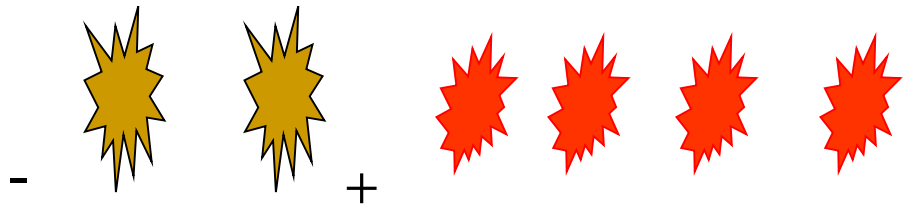
Cori cycle - removing lactate from tissues to the liver and utilization for gluconeogenesis



Coriovi – Nobel price 1947

Energy gain of glycolysis

1. direct profit ATP - substrate phosphorylation



consumption ATP

gain ATP ⇒

2 ATP for on glucose

This gain is true for aerobic and anaerobic process.

For anaerobic glycolysis is only profit of ATP .

2. ATP further gains in aerobic glycolysis:

- NADH reoxidation reaction of 5 (glyceraldehyd-P→1,3-bisP-glycerate) :

- transmission with " shuttles " to the respiratory chain - profit reoxidace NADH - profit 2x 2-3 ATP

- transmission of pyruvate to acetylCoA (2 NADH) 2x3 ATP

- transmission of acetylCoA in CAC 2x12 ATP

The overall balance of aerobic glycolysis

After aerobic glycolysis pyruvate :

Reaction	profit ATP
glucose →2 pyruvate (substrate phos.) 2 NADH → 2NAD^+	2 4-6*

** It depends on the type of the shuttle*

Further conversion of pyruvate :

Reaction	profit ATP
2 pyruvate → 2 acetylCoA + 2 NADH	6*
2 acetyl CoA → 2 CO_2 + 6 NADH + 2 $FADH_2$	2x 12
The total maximum gain of glycolysis	**36-38 ATP**

* (2x NADH to RCh)

Balance anaerobic glycolysis

After anaerobic glycolysis pyruvate :

Reaction	profit ATP
glukosa →2 x pyruvát (substrátová f.)	2
2 NADH → 2NAD+	0

Creation and consumption of NADH in anaerobic glycolysis

Reaction	profit/ loss NADH
2 glyceraldehyd-P → 2 1,3-bisP-glycerát	+2
2 pyruvát → 2 laktát	- 2
total	0

- **During anaerobic glycolysis , the net energy yield 2 ATP phosphorylation of substrate**
- it's just a small fraction of the energy stored in the molecule of glucose
- but has a role in process when supply of oxygen is limited
- tissue does not have mitochondria (ERCS , leukocytes , ..)
- it is necessary that lactate is saved for gluconeogenesis

Oxidative decarboxylation of pyruvate

- Pyruvate dehydrogenase complex
- Conversion of pyruvate to acetyl-CoA

matrix of mitochondria

Summary equation:

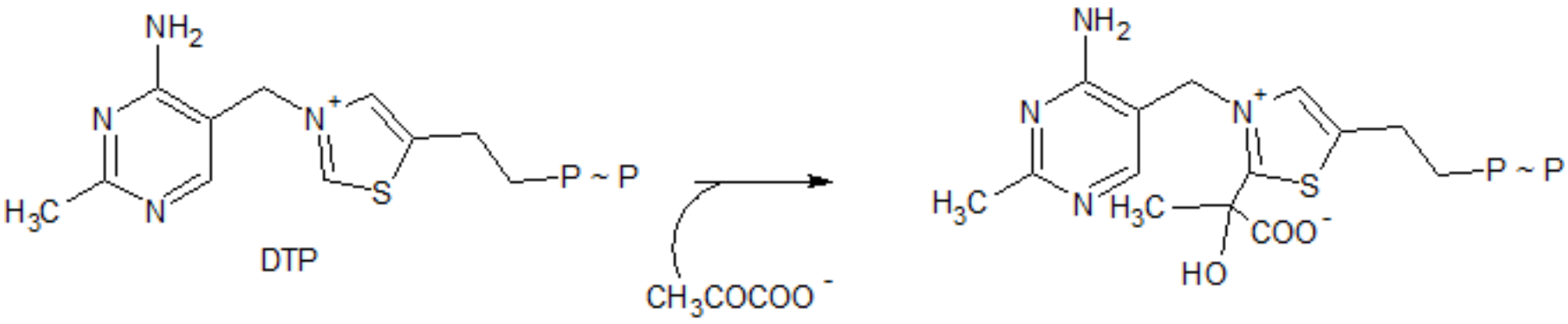
$$CH_3COCOOH + CoA\text{-}SH + NAD^+ \longrightarrow CH_3COSCoA + CO_2 + NADH + H^+$$

acetyl-CoA

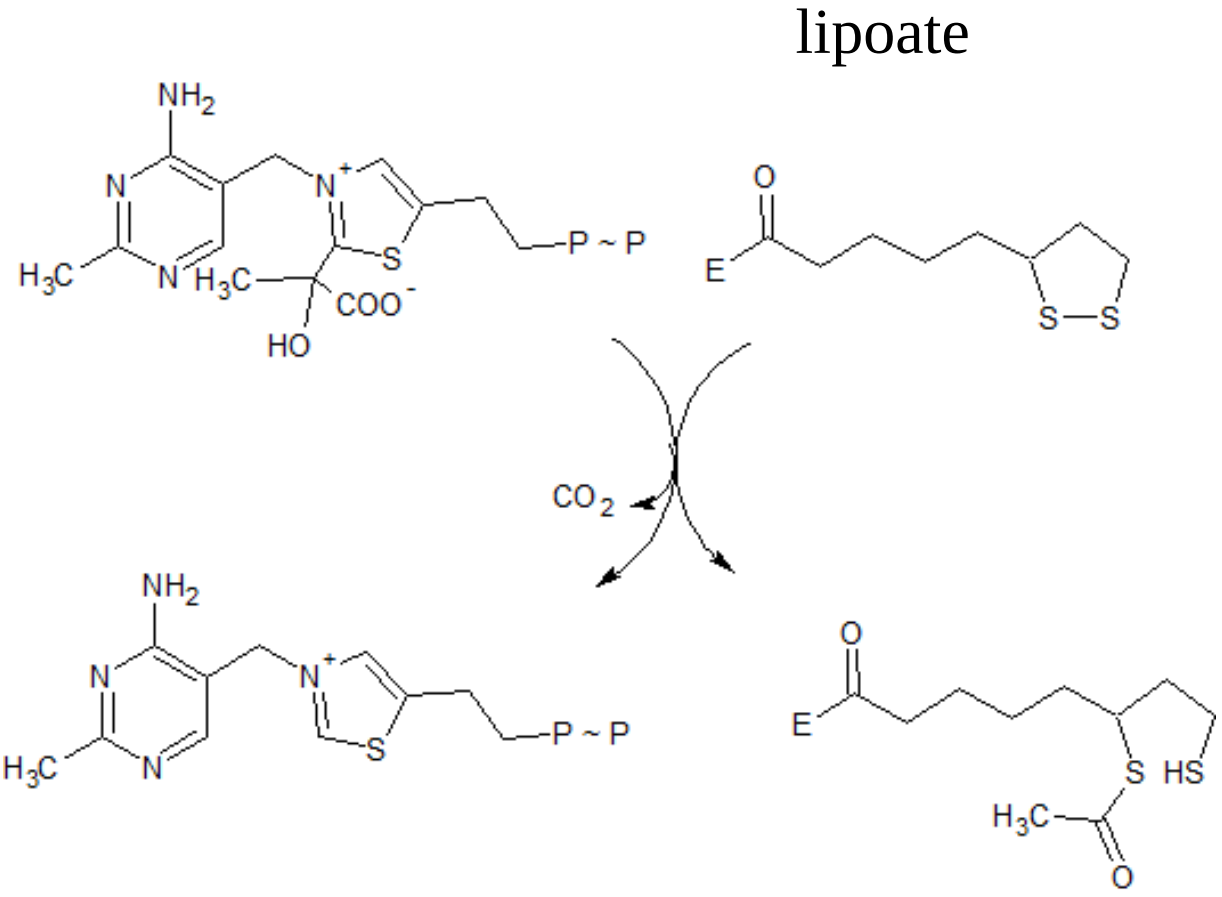
cofactors: thiamine pyrophosphate, lipoic acid, CoA, FAD, NAD^+

