Basic concept and design of metabolism

The glycolytic pathway

Metabolism

Living organisms require a **continual input of free energy** for three major purposes:

– the performance of **mechanical work** in cellular movements,
– the **active transport of molecules and ions** across membranes,
– the **synthesis of macromolecules and other biomolecules** from simple precursors.

Metabolism – processes at which living organism utilizes and produces energy.

Roles of Metabolism

- to provide energy (catabolic processes)
- to synthesize molecules (anabolic processes)
- both types of processes are tightly connected

The metabolic interplay of living organisms in our biosphere

Two large groups of living organisms according to the chemical form of carbon they require from the environment.

Autotrophic cells ("self-feeding" cells)

– **green leaf cells of plants** and photosynthetic bacteria – **utilize CO_2** from the atmosphere as the sole source of carbon for construction of all their carbon-containing biomolecules.

They absorb **energy of the sunlight.** The synthesis of organic compounds is essentially the **reduction (hydrogenation) of CO_2** by means of hydrogen atoms, produced by the photolysis of water (generated dioxygen O_2 is released).

Heterotrophic cells

– cells of higher **animals** and most microorganisms – must obtain carbon in the form of relatively complex **organic molecules** (nutrients such as glucose) formed by other cells. They obtain their **energy from the oxidative (mostly aerobic) degradation of organic nutrients** made by autotrophs and return CO_2 to the atmosphere.

Carbon and oxygen are constantly cycled between the animal and plant worlds, solar energy ultimately providing the driving force for this massive process.

The metabolic interplay in human

Absorb energy of the sunlight

O_2

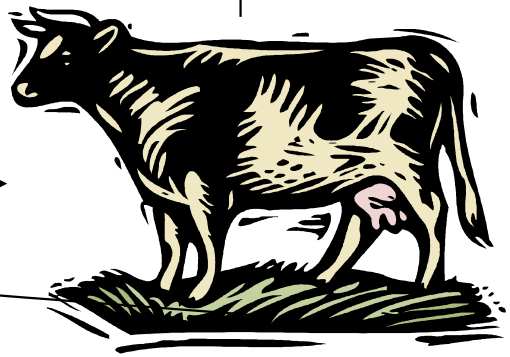
macromolecules

energy

CO_2

Small molecules

Proteins, lipids , saccharides



CO_2

Autotrophs

Heterotrophs

Energy in chemical reactions

Gibbs free energy (Δ G)

The maximal amount of useful energy that can be gained in the reaction (at constant temperature and pressure)

$$aA + bB \rightarrow cC + dD$$

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

$\Delta G^{0'}$ pH = 7,0, T=25 °C)

$$\Delta G^{o'} = -RT \ln K$$

The ΔG of a reaction depends on the **nature** of the reactants (expressed by the ΔG^o term) and on their **concentrations** (expressed by the second term).

Living organisms as open systems

- They permanently take up nutrients with the high enthalpy and low entropy
- Nutrients are converted to waste products with low enthalpy and high entropy
- Energy extracted from nutrients is used to power biosynthetic processes and keep highly organised cellular structure
- A part of energy is converted to heat
- Living organism can never be at equilibrium
- Steady state - open systems in which there is a constant influx of reactants and removal of products
- Reactions are arranged in series, product of one reaction is a substrate of the following reaction

Biochemical processes

exergonic **endergonic**

Endergonic reactions can proceed only in coupling with exergonic reactions

Transfer of energy from one proces to another process in enabled by „high-energy“ compounds

Mostly ATP is used.

Phosphoryl group PO_3^{2-} is transferred from one to another compound in process of coupling

Principles of coupling

Example 1:

Formation of glucose-6-phosphate

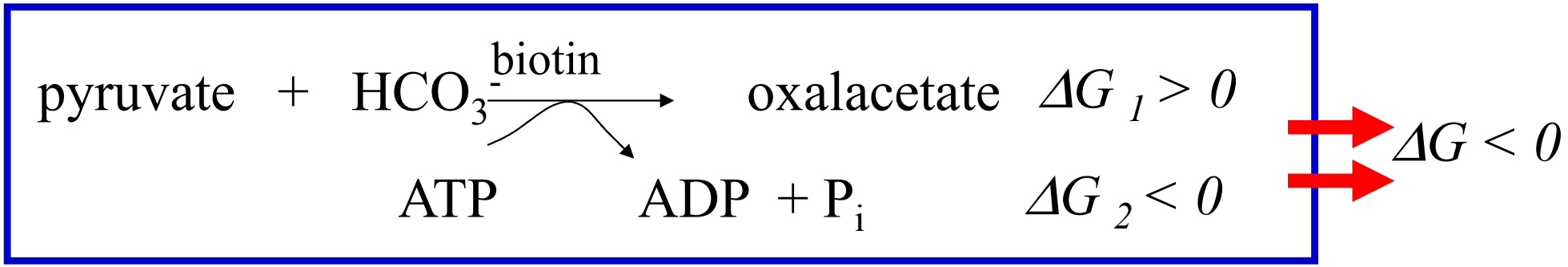
$$\text{glucose} + P_i \rightarrow \text{glucose-6-P} + H_2O \qquad \Delta G^{o\prime} = +13{,}8 \text{ kJ/mol}$$

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

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

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

Example 2: Carboxylation of pyruvate



Partial reactions:

HCO_3^- + ATP → ADP + $^-OCO\text{-}O\text{-}PO_3^{2-}$

phosphocarbonate

$^-OCO\text{-}O\text{-}PO_3^{2-}$ + biotin → ^-OOC-biotin + P_i

^-OOC-biotin + pyruvate → oxalacetate

$\Delta G < 0$

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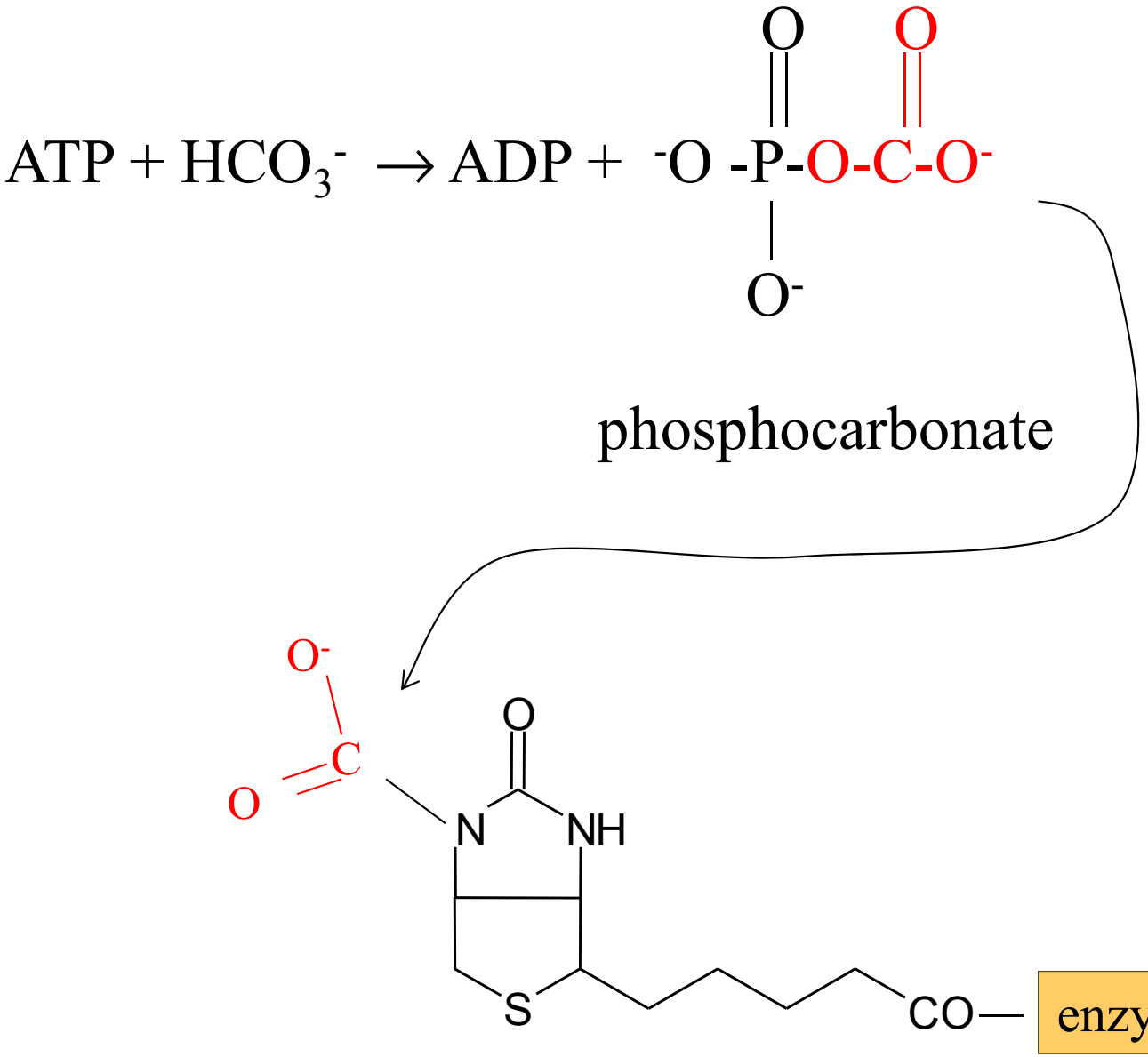
biotin + ATP + HCO_3^- → carboxybiotin + ADP + Pi

carboxybiotin + pyruvate → biotin + oxalacetate

Carboxylation of biotin - formulas



Remember:

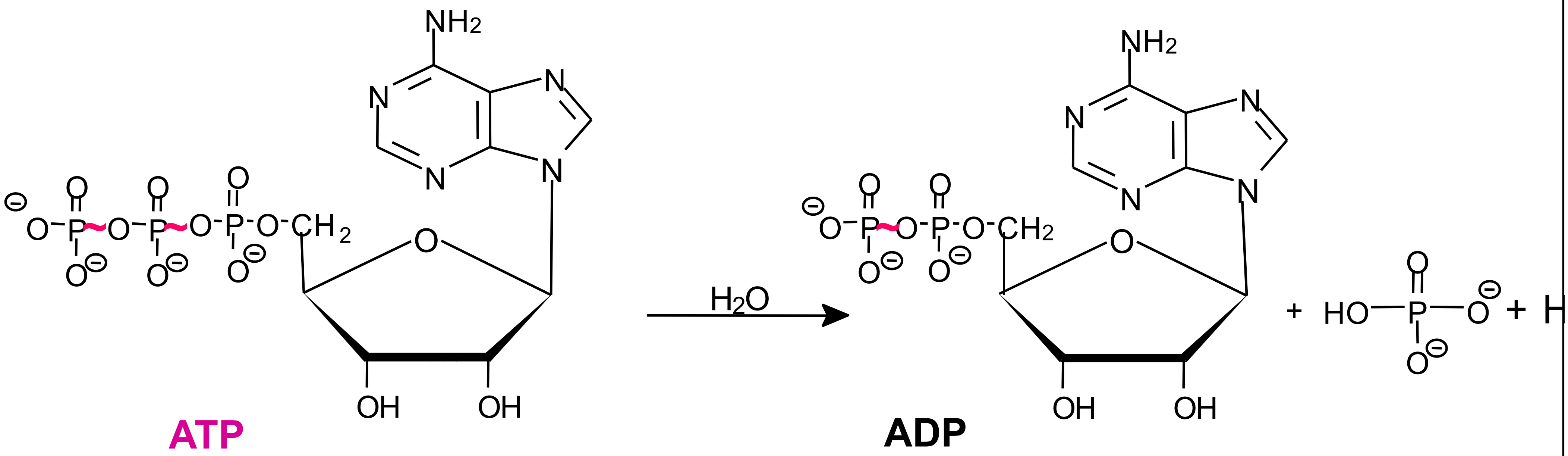
- Biotin is necessary for carboxylation reactions
- Carboxylation is always connected with ATP cleavage

Carboxylate anion is activated by binding P_i and by means of biotin attached to enzyme is transferred to pyruvate

The term „high-energy compound“

(also „macroergic compound“ or „energy rich compounds“)

The most important is ATP



ATP provides energy in two reactions:

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

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

Reactions are catalyzed by enzymes

Similarly GTP, UTP a CTP can provide energy

The other high-energy compounds

Compounds that by hydrolytic cleavage provide energy that is comparable or higher than $\Delta G^{0\prime}$ of ATP hydrolysis

Most often derivatives of phosphoric acid containing phosphate bonded by:

- anhydride
- amide
- enolester bond

(esters of phosphoric acid are not macroergic compounds)

Most important macroergic phosphate compounds

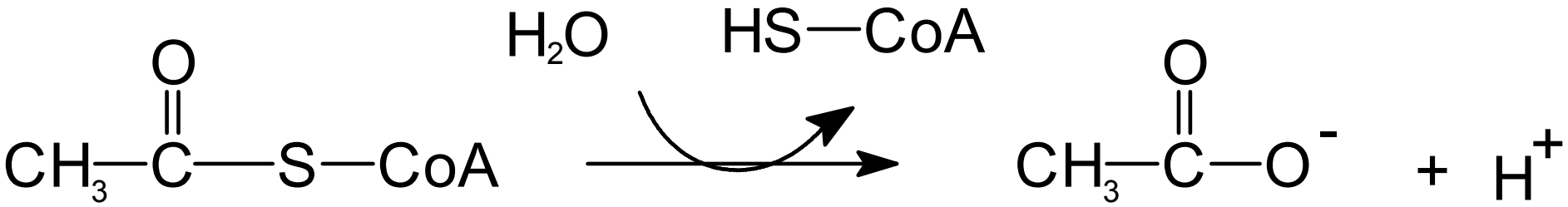
Compound	ΔG^0 (kJ/mol)	typ compound
phosphoenolpyruvate	-62	enolester
carbamoyl phosphate	-52	mixed anhydride
1,3-bisphosphoglycerate	-50	mixed anhydride
phosphocreatine	-43	amide

These compounds are formed in metabolic processes.

Their reaction with ADP can provide ATP = **substrate phosphorylation**

Energy- rich compounds may be also thioesters

(e.g. acyl group bonded to coenzym A)



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

How are formed energy-rich compounds during metabolism ?

„combustion of nutrients“

• nutrients in food (lipids and saccharides, partially proteins) contain carbon atoms with low oxidation number

• they are continuously degraded (oxidized) to various intermediates, that in decarboxylation reactions release CO_2

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

•energy released by their reoxidation is utilized for synthesis of ATP

(**oxidative phosphorylation**)

•several high energy compounds are formed directly during the metabolism of nutrients – they provide ATP in a reaction with ADP (**phosphorylation of ADP on substrate level)**

Formation of ATP in the cell

➢Oxidative phosphorylation

Accounts for more than 90% of ATP generated in animals

= the synthesis of ATP from ADP and Pi

$ADP + P_i \rightarrow ATP$ catalysed by ATP-synthase

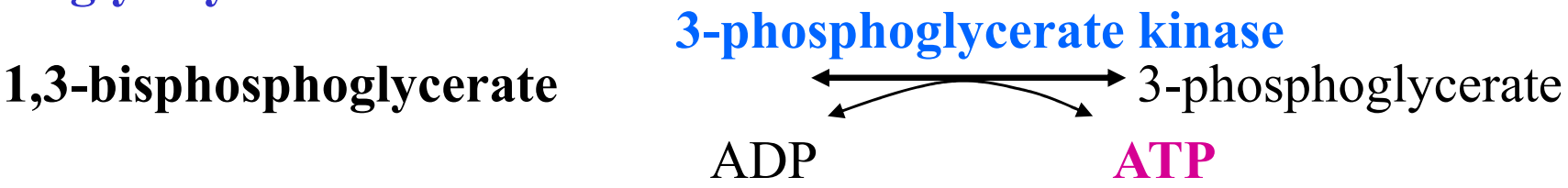
Reaction is driven by the **electrochemical potential of proton gradient** across the inner mitochondrial membrane. This gradient is generated by the **terminal respiratory chain**, in which **hydrogen atoms**, as NADH + H^+ and $FADH_2$ produced by the oxidation of carbon fuels, **are oxidized to water**.
The oxidation of hydrogen by O_2 is coupled to ATP synthesis.