Reactions

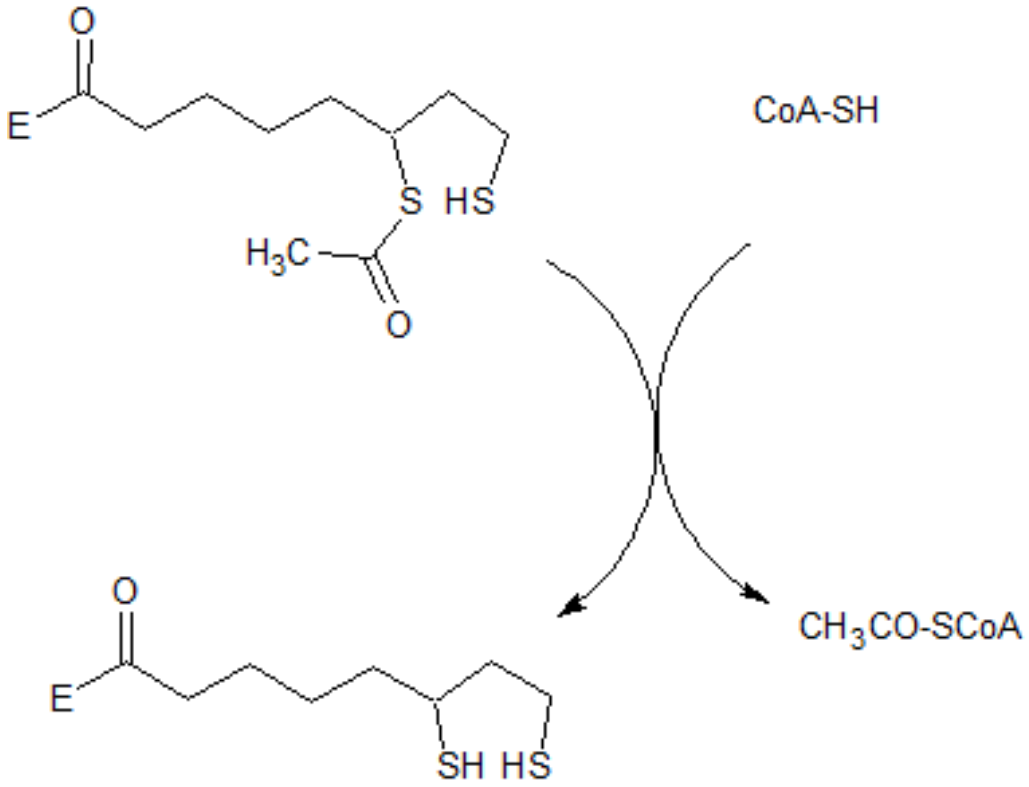
1. binding of pyruvate to thiamine pyrophospate



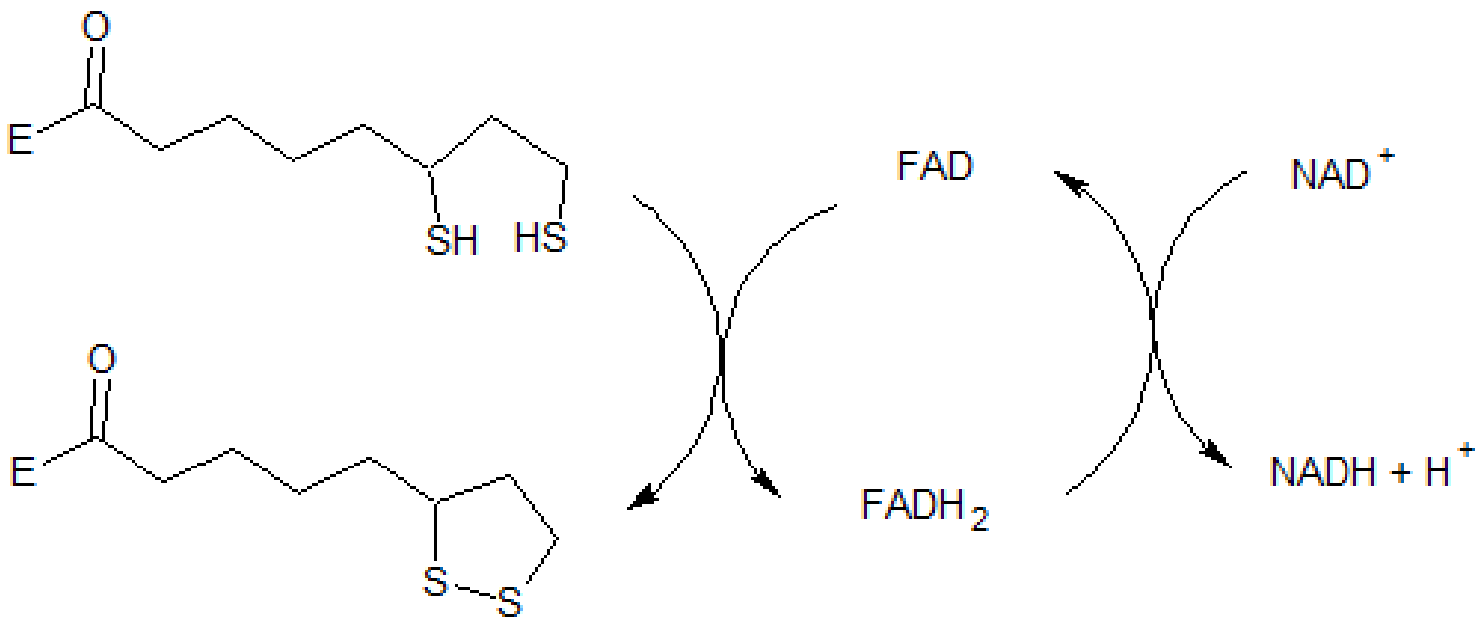
2. decarboxylation and acetyl transfer to lipoate



3. transfer of acetyl to Coenzyme A



4. re-oxidation of lipoate, transfer of hydrogen via FAD to NAD^+



Gluconeogenesis

- Glucose is not an essential nutrient. Human body can produce it through a series of reactions which are called gluconeogenesis
  (GNG) .
- GNG is one of the factors that postresorption phase (starvation) is ensured the maintenance of blood glucose levels within the physiological range from 3.1 to 5.0 mmol / l .
- Other factors are glycogenolysis (a major factor in postresorption phase) and consumption of carbohydrates (the main factor in the resorptive phase - phase after a meal) .

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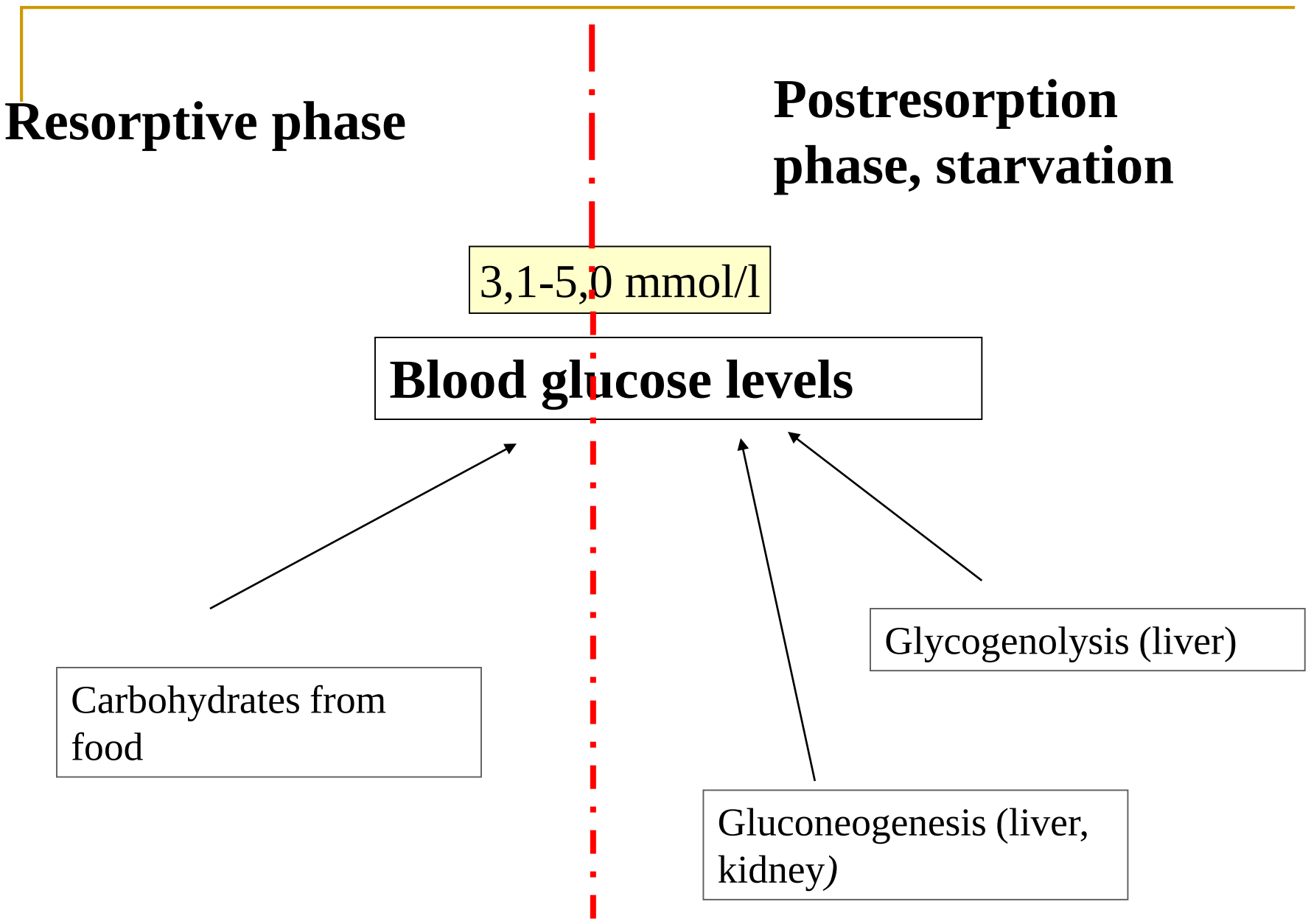
- **Glucose metabolism** – and its amount in the blood – the influence of hormones.
- Insulin, glucagon, adrenaline (instantaneous stress hormone) and cortisol (primary stress hormone). Basic information on individual hormones are given in table.

- **Gluconeogenesis - in the liver (to a small extent in the kidney)**, specifically in the cytosol of cells.
- Glucose is synthesized from simpler non-sucrose substances :
- lactate
- pyruvate
- glucogenic AA
- glycerol


Resorptive phase
Postresorption phase, starvation
3,1-5,0 mmol/l
Blood glucose levels
Carbohydrates from food
Glycogenolysis (liver)
Gluconeogenesis (liver, kidney)

The main hormones in the metabolism of glucose

Hormone	Source	Effect on Glc level in blood
Insulin	β-cells of pancreas	↓
Glucagon	α-cells of pancreas	↑
Adrenaline	Adrenal medulla	↑
Cortisol	Adrenal cortex	↑

The different reactions of glycolysis and GNG

- For the synthesis of glucose are used **enzymes and reactions of glycolysis** - but not all , since **three reactions of glycolysis are irreversible** and need to be replaced .
- These are the reactions:
- 1) Glucose + ATP → glucose-6- phosphate + ADP
- 2) fructose - 6-phosphate + ATP → fructose 1,6-bisphosphate + ADP
- 3) PEP + ADP → pyruvate + ATP

- These reactions cannot be carried out in reverse for a simple reason – the reverse reaction in neither of the cases releases enough energy to produce the products – e.g. substrate phosphorylation (originated by ATP) - reverse reaction is not possible because the cleavage of glucose-6 -phosphate does not provide enough energy needed for the synthesis of ATP.

Gluconeogenesis - synthesis of glucose de novo

- Organ : liver (kidney)
- Location: cytoplasm of cells
- Source for synthesis : a non-sugar substances
- (lactate, pyruvate , glucogenic amino acids, glycerol)

- enzymes of glycolysis , only 3 irreversible reactions are replaced by other enzymes

Irreversible reactions of glycolysis (kinase reaction)

1. Glc + ATP $\rightarrow$ Glc-6-P + ADP

(replaced by another enzyme)