➢Phosphorylation of ADP on substrate level

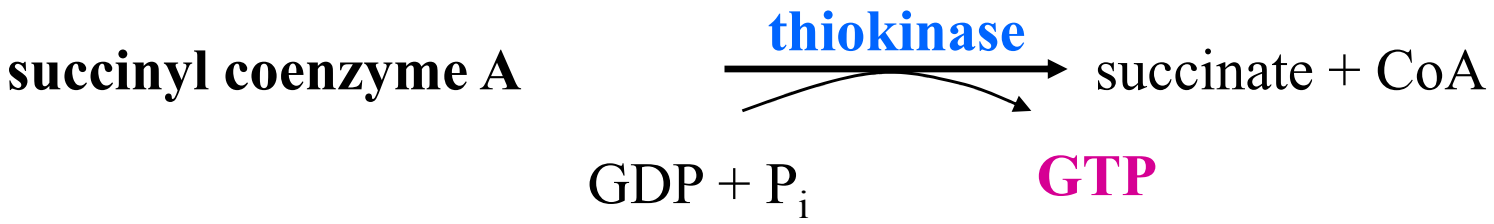
Transfer of $-PO_3^{2-}$ from energy rich compound to ADP

Examples of substrate-level phosphorylations

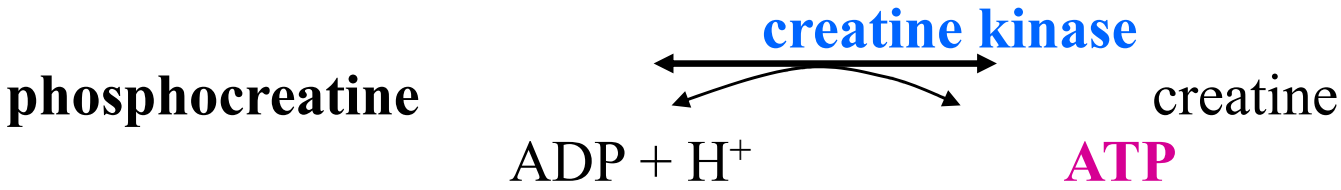
In glycolysis



In the citrate cycle



In skeletal muscle phosphocreatine serves as a reservoir of high-potential phosphoryl groups that can be readily transferred to ATP:



ATP in cells

- Life expectancy of an ATP molecule is about 2 min.
- It must be permanently synthesized
- Momentary content of ATP in a human body is about 100 g, but 60-70 kg is produced daily
- Adenylate kinase maintains the equilibrium between ATP, ADP a AMP

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

Energy status of a cell

[ATP]/[ADP] ratio (in most cells 5-200)

Energy charge of the cell:

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

The energy charge of most cells ranges **from 0.80 to 0.95**

Control of metabolism

Metabolism is regulated by controlling

- **catalytic activity of enzymes**

  allosteric and cooperative effects, reversible covalent modification, substrate concentration

- **the amount of enzymes**

  synthesis of adaptable enzymes

- **the accessibility of substrates**

  compartmentalization segregates biosynthetic and degradative pathways the flux of substrates depends on controlled transfer from one compartment of a cell to another

- **the energy status of the cell**

  of which the energy charge or the phosphorylation potential are used as indexes

- **communication between cells**

  hormones, neurotransmitters, and other extracellular molecular signals often regulate the reversible modification of key enzymes

Metabolism of sacharides 1

Celular metabolism of glucose

Transport of Glucose into the cells

Molecules of glucose are strongly polar, they cannot diffuse freely across the hydrophobic lipid bilayer

Glucose transporters

Transmembrane proteins facilitating a transport of glucose

2 main types: GLUT (1-14)* and SGLT**

* *glucose transporter*

** *sodium-coupled glucose transporter*

GLUT 1-GLUT 14, family of transporters with common structural features (isoforms) but a tissue specific pattern of expresion:

~ 500 AA, 12 transmembrane helices

Mechanism of transport:

facilitated diffusion (follows concentration gradient, do not require energy)

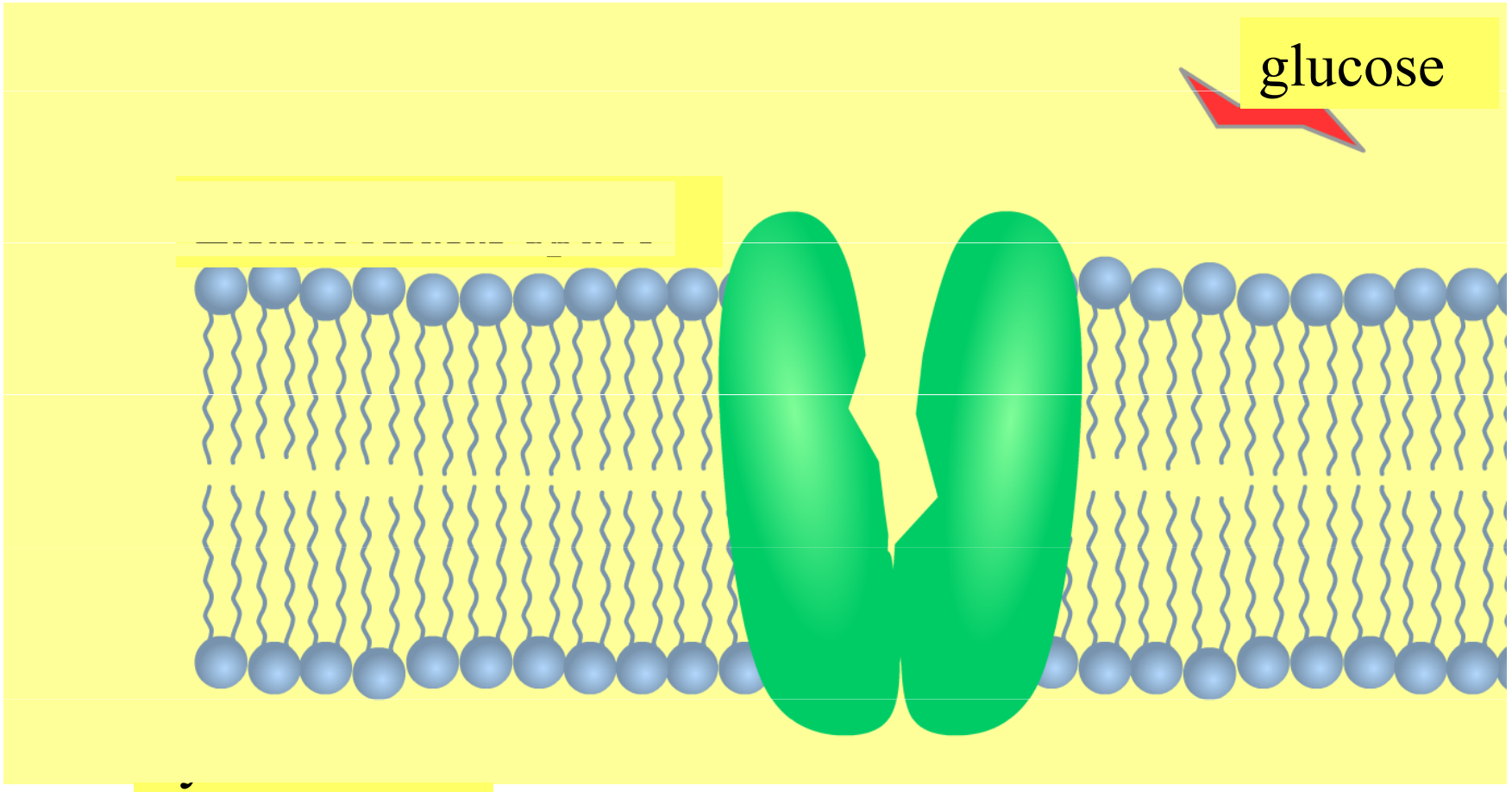
Differences between the GLUT transporters

- affinity to glucose
- different way of regulation
- tissue specific occurence

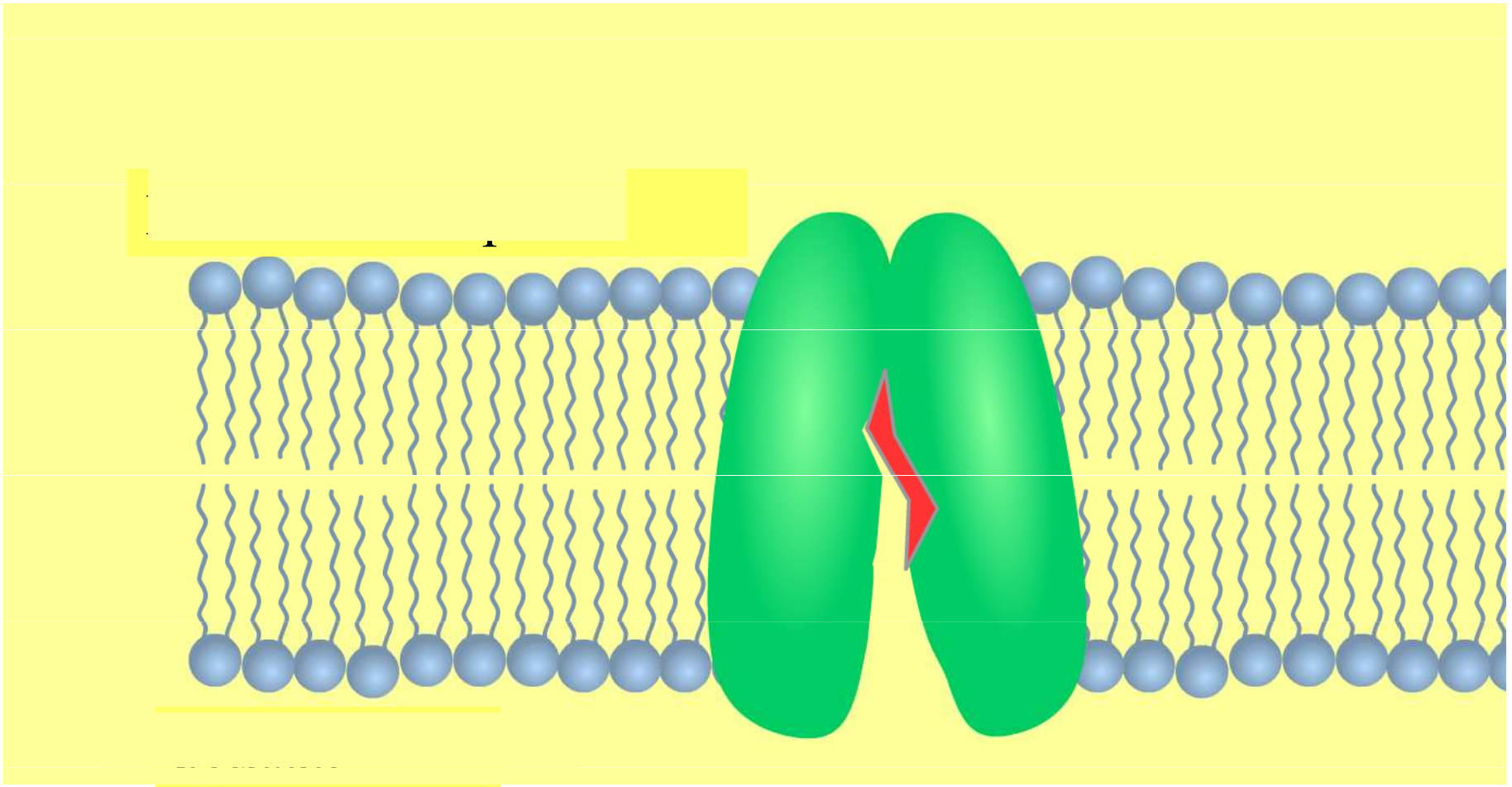
Glucose transporters

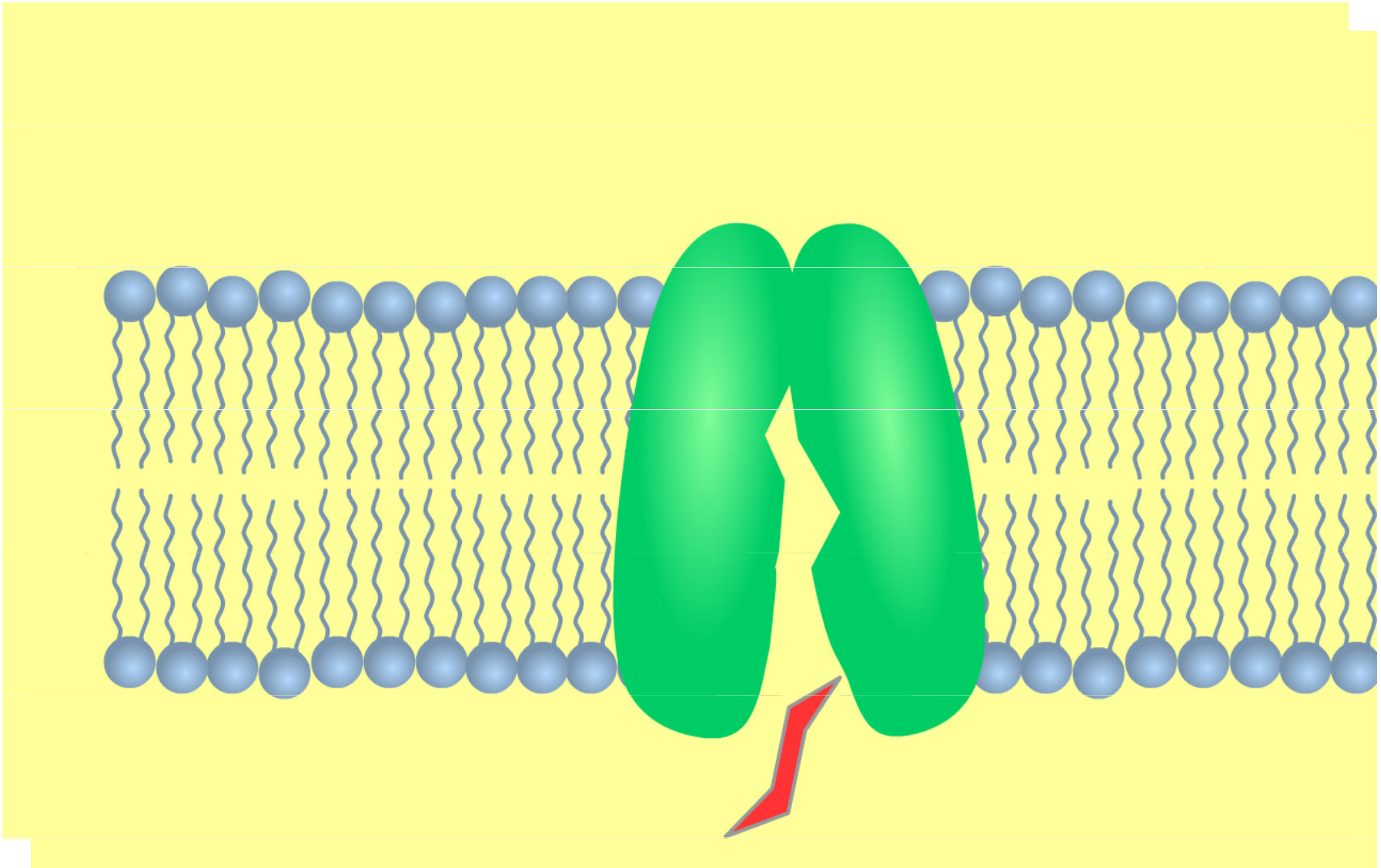
Typ	characteristics
GLUT 1	Basal glucose uptake (ercs, muscle cells at resting conditions, brain vessels ..)
GLUT 2	Liver, β cells of pancreas , kidney
GLUT 3	Neurons, placental cells
GLUT 4	Muscle, adipocytes – dependent on insulin
GLUT 5	Transport of fructose, small intestine
GLUT 7	Intracelular transport liver

Transport of glucose by GLUT



Two conformational states of transporters





GLUT 1 deficiency

Inherited deficiency of glucose transporter

Transport across the blood brain barrier is reduced

The glucose concentration in cerebrospinal fluid is decreased.

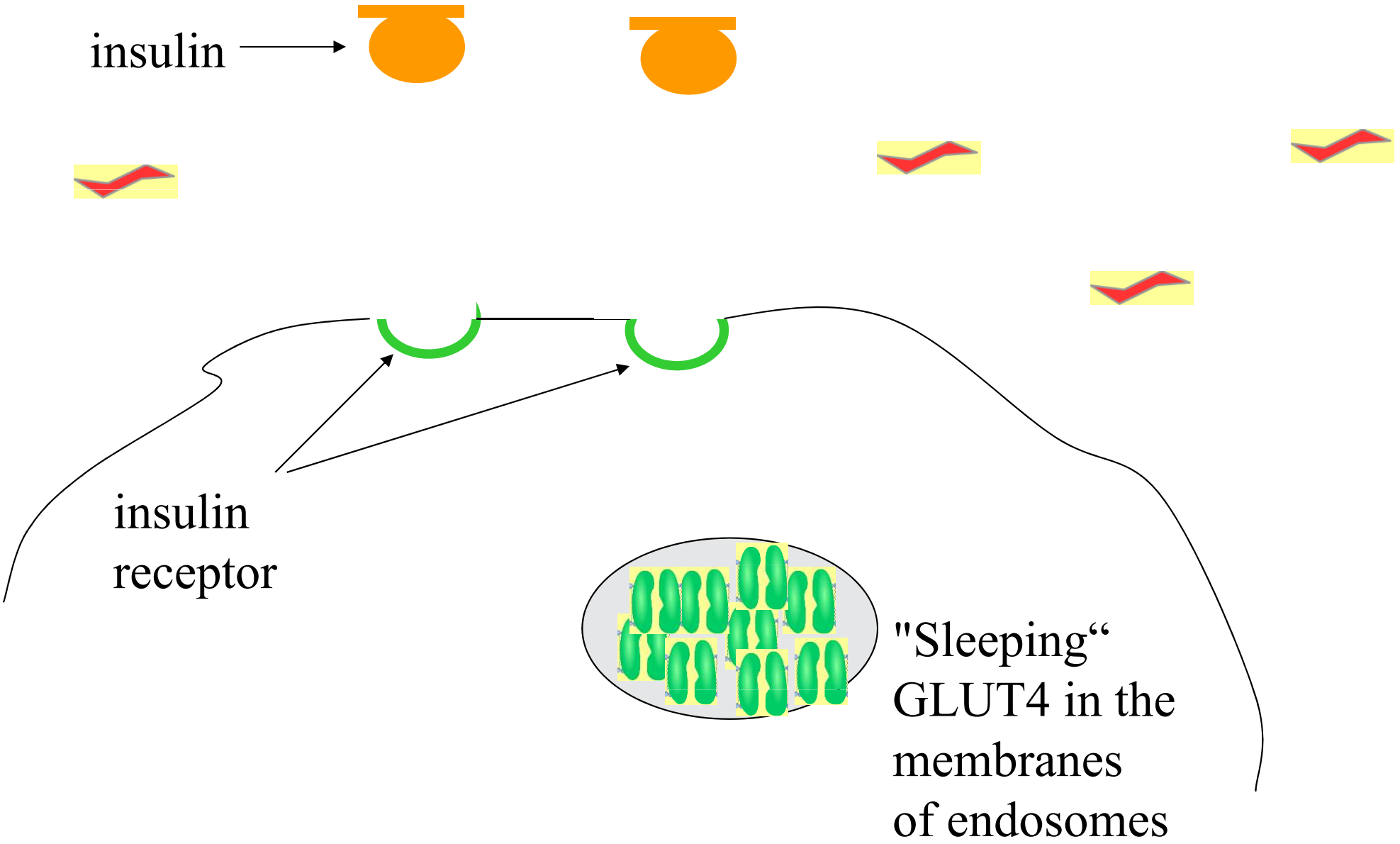
Moreover GLUT1 is essential also for transport of glucose into neurons and glial cells

As glucose is the principal source of fuel to the brain, GLUT1 deficiency causes impaired provision of energy → epileptic encephalopathy

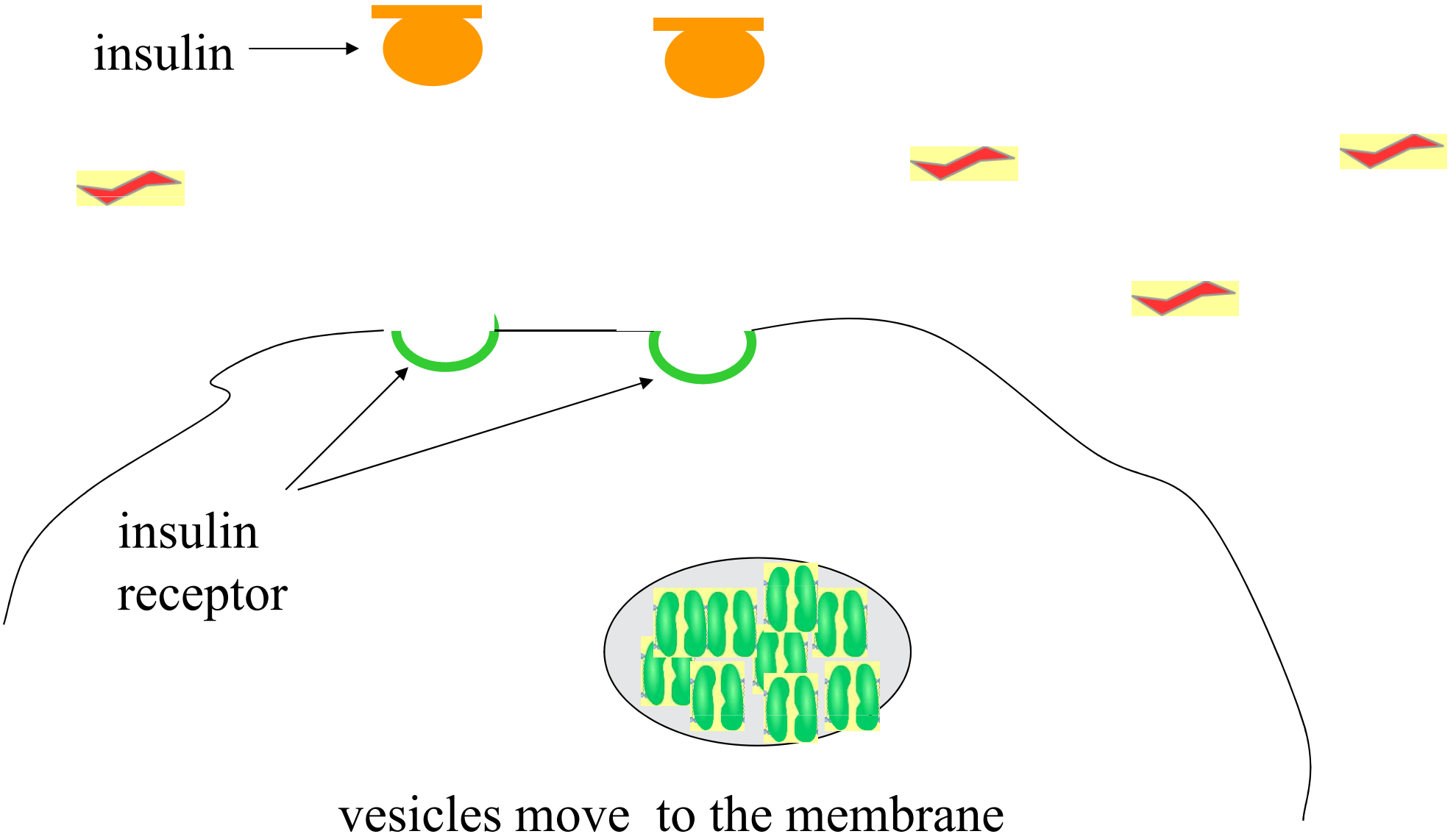
Observation: decreased level of glucose in liquor

Treatment: The only known treatment to date is a very restrictive diet called the ketogenic diet

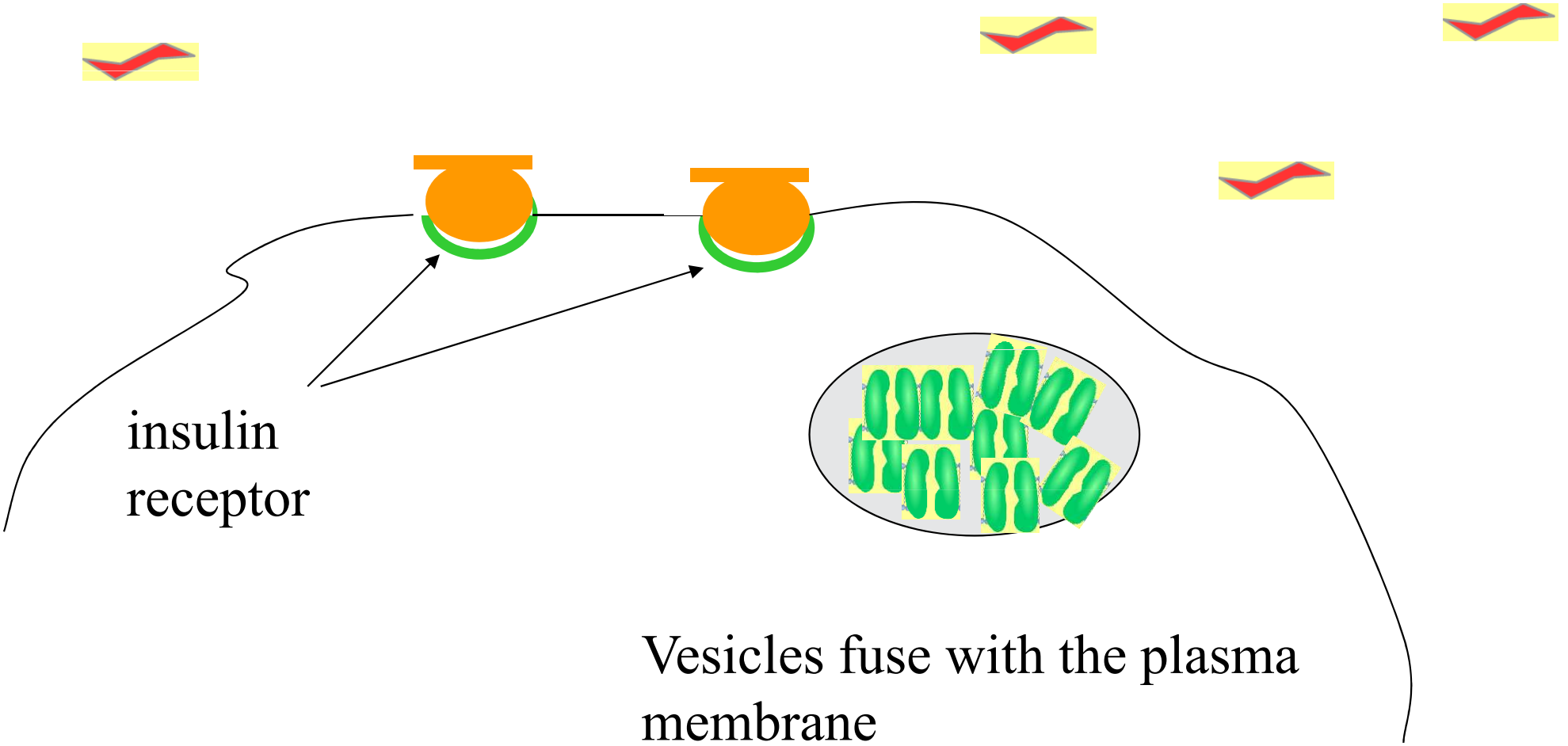
GLUT 4 carriers are regulated by insulin (muscle, adipocytes)



Binding of insulin to its receptor

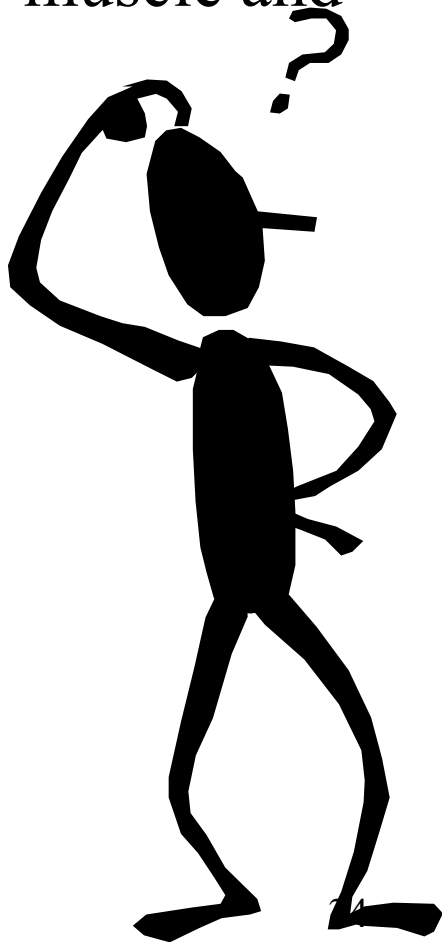


Transport of glucose into the cell



GLUT 4 receptors– conclusion:

The presence of **insulin** leads to a **rapid increase in the number of GLUT4** transporters in the plasma membrane. Hence, insulin promotes the uptake of glucose by muscle and adipose tissue.



Transport of glucose into the epithelial cells of the small intestine and renal tubules cells