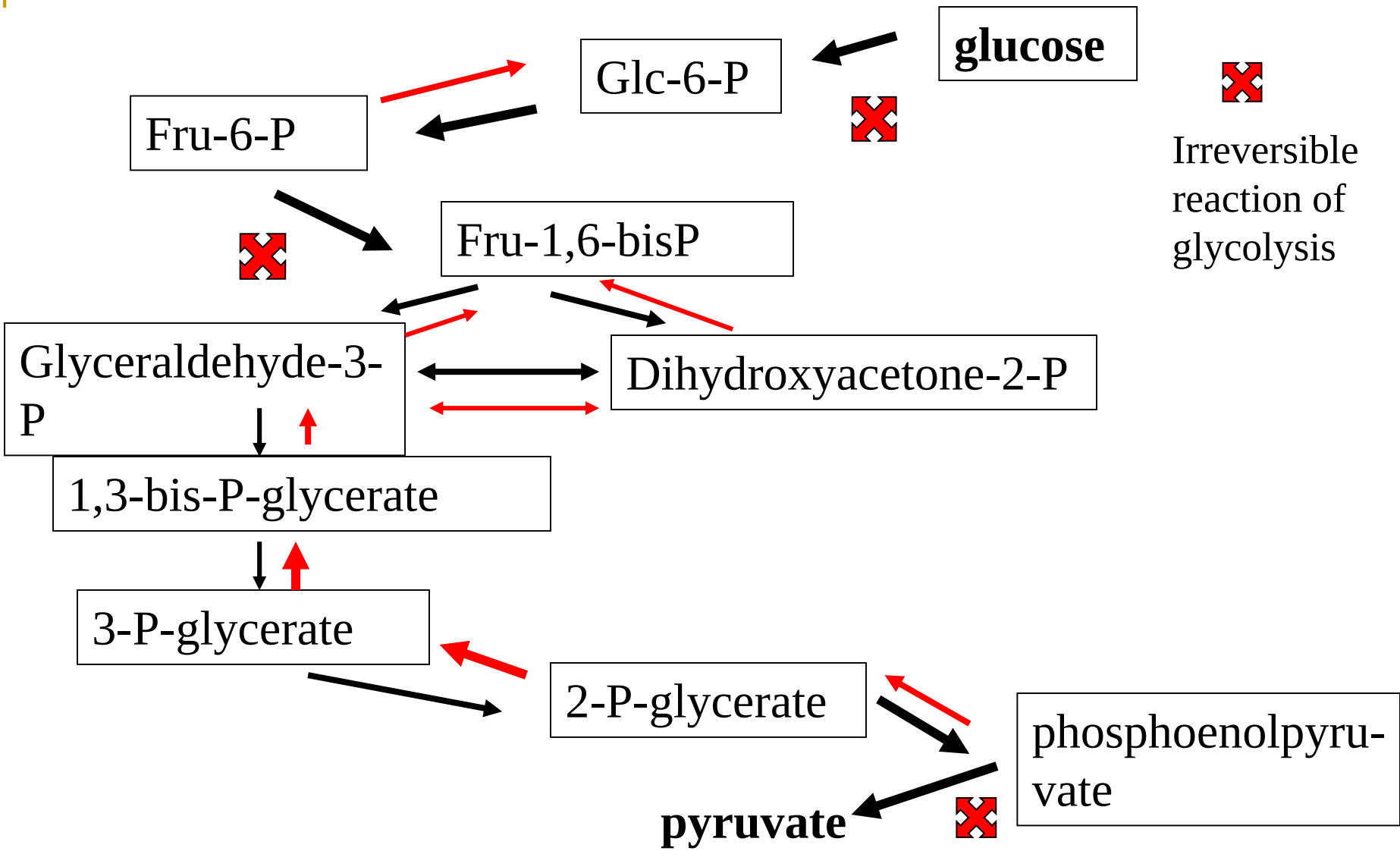
2. Fru-6-P + ATP $\rightarrow$ Fru-1,6-bisP

(replaced by another enzyme)

3. PEP + ADP $\rightarrow$ pyruvate + ATP

(replaced by " bypass ")

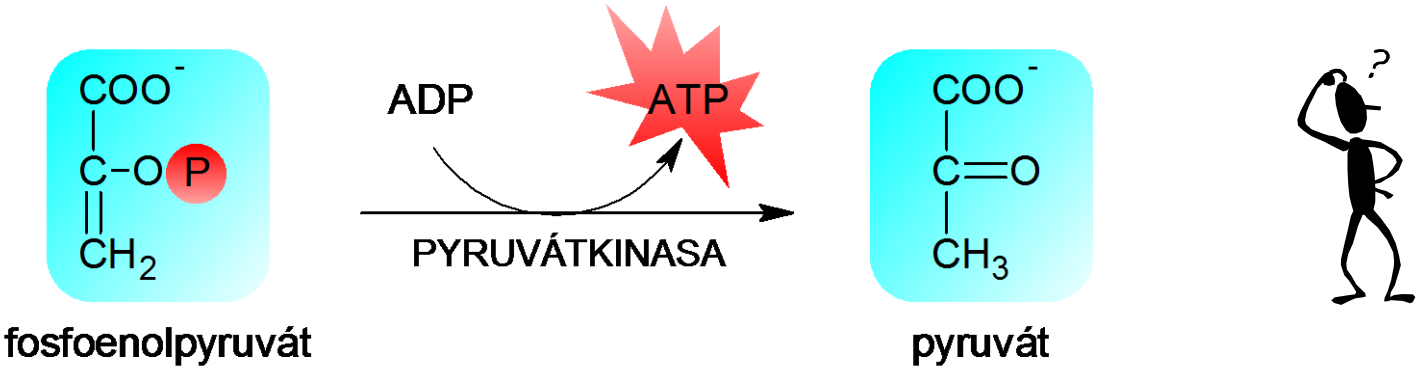
Glycolysis x gluconeogenesis



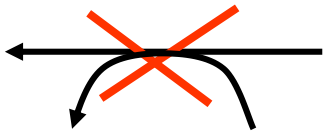
Specific reactions of gluconeogenesis

1. Synthesis of phosphoenolpyruvate

Why does this reaction not occur in reverse?



fosfoenolpyruvát pyruvát



ADP ATP

Δ G° = -61,9 kJ/mol

ATP conversion to ADP does not provide enough energy for a reverse reaction

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The emergence of phosphoenolpyruvate is divided into two stages :

1. the establishment of oxaloacetate decarboxylation to pyruvate *

localization : liver and kidney mitochondria

enzyme: pyruvate carboxylase

Energy : consumption of 1 ATP

2. The conversion of oxalacetate to phosphoenolpyruvate

localization : cytoplasm (mitochondria)

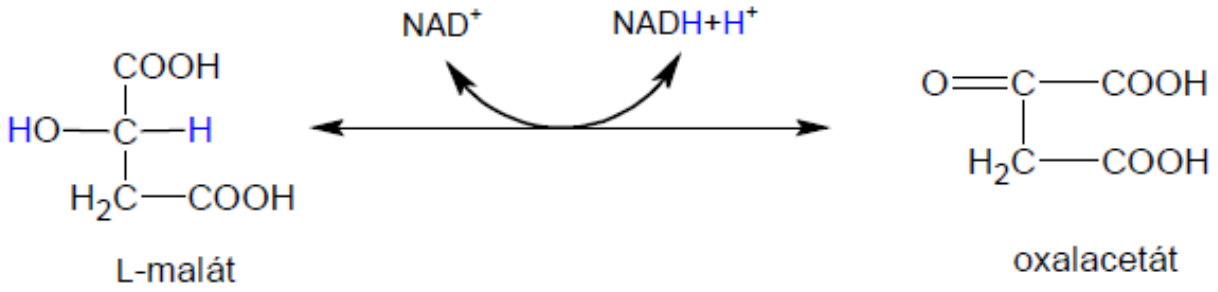
enzyme: phosphoenolpyruvatecarboxykinase

Energy : consumption of 1 GTP

* Note .: carboxylation of pyruvate is also anaplerotical reaction of the citric acid cycle

Other ways the formation of oxaloacetate in mitochondria

•dehydrogenation of malate (CAC)



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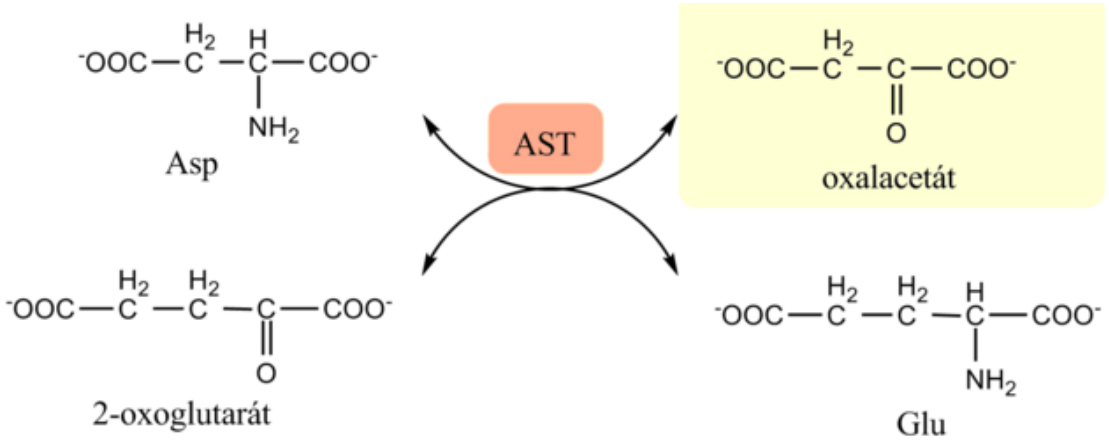
•aspartate transamination