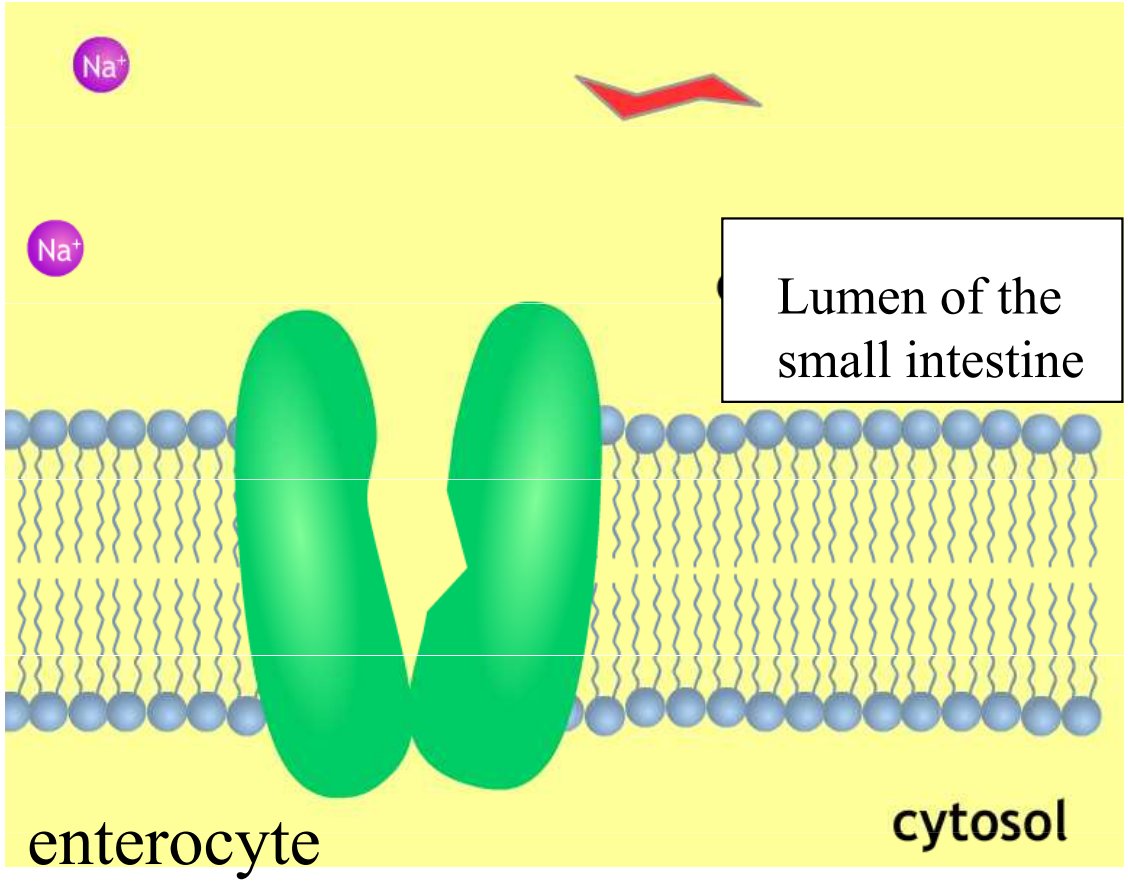
SGLT - transporters

- Mechanism: cotransport with Na^+

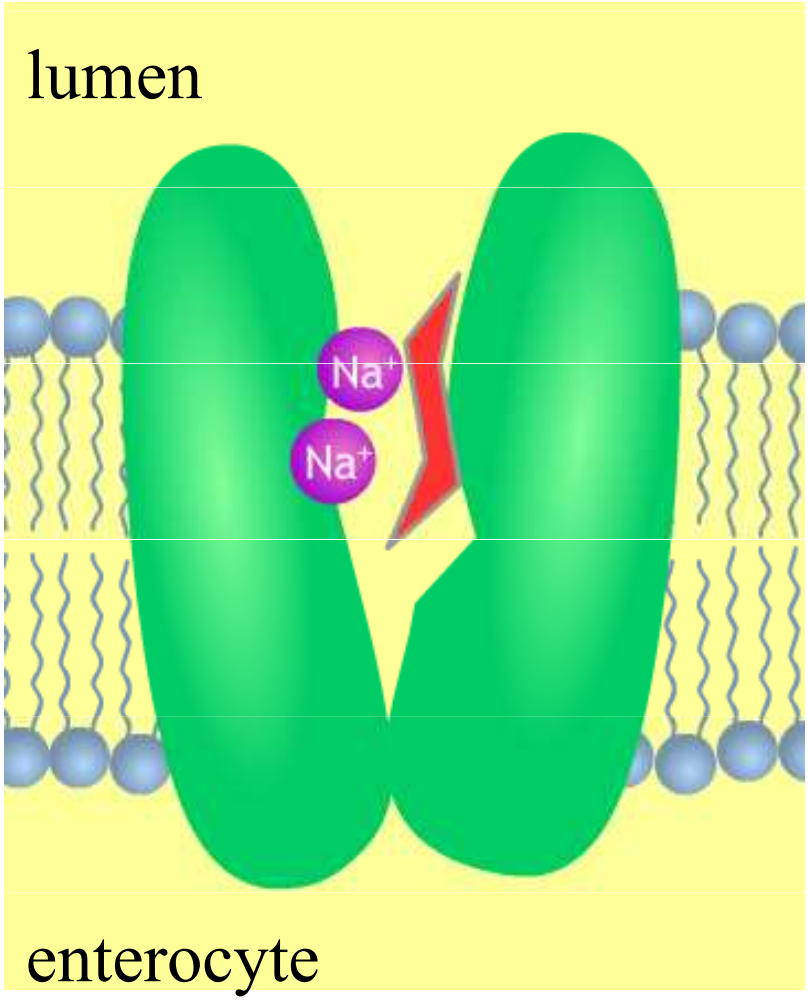
secondary active transport

- glucose and Na^+ bind to two specific sites of the carrier
- they are transported into the cell at the same time (without energy requirement)
- Na^+ is consequently transported outside the cell by ATPase (active transport - consumption of ATP)
- glucose is consequently transported outside the cell by GLUT2

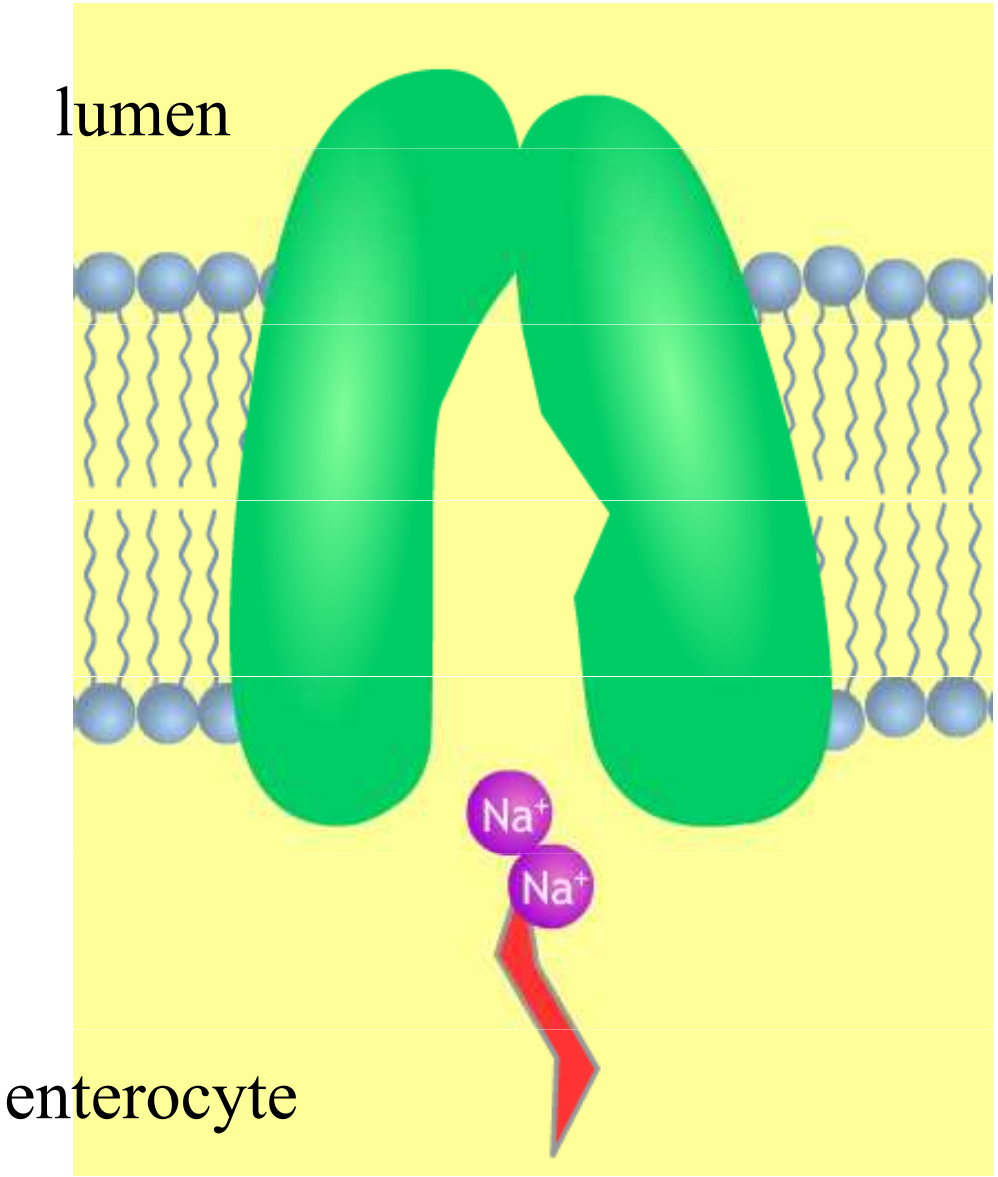
Cotransport of glucose with Na^+



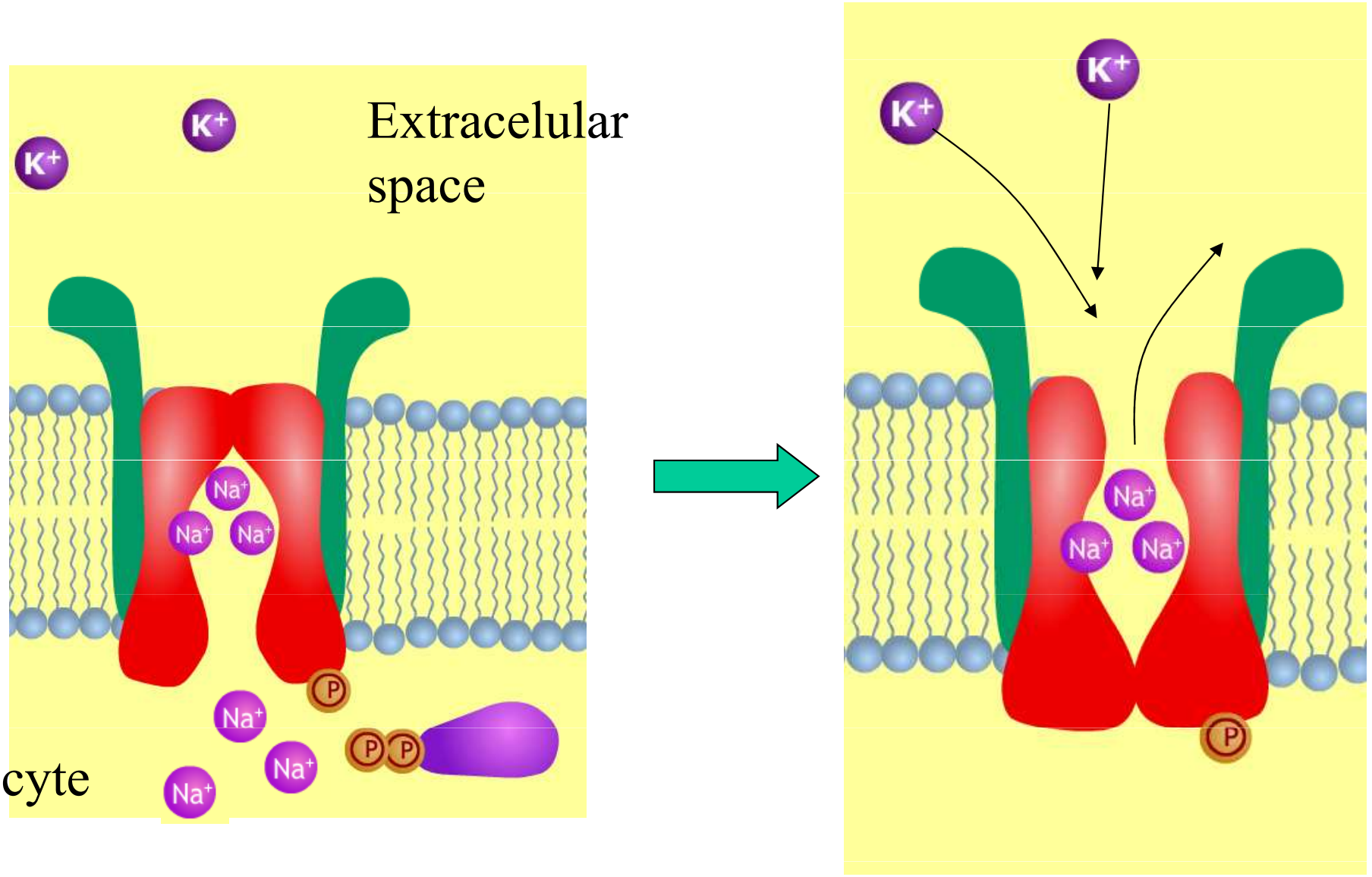
Transporter changes conformation after the binding glucose and Na^+



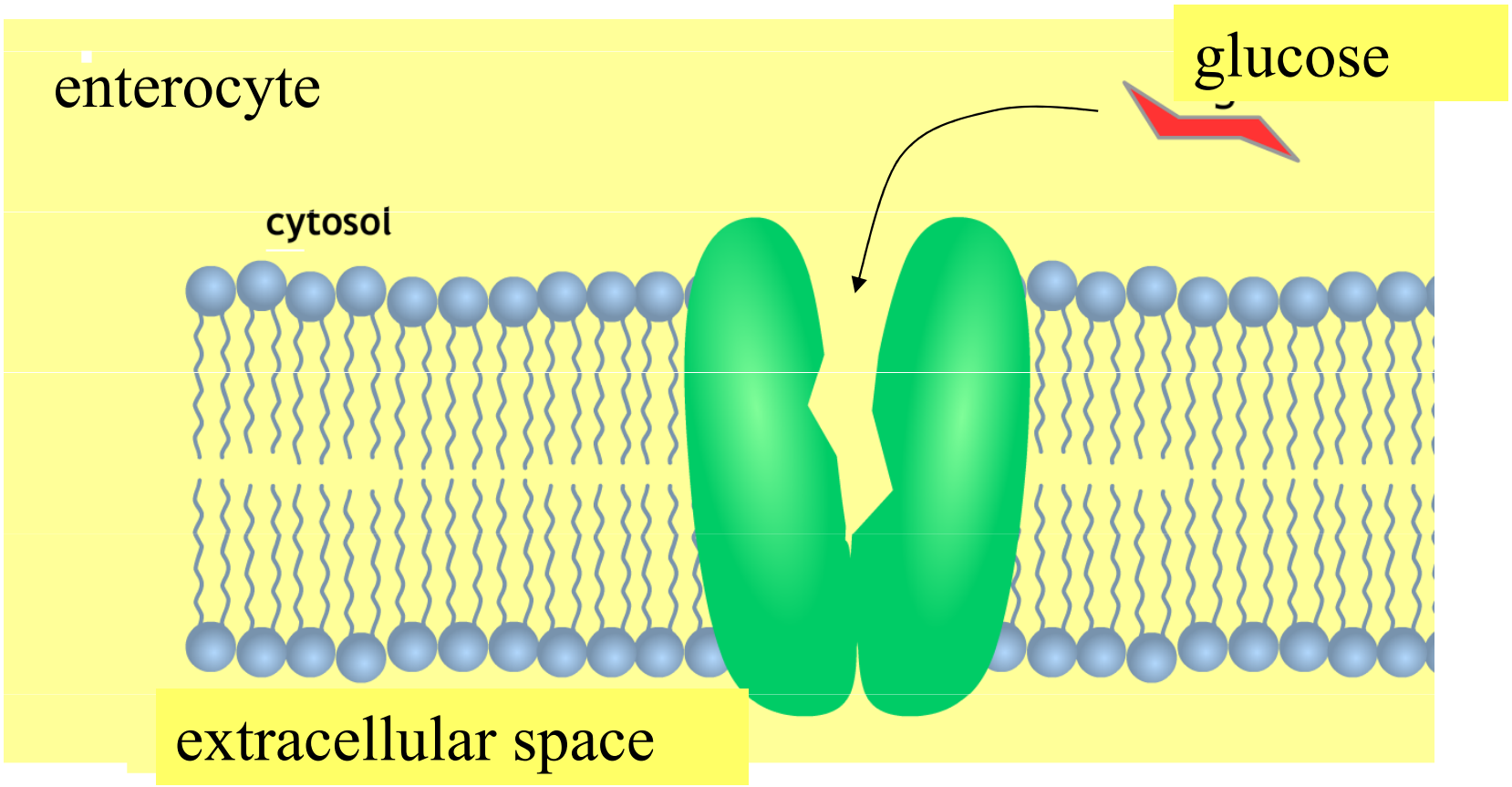
Na^+ and glucose enter the cell (symport)



Na^+/K^+-ATPase is located on the capillary side of the cell and pumps sodium outside the cell (active transport)



glucose is exported to the bloodstream via uniport system GLUT-2 (pasive transport)



SGLT1 deficiency

Hereditary disturbance in the transport of glucose and galactose

Rare disorder (autosomal recessive patern).

This failure of active transport prevents the glucose and galactose from being absorbed.

Symptoms become apparent in the first weeks of a baby's life.

Severe diarrhea leading to life-threatening dehydration, destabilization of the acidity of the blood and tissues (acidosis), stomach cramps.

Why diarrhea?

The water that normally would have been transported across the brush border with the sugar instead remains in the intestinal tract to be expelled with the stool, resulting in dehydration of the body's tissues and severe diarrhea.

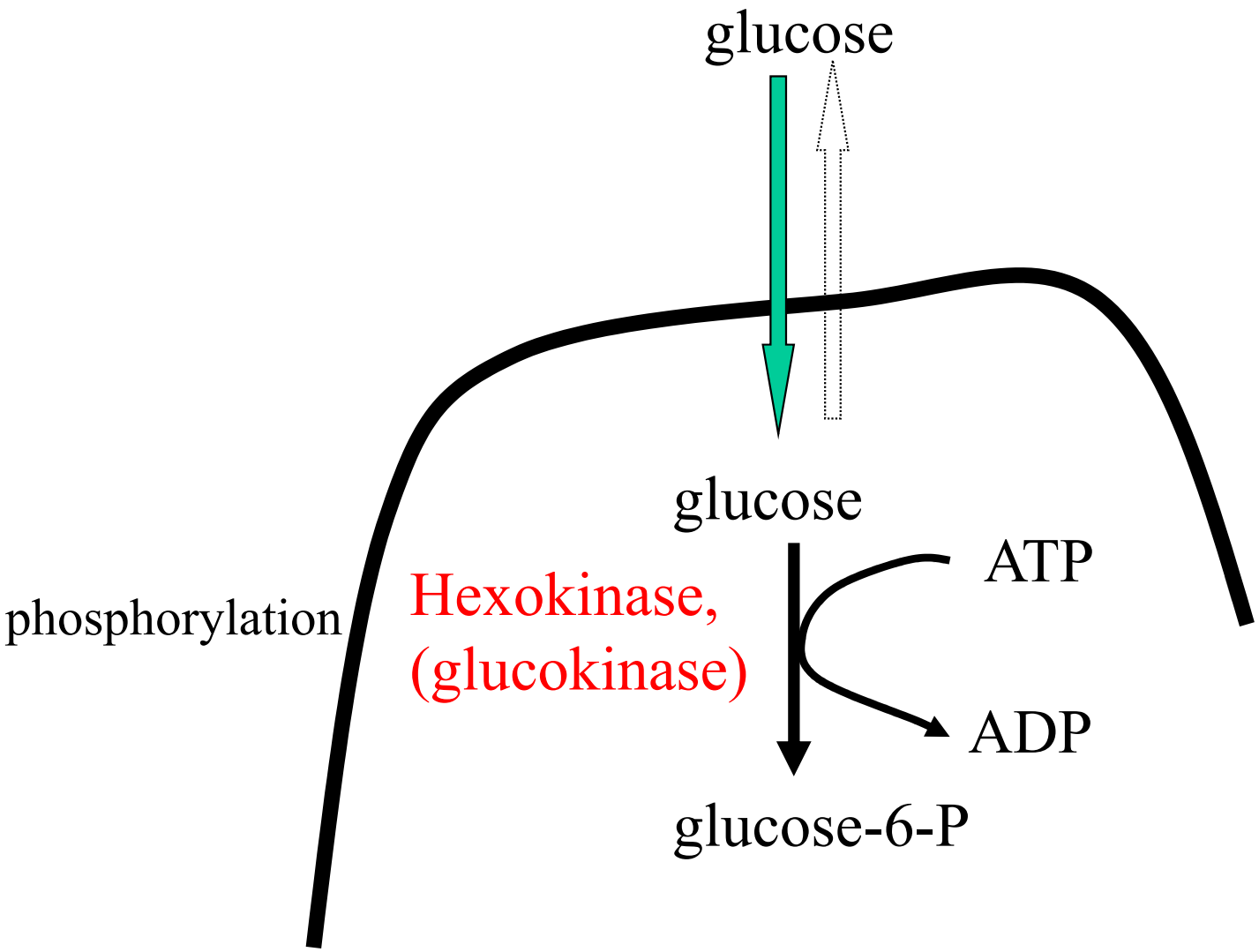
Glucose metabolism in cells

Glucose-6-phosphate is formed immediately after the glucose enters a cell:

glucose + ATP $\longrightarrow$ glucose-6-P + ADP

Enzymes hexokinase or glucokinase

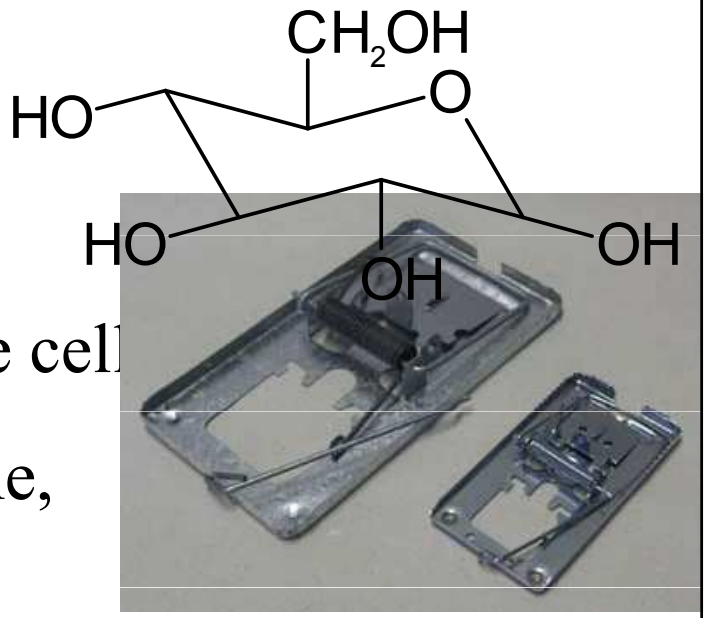
Glucose phosphorylation



The reverse reaction is catalyzed by glucose-6-P phosphatase

This occurs only in liver (and in less extent in kidney).

Consequence:

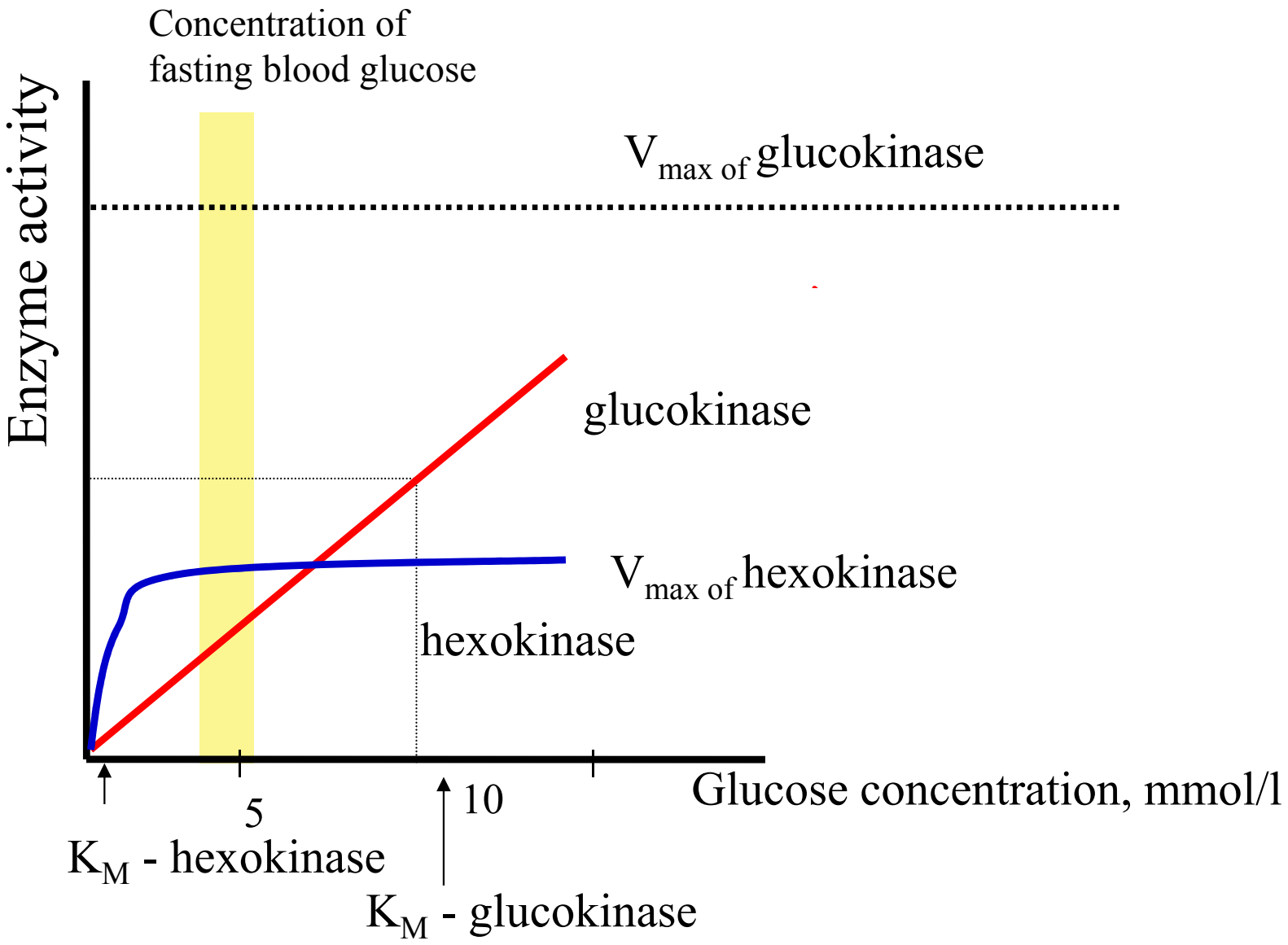


- Phosphorylation reaction traps glucose in the cell
- Glc-6-P cannot diffuse through the membrane, because of its negative charges.
- Formation of Glc-6-P maintains the glucose concentration gradient and **accelerates the entry of glucose into the cell**.
- Only liver (kidney) can convert Glc-6-P back to glucose and release it to blood
- The addition of the phosphoryl group begins to **destabilize glucose**, thus **facilitating its further metabolism**

GLUCOKINASE X HEXOKINASE

Characteristics	Hexokinase	Glucokinase
Occurence	In many tisues	liver, pancreas
Specifity	broad (hexoses)	glucose
Inhibition	Glc-6-P	Is not inhibited
Afinity to Glc	high	low
Inducibility	no	by insulin
K_M (mmol/l)	0,1	10

Glucose concentration and rate of phosphorylation reaction



•**glucokinase** functions only when the intracellular concentration of glucose in hepatocyte is elevated (after a carbohydrate rich meal)

• **hexokinase in liver** functions at lower concentrations of glucose

• **Hexokinase is** inhibited by glucose 6-phosphate, the reaction product. High concentration of this molecule signals that the cell no longer requires glucose for energy, for storage in the form of glycogen, or as a source of biosynthetic precursors, and the glucose will be left in the blood.

Role of glucokinase in pancreas

Glucokinase in β- cells of pancreas functions as a **blood glucose sensor**

When blood glucose level is high (after the saccharide rich meal), glucose enters the β-pancreatic cells (by GLUT2) and is phosphorylated by glucokinase

Increase of energy status in the cell enhances the release of insulin

Conversions of Glc-6P in the cells and their significance

Pathway	Význam
Glycolysis	Energy gain, synthesis of fatty acids from acetyl-CoA
Synthesis of glycogene	Formation of glucose stores
Pentose phosphate path.	Source of pentoses, source of NADPH
Synthesis of derivatives	Synthesis of glycoproteins, proteoglycans

Glycolysis

•Significance: energy gain, formation of intermediates for other processes, includes also the metabolism of galactose and fructose

- Occurs in most of tissues
- Location: cytoplasma
- Reversible, enzyme catalyzed reactions
- Three reactions are irreversible

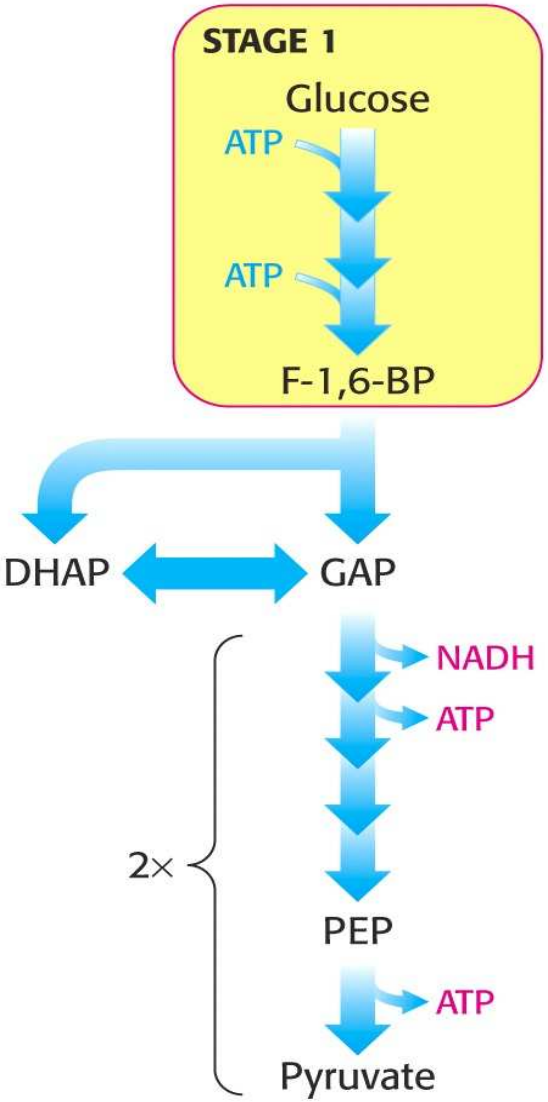
Aerobic glycolysis

At adequate supply of oxygen, pyruvate is converted to acetylCoA

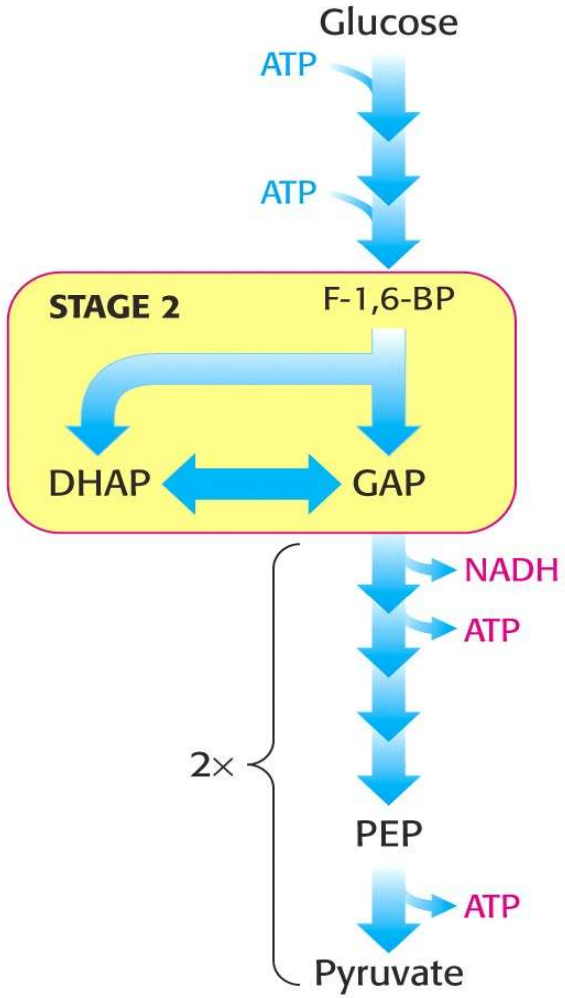
Anaerobic glycolysis

When oxygen is lacking, pyruvate is converted to lactate

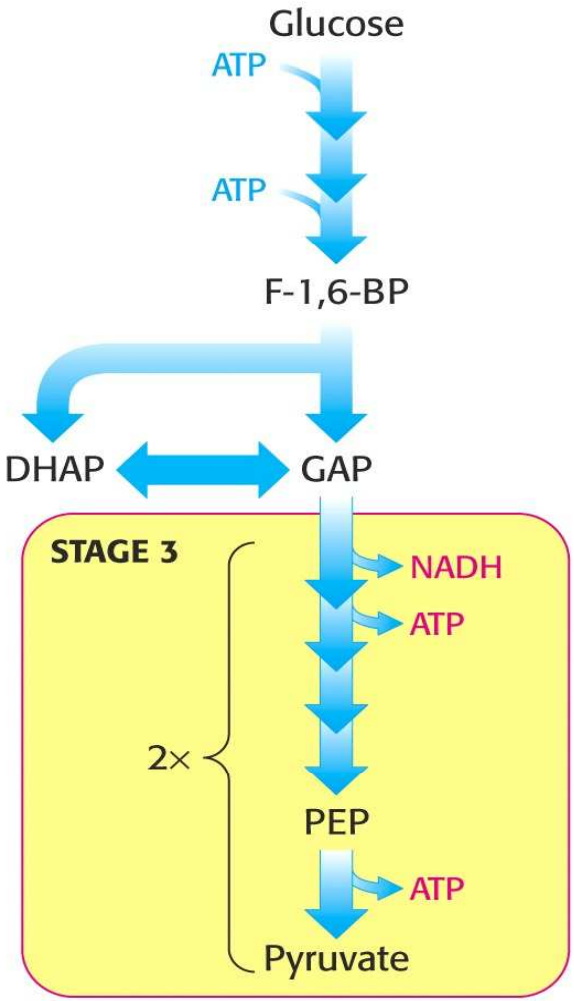
Three stages of glycolysis:



Trapping the glucose in the cell and destabilization by phosphorylation.