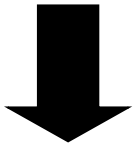


WikiSkripta: Aminotransferázy [online]. [cit. 2014-07-17]. Dostupné z: http://www.wikiskripta.eu/index.php/Aminotransfer%C3%A1zy

Oxaloacetate formed in the mitochondrial matrix . The decarboxylation takes place partly in the cytoplasm :

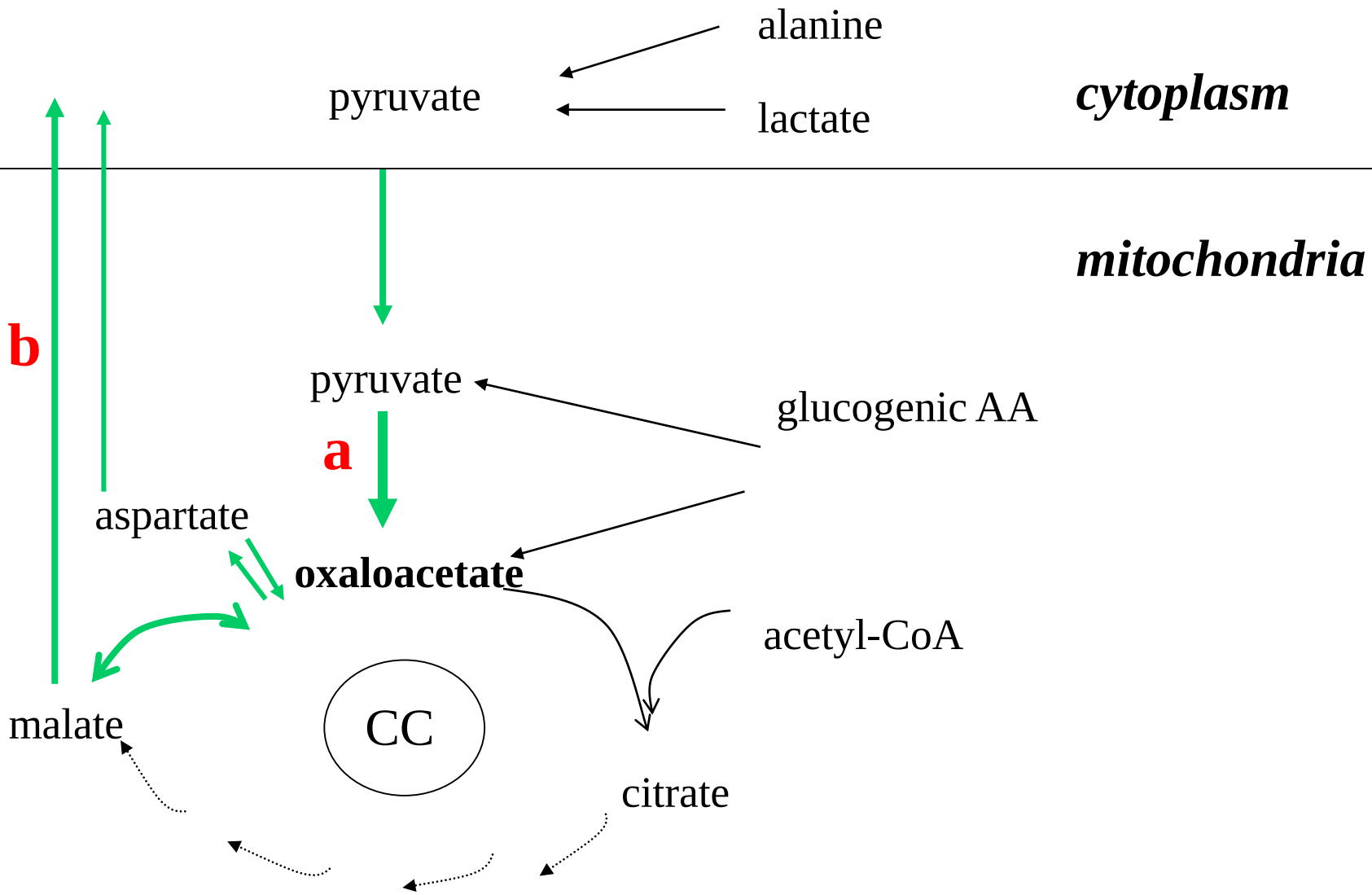
The mitochondrial membrane is not permeable to oxaloacetate .

How to get oxalacetate from mitochondria to the cytoplasm ?

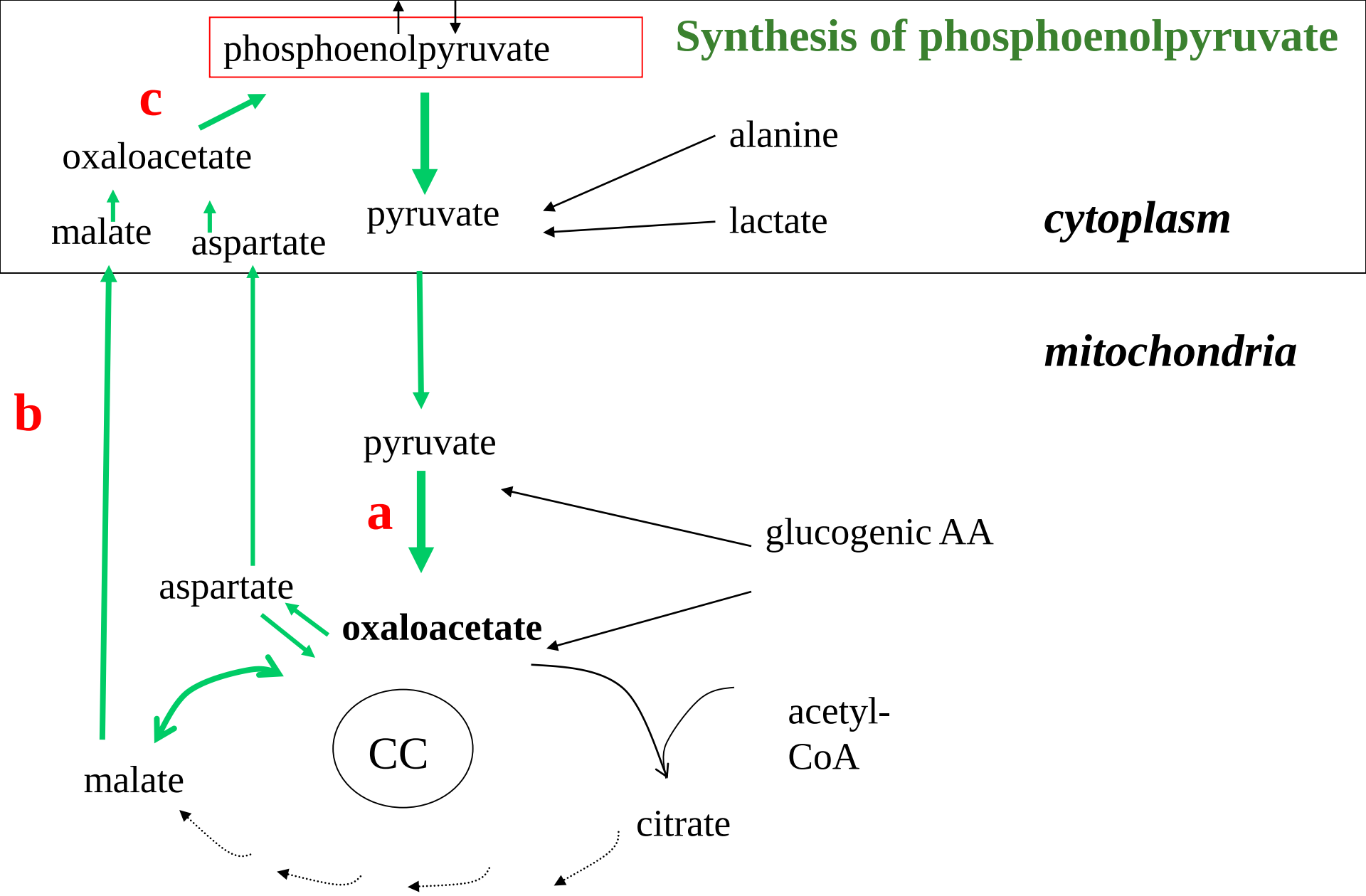


It is transported in the form of malate or aspartate.

Synthesis and transport of oxaloacetate

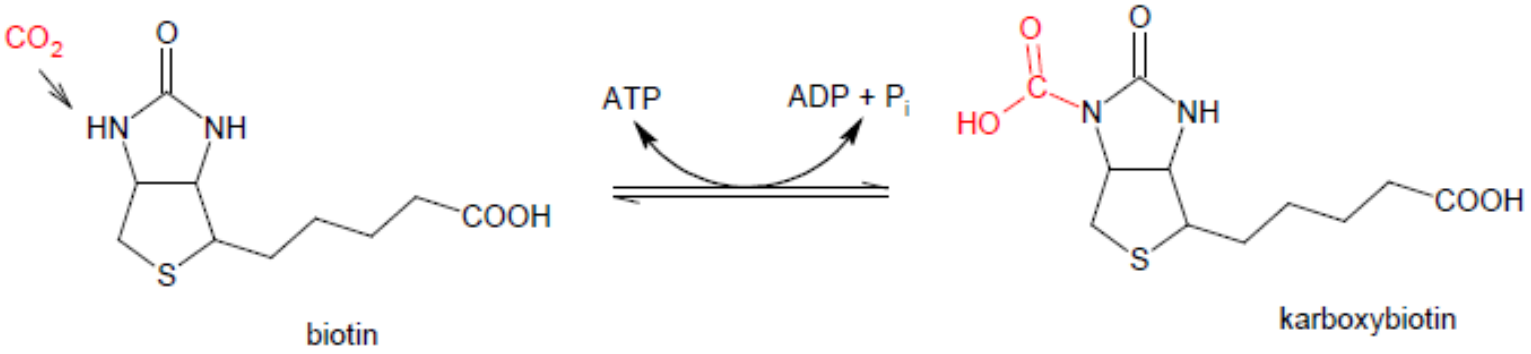


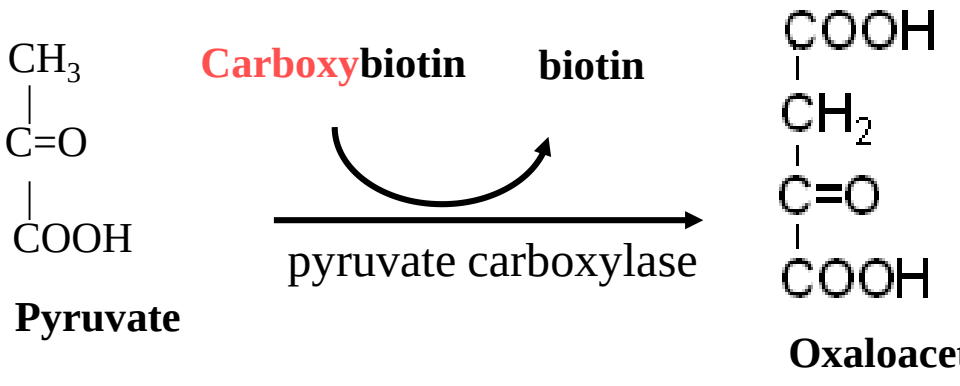
Synthesis of phosphoenolpyruvate



1. Conversion of pyruvate to phosphoenolpyruvate a

- **Carboxylation of pyruvate (matrix of mitochondria)**





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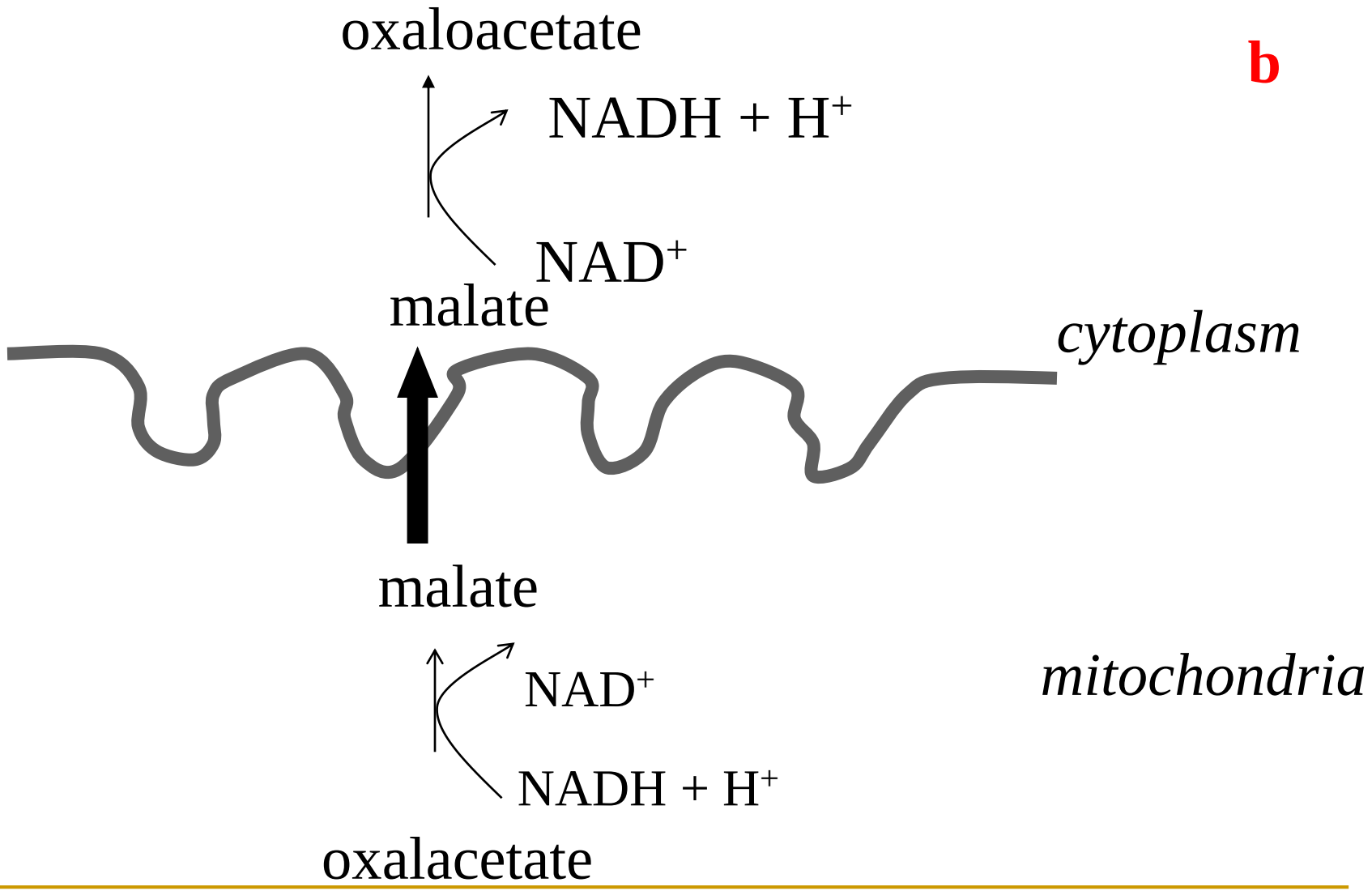
- **Transport of oxaloacetate into cytoplasm in form of malate**