Cleavage into two three-carbon units.

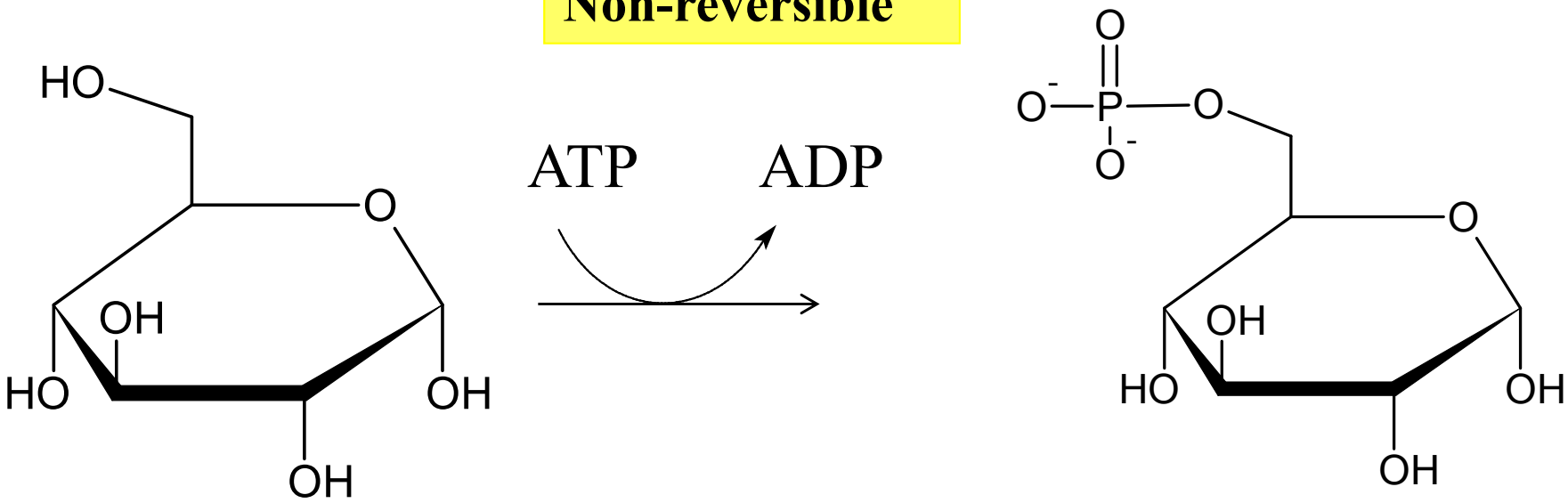


Oxidative stage in which new molecules of ATP are formed by substrate-level phosphorylation of ADP.

Reactions of glycolysis

1. Formation of Glc-6-P

Non-reversible



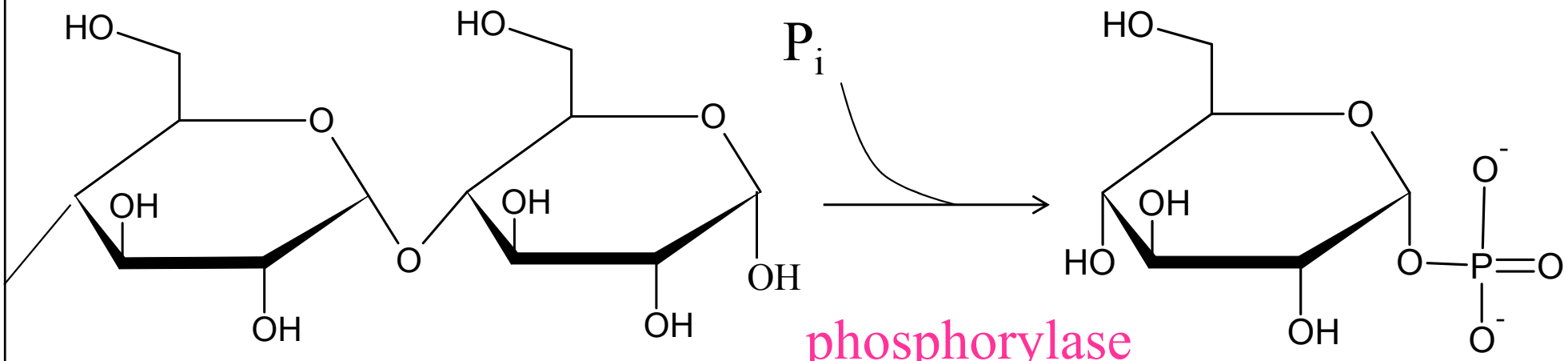
hexokinase, glucokinase

ATP

Formation of glucose-6-P from glycogen

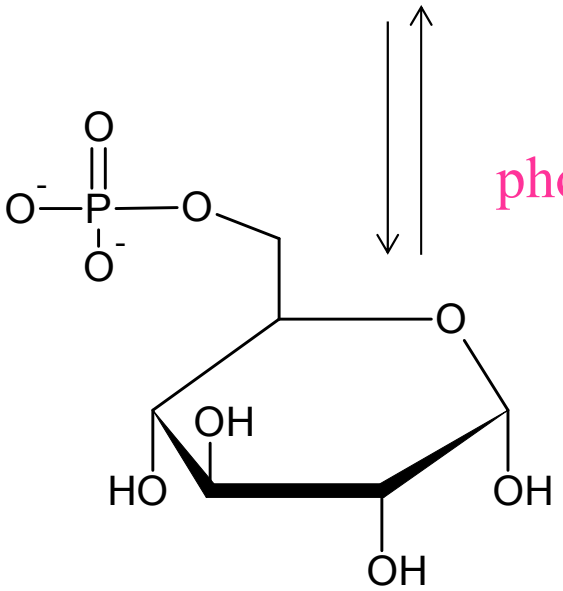
(no consumption of ATP)

glukosa-1-P



phosphorylase

glycogen

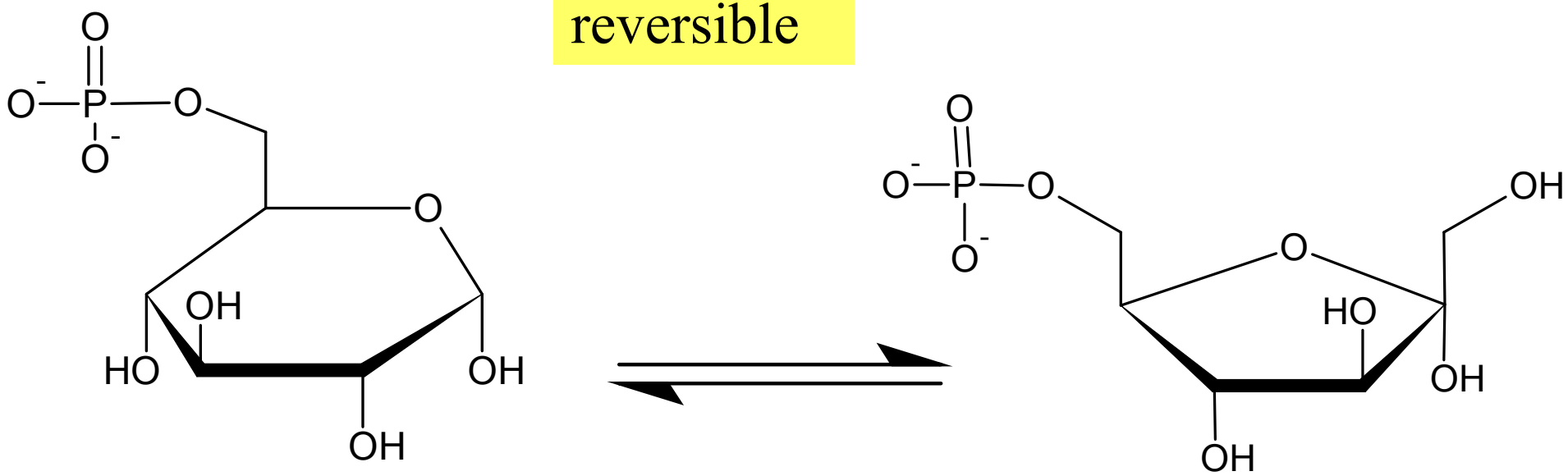


phosphoglucomutase

glucose-6-P

2. Izomerization of glucose-6-P

reversible

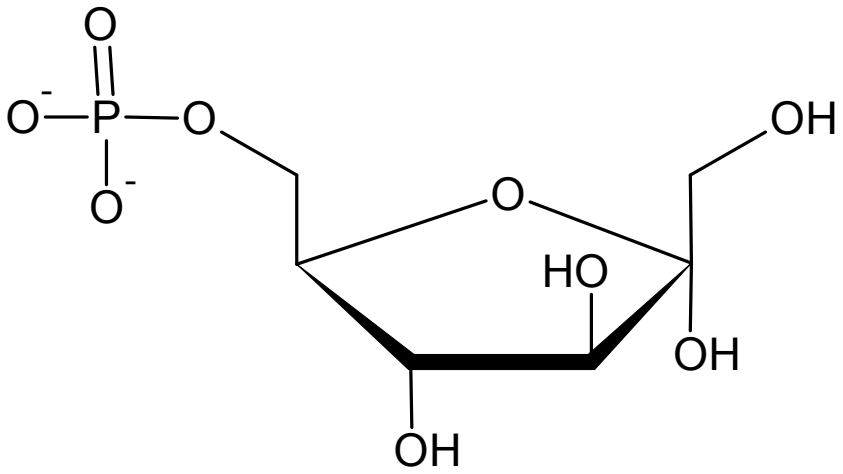


phosphoglucoisomerase

Fructose-6-P

3. Formation fructose-1,6-bisphosphate

ATP

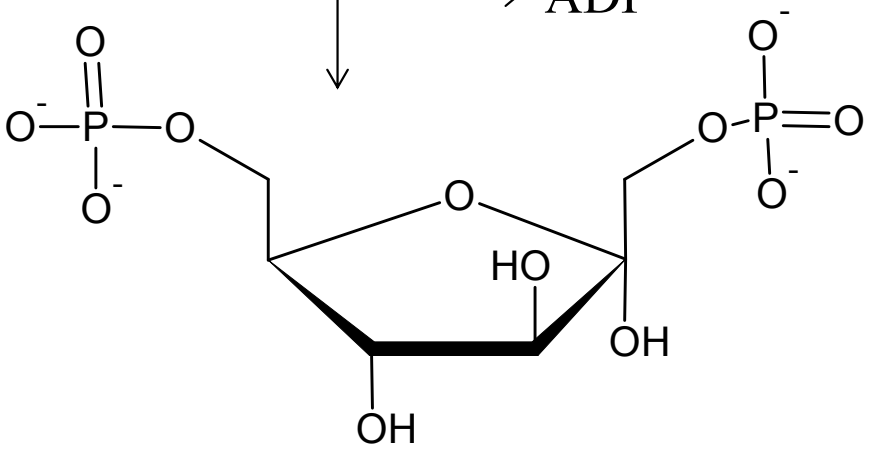


ATP

phosphofructokinase

ADP

Non-reversible



<u>The rate of this reaction determines the rate of the whole glycolysis</u>

Properties of enzymes that are rate limiting step of a metabolic pathway

- The molar activity (turnover number, k_{cat}) of the particular enzyme is smaller than those of other enzymes taking part in the metabolic pathway.
- The reaction rate does not usually depend on substrate concentration [S] because it reaches the maximal value V_{max}.
- The reaction is practically irreversible. The process can be reversed only by the catalytic action of a separate enzyme.

Regulation of phosphofructokinase

- **allosteric** inhibition by ATP and citrate
- **allosteric activation by** AMP, ADP and by fructose-2,6-bisphosphate in liver*

* The formation of fructose-2,6-bisP is controled by hormones

Regulatory effect of fructose-2,6-biP on glycolysis and gluconeogenesis in liver

2,6-biP is allosteric efector of

phosphofructokinase

(glycolysis)

activation

fructose 1,6-bisphosphatase

(gluconeogenesis)