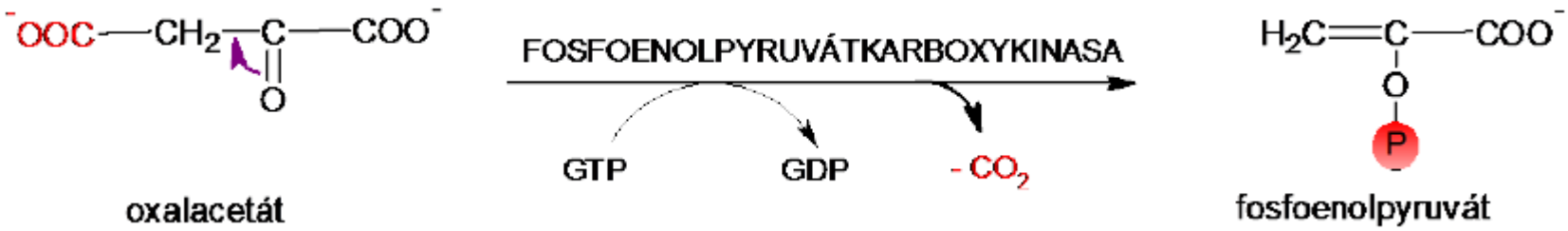


- **Transport of oxaloacetate into cytoplasm in form of aspartate**

(*lecture Respiration – aspartate/malate shuttle).

- **decarboxylation of oxaloacetate**

C



(PEP)

participates in reversible reactions of glycolysis

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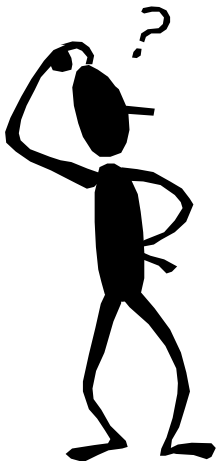
Synthesis of phosphoenolpyruvate from pyruvate or lactate requires 2 ATP



Coupled carboxylation and subsequent decarboxylation enables the occurence of otherwise energetically unfavorable reaction.

(also in the synthesis of fatty acids)

Why is pyruvate carboxylation preferred instead of decarboxylation during GNG?

Organisms is under the influence of glucagon

Fatty acids are released from adipose tissue

β-oxidation of fatty acids takes place in liver

Acetyl-CoA is present in liver

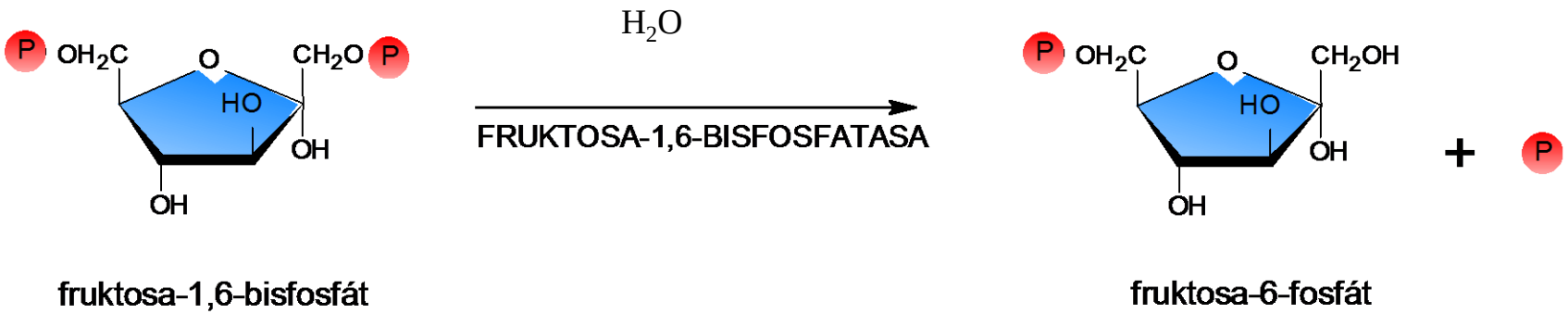
↓

Acetyl-CoA: inhibits pyruvatedehydrogenase
activates pyruvatecarboxylase

Second special reaction in GNG

2. Dephosphorylation of fructose-1,6-bisphosphate

hydrolytic cleavage



Fructose-1,6-bisphosphatase

Allosteric inhibition by AMP, activation by ATP

Inhibition by fructose-2,6-bisphosphate (glucagon downregulates its level)

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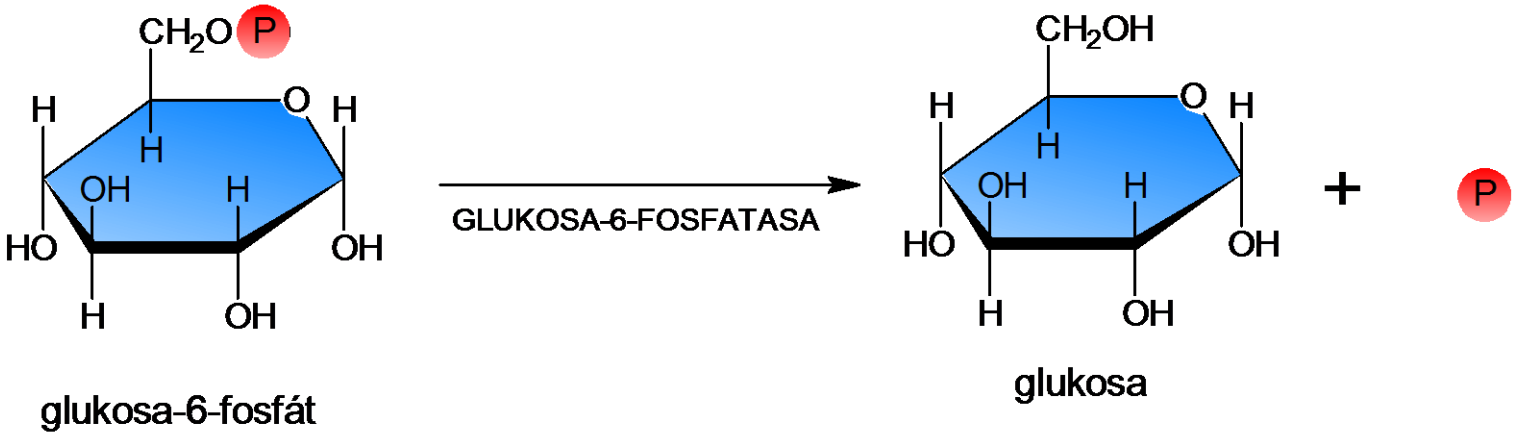
Third special reaction in GNG

3. Dephosphorylation of glucose-6-P



glukosa-6-fosfát

glukosa

The enzyme is located in liver and kidneys, **not in muscles**.

Enzyme is localized in ER

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Energetic requirements of GNG

reaction	ATP/glucose
2 pyruvate → 2 oxaloacetate	-2
2 oxaloacetate → 2 phosphoenolpyruvate	-2 (GTP)
2 3-phosphoglycerate → 2 1,3-bisphosphoglycerate	-2

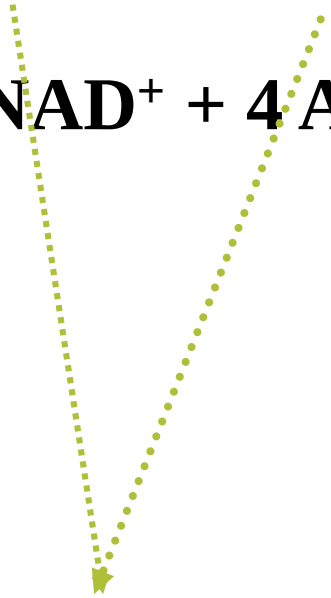
-6 ATP/glucose

The source of energy is mainly β-oxidation of FA

Summary equation of GNG

2 pyruvate + 4 ATP + 2 GTP + 2 NADH + $2H^+ \rightarrow$

glucose + 2 NAD^+ + 4 ADP + 2 GDP + 6 P_i



Consumption: -6 ATP

Substrates for GNG - origins

Lactate

created in tissues, transported via blood into liver

lactate + $NAD^+ \rightarrow$ **pyruvate** + NADH + H^+ (cytoplasm)

(Cori cycle)

Glycerol

- created in adipocytes during the cleavage of triacylglycerols
- transported via blood to liver
- liver (cytoplasm):

glycerol + ATP → glycerol-3-P + ADP

glycerol-3-P + NAD^+ ⇆ **dihydroxyacetone-P** + NADH + H^+

What is the energy consumption for synthesis of 1 mol glucose from glycerol?

Glucogenic amino acids

their metabolism yields pyruvate or other byproducts of citric acid cycle, which can be transformed to oxaloacetate

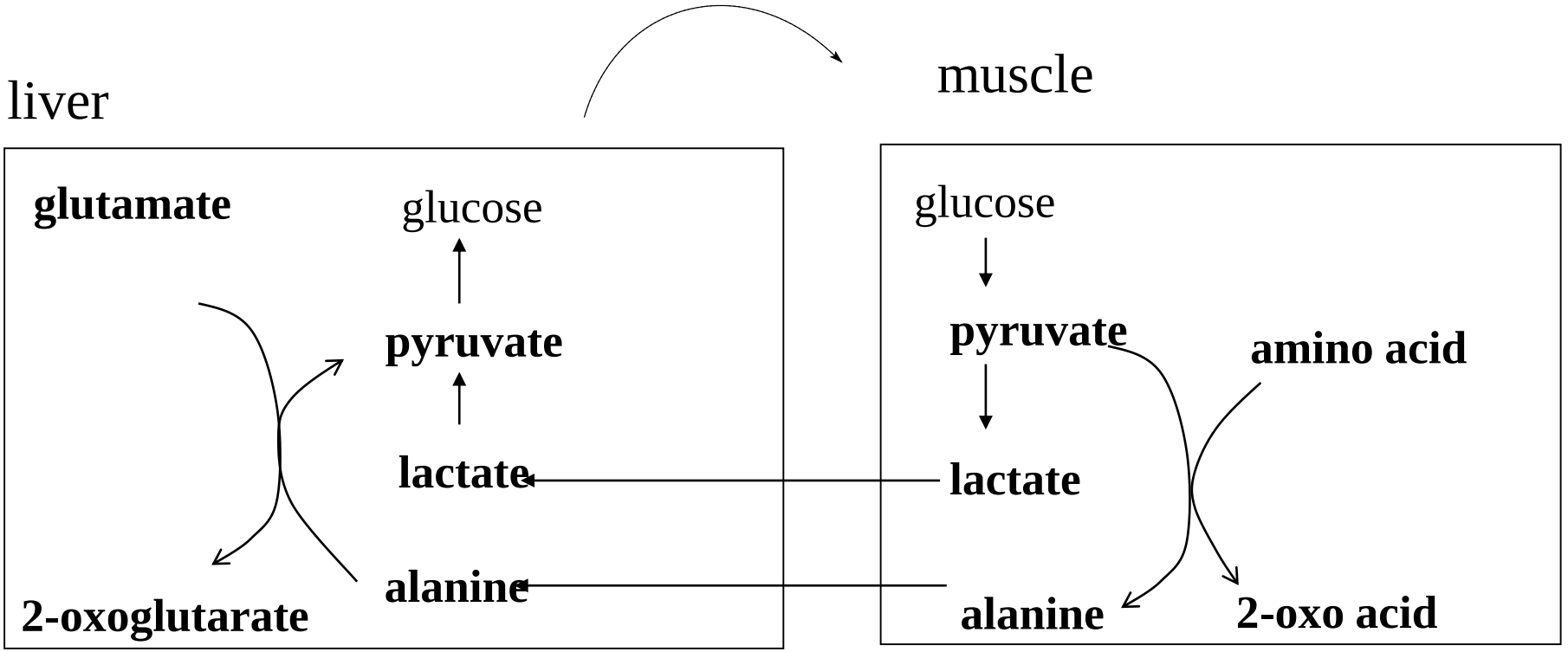
Acetyl-CoA – it is not a direct source in gluconeogenesis !!!

it is metabolised in CC to CO_2

in animals, fatty acids are not transformed into glucose

The most important glucogenic amino acid is alanine

It is released form muscle where it is created by transamination from pyruvate, then it is transported into liver where it is trasformed back into pyruvate



Glukoneogenesis from lactate and glycerol requires NAD^+

Sometimes during metabolism the $NADH/NAD^+$ ratio can be high – glukoneogenesis does not take place

$NADH/NAD^+$ ratio rises e.g. during ethanol metabolism (alcohol dehydrogenase), therefore the intake of alcohol can inhibit gluconeogenesis $\Rightarrow$ hypoglycemia

The main factors in gluconeogenesis regulation

Availability of substrates.

Regulation of irreversible reactions allosterically or by hormones.

Allosteric effects are fast (immediate response)

Hormones can influence GNG by:

- direct effect by second messenger – activation or inhibition (fast)
- regulation of synthesis by induction or repression (slow – hours to days)

Effects of activators and inhibitors on enzymes in glycolysis and gluconeogenesis

Enzyme	**Activator**	**Inhibitor**
Hexokinase		Glucose-6-phosphate
Phosphofructokinase	5´AMP, fructose-6-phosphate, fructose-2,6-bisphosphate	Citrate, ATP, glucagon
Pyruvatekinase	fructose-1,6-bisphosphate, insulin	ATP, alanine, glucagon, noradrenalinw
Pyruvatedehydrogena-se	CoA, NAD^+, insulin, ADP, pyruvate	Acetyl-CoA, NADH, ATP
Pyruvatecarboxylase	Acetyl-CoA	ADP
Phosphoenolpyruvate carboxykinase	Glucagon ?	

Effects of hormones on enzymes in glycolysis and gluconeogenesis

Enzyme	**Inductor**	**Repressor**
Glucokinase	Insulin	Glucagon
Fosfofruktokinasa	Insulin	Glucagon
Pyruvatekinase	Insulin	Glucagon
Pyruvatecarboxylase	Glucokorticoids Glucagon Adrenaline	Insulin
Phophoenolpyruvate carboxykinase	Glucocorticoids Glucagon Adrenaline	Insulin
Glucose-6-phosphatase	Glucocorticoids Glucagon Adrenaline	Insulin

Gluconeogenesis in kidneys

Kidneys can produce glucose by GNG

They can release it into bloodstream – during postresorption phase, fasting or acidosis

Substrates - lactate, glycerol a glutamine