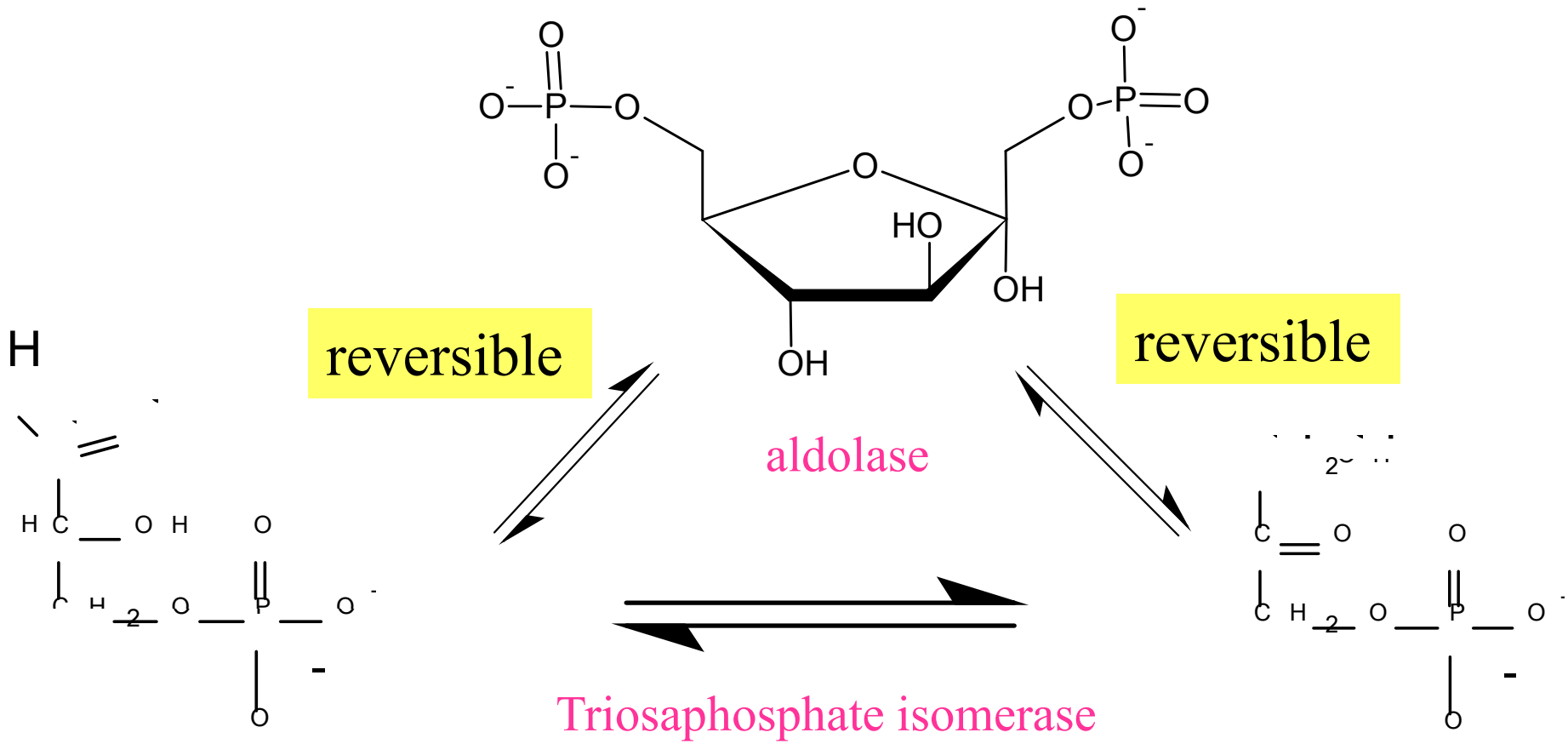
inhibition

Formation of fructose-2,6-biP

↑ Stimulation by fructose-6P

↓ inhibition by glucagone

4.Formation of triose-phosphates

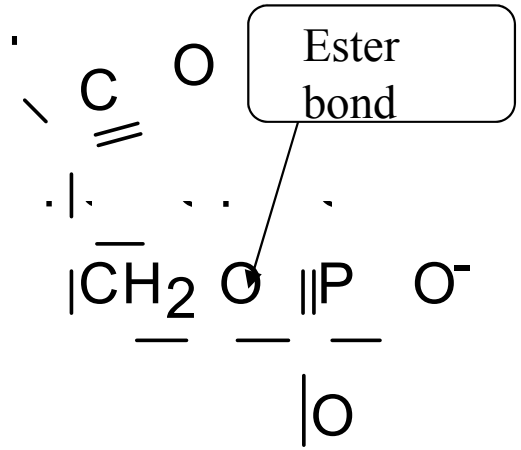


Glyceraldehyde-3-P

reversible

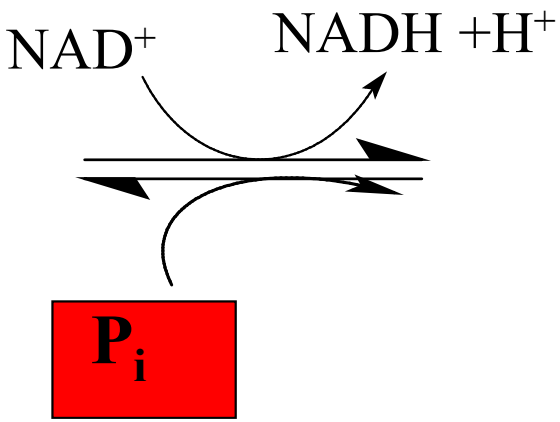
Dihydroxyaceton-P

5. Oxidation and phosphorylation of glyceraldehyde-3-P

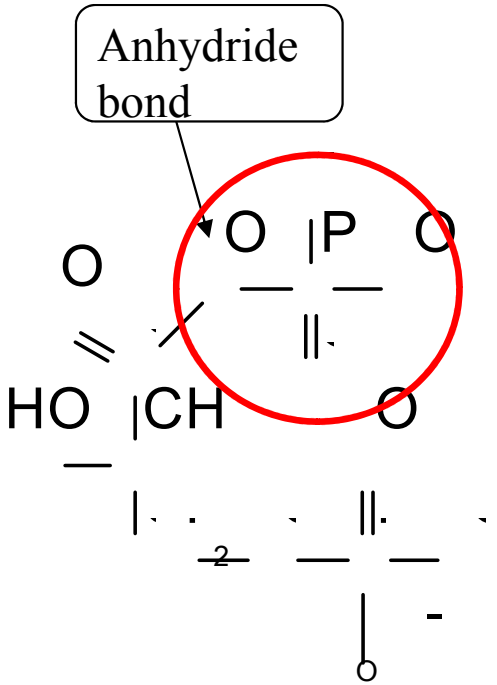


glyceraldehyde-3-P

reversible !!



glyceraldehyde-3-P dehydrogenase

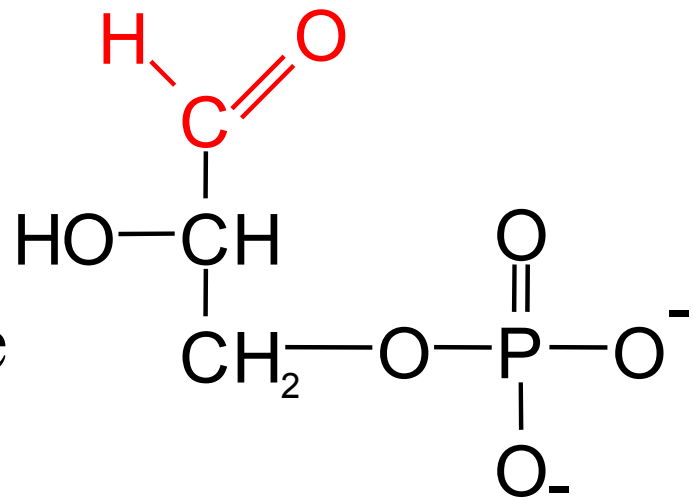


1,3-bisphospoglycerate

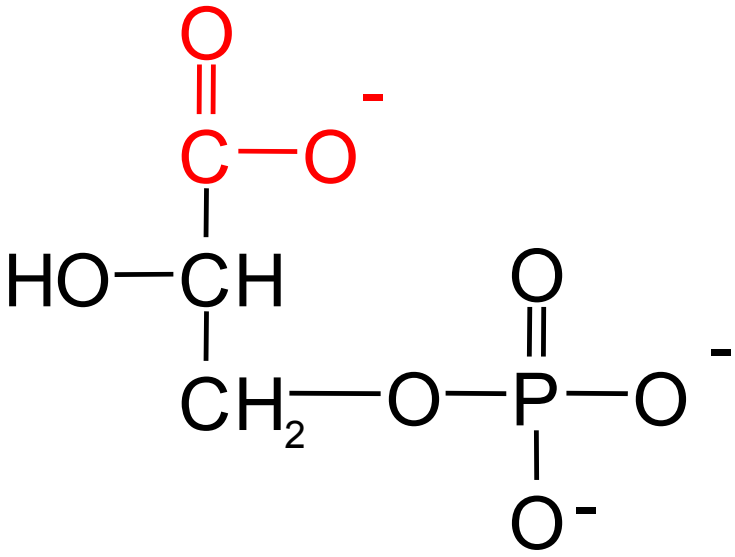
Note, that reaction enters P_i, not ATP !!!!

Compare:

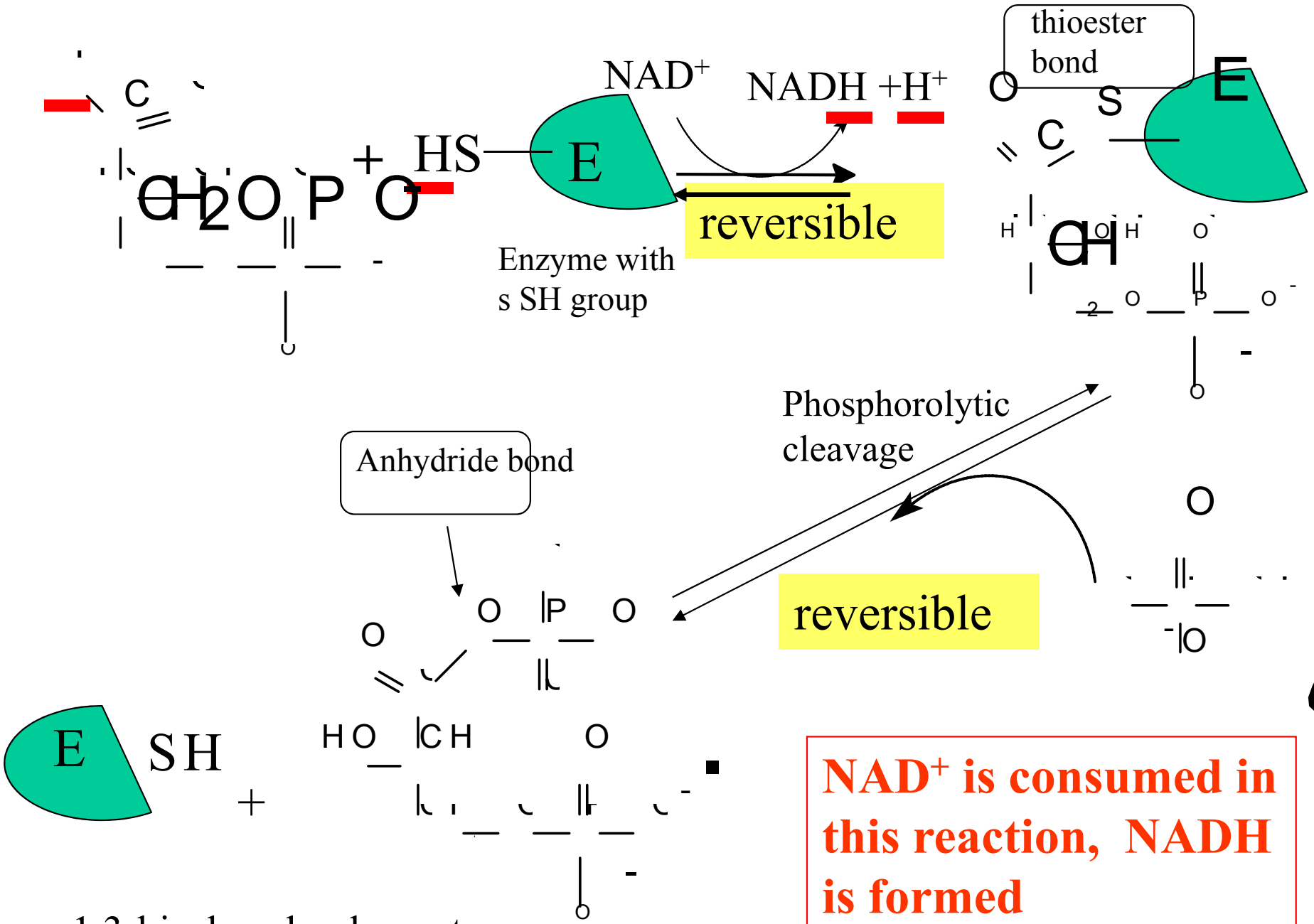
glyceraldehyde-3-phosphate



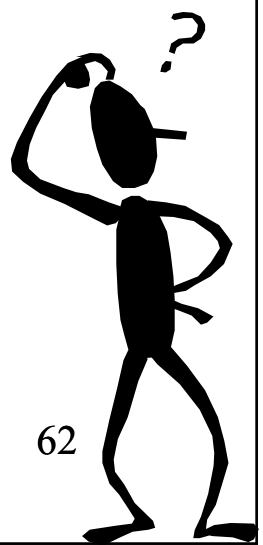
3-phosphoglycerate



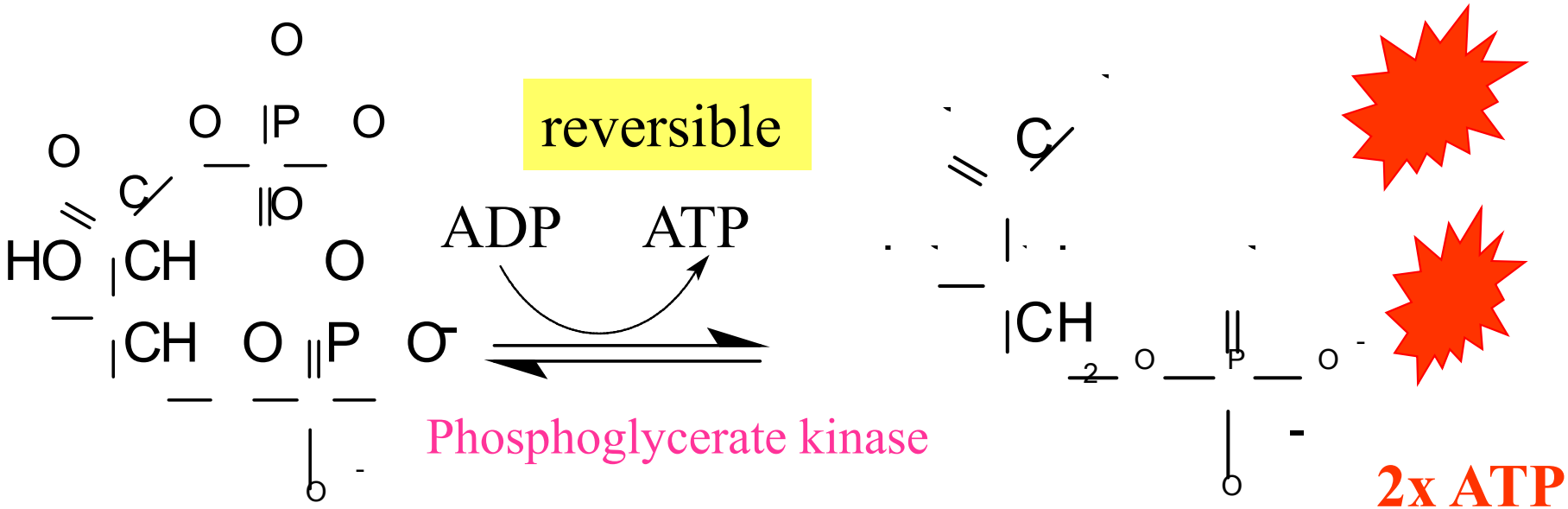
Oxidation and phosphorylation of glyceraldehyde-3-P



NAD^+ is consumed in this reaction, NADH is formed



6.Formation of 3-phosphoglycerate and ATP



1,3-bisphosphoglycerate

3-phosphoglycerate

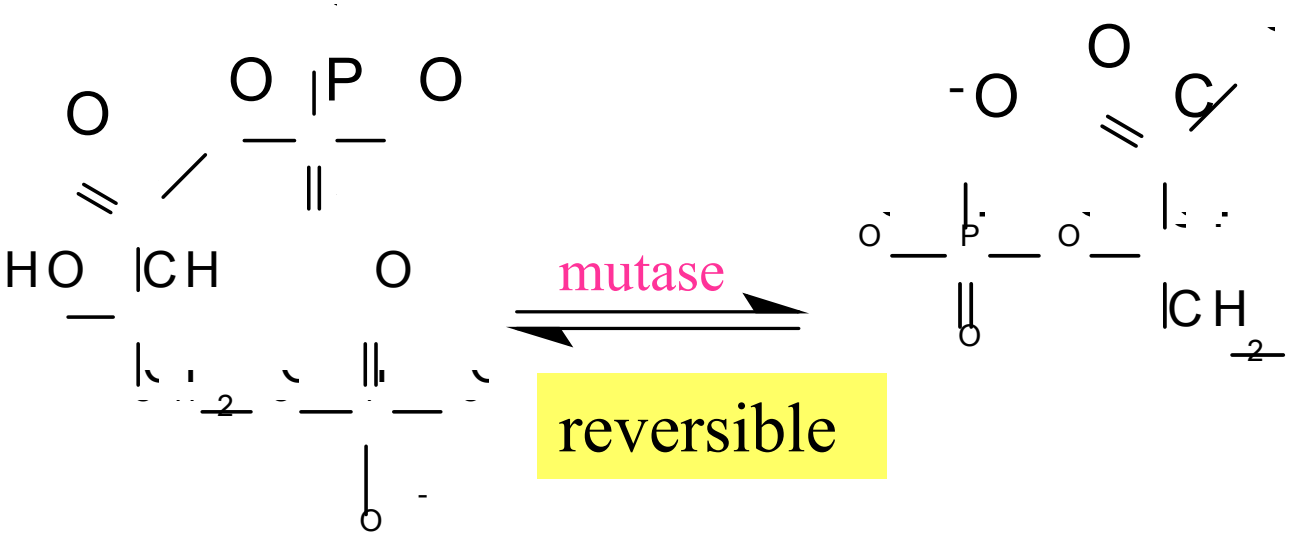
Formation of ATP by substrate-level phosphorylation:

1,3 BPG is high-energy compound (mixed anhydride),

Energy released during PO_3^{2-} transfer is utilized for ATP synthesis

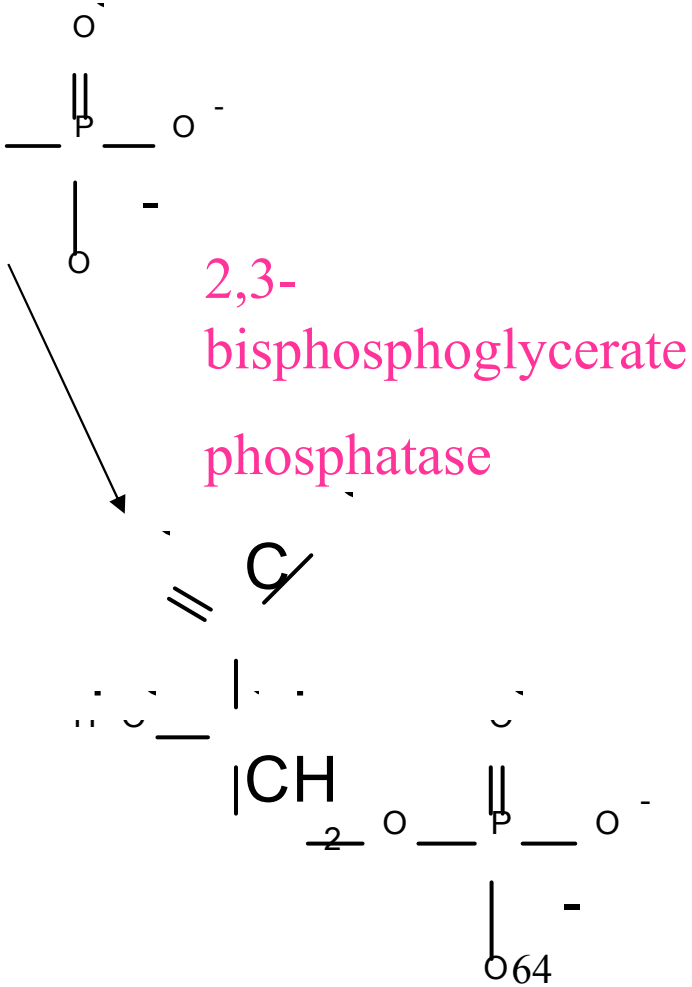
Formation of 2,3-bisphosphoglycerate, side reaction in erytrocytes

Binding to Hb, significance for release of O_2



reversible

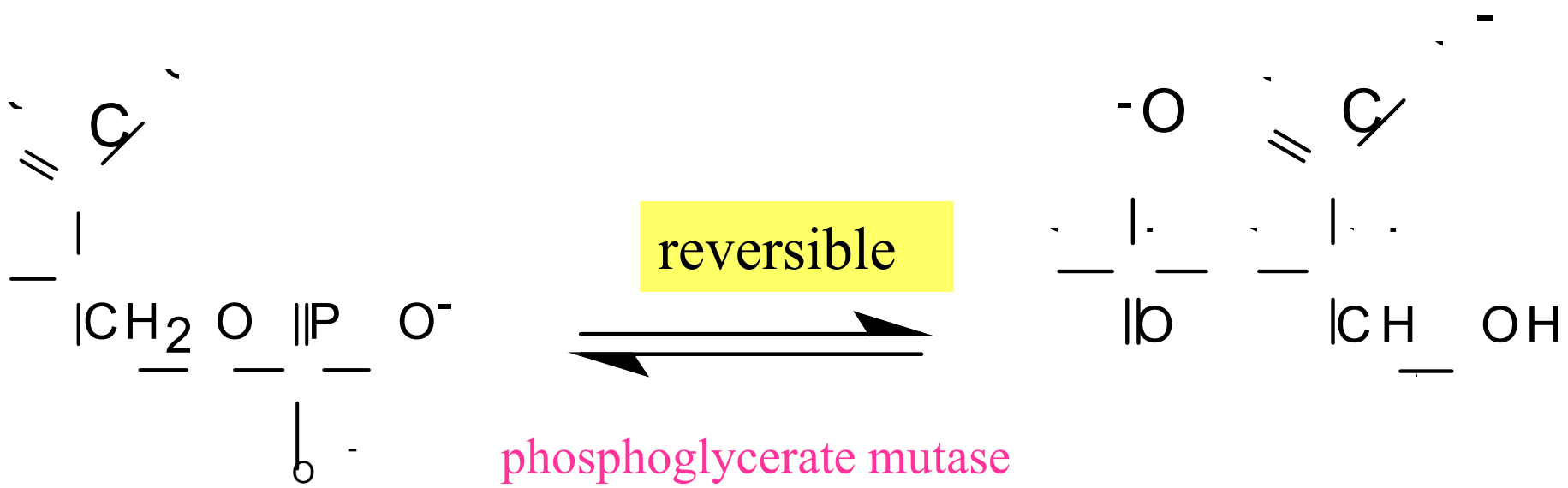
1,3-bisphosphoglycerate



This reaction does not provide ATP !!!

3-phosphoglycerate + P_i

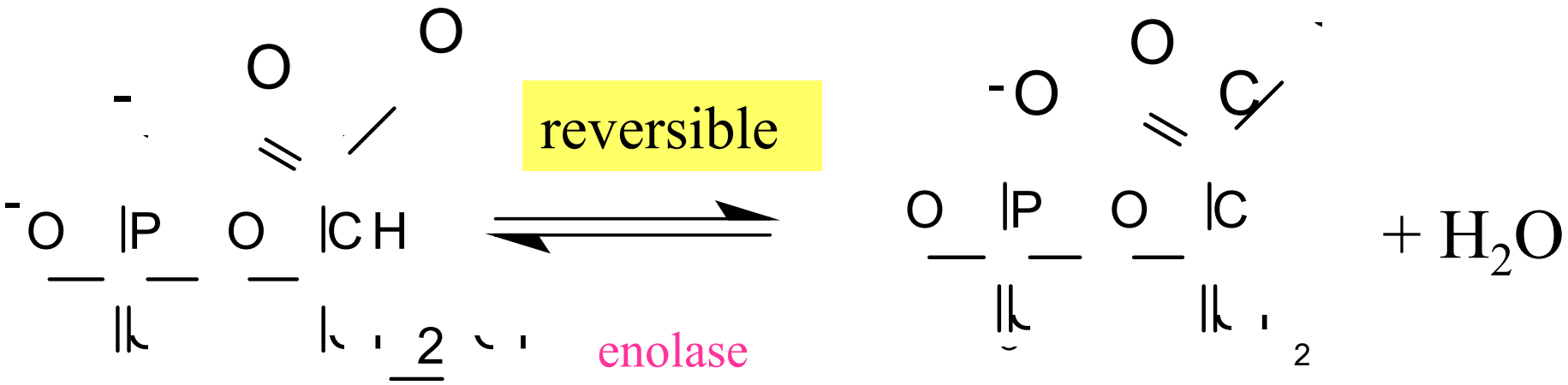
7. Formation of 2-phosphoglycerate



3-phosphoglycerate

2-phosphoglycerate

8. Formation of phosphoenolpyruvate

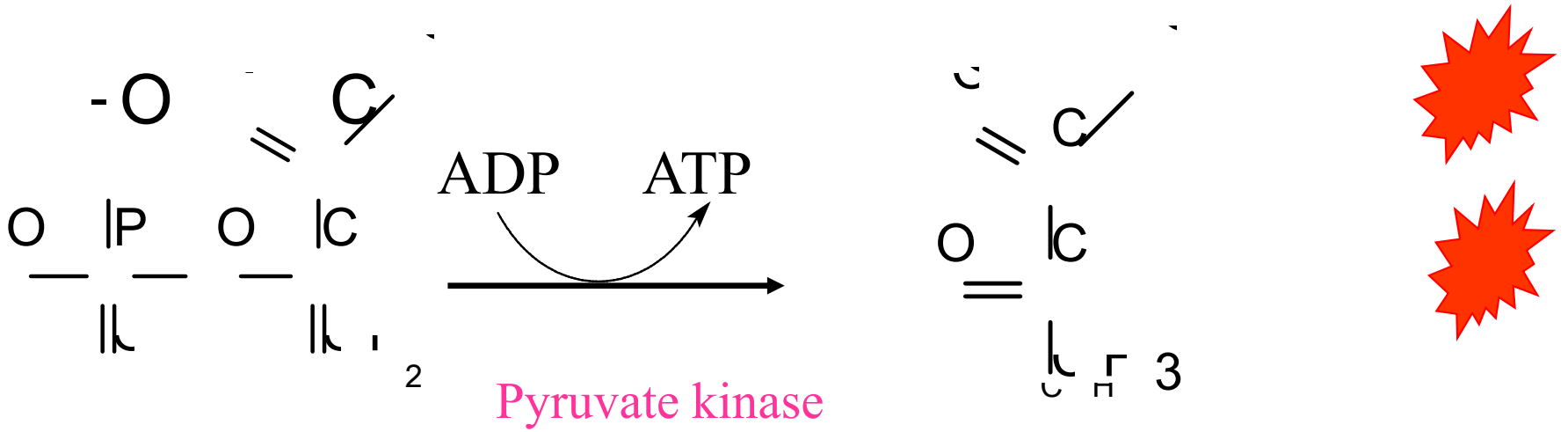


2-phosphoglycerate phosphoenolpyruvate

enolase (inhibition by F^-)

Whem blood samples are taken for mesurement of glucose, it is collected in tubes containing fluoride

9. Formation of pyruvate



phosphoenolpyruvate

Non reversible !!!

2x ATP

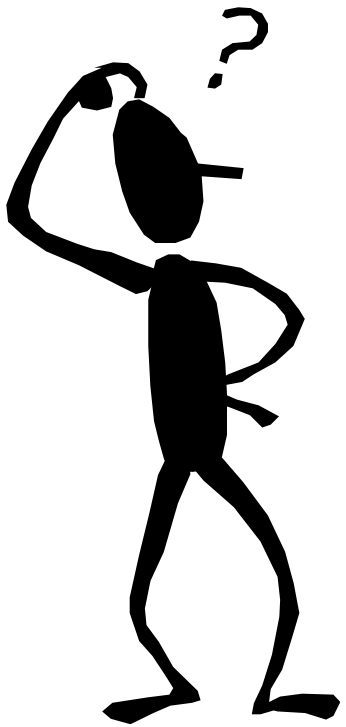
(substrate-level phosphorylation)

Pyruvate kinase

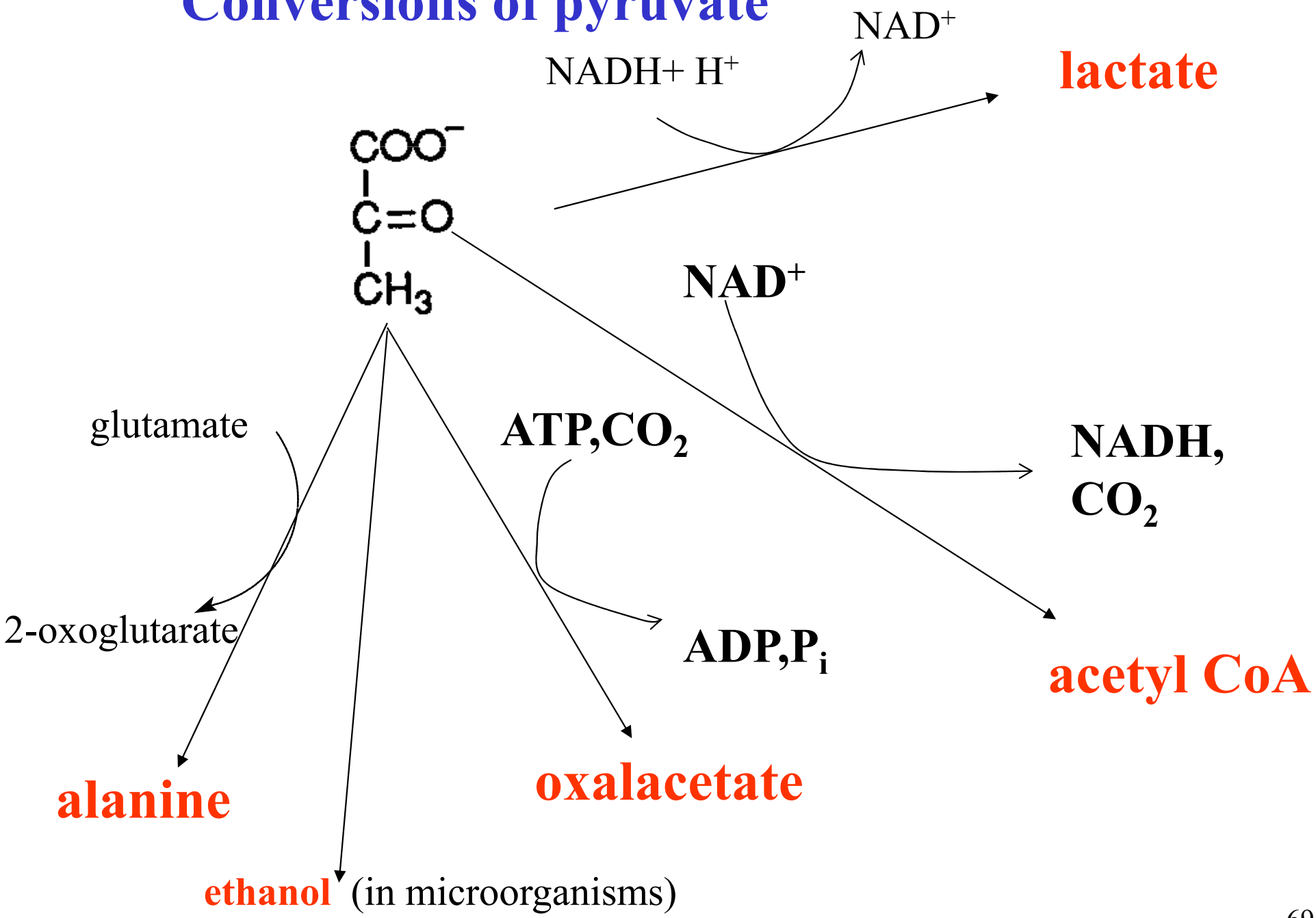
Activation by fructose-1,6-bisP

Inactivation by glucagon

Which reactions of glycolysis are non-reversible?

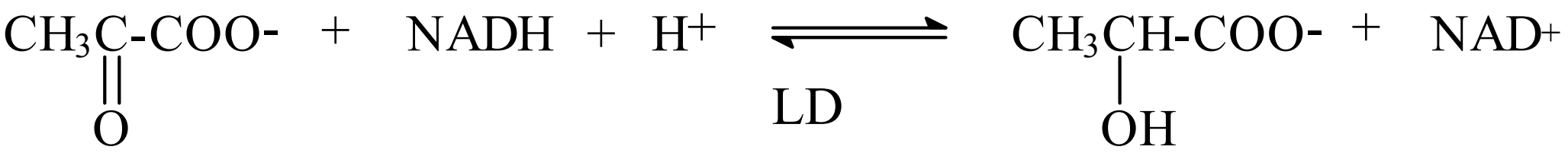


Conversions of pyruvate



Formation of lactate - anaerobic glycolysis

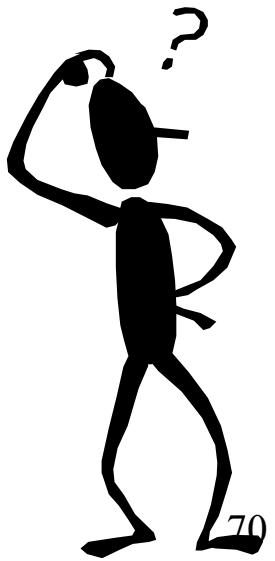
NADH cannot be reoxidized in respiratory chain



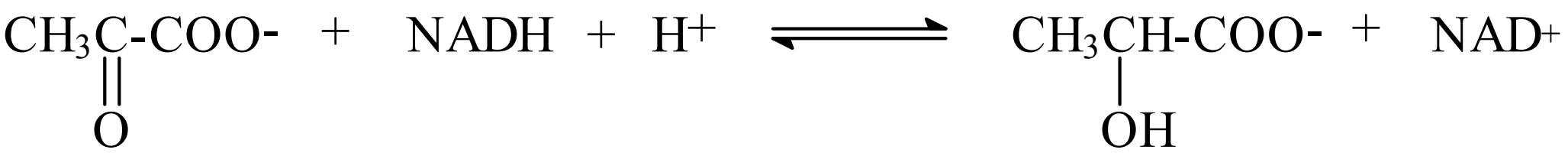
Significance of this reaction:

Regeneration of NAD^+ consumed in formation 2,3-bisP-glycerate

when NAD^+ is lacking, the glycolysis cannot continue



Lactate dehydrogenase (LD)

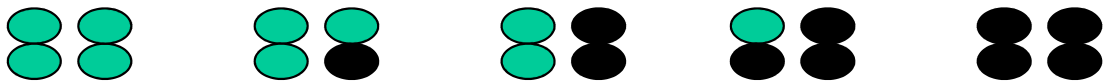


Enzyme catalyzes the reaction in both directions

Isoenzymes LD_1 - LD_5

Subunit H (heart)

Subunit M (muscle)



LD_1 LD_2 LD_3 LD_4 LD_5

Formation of lactate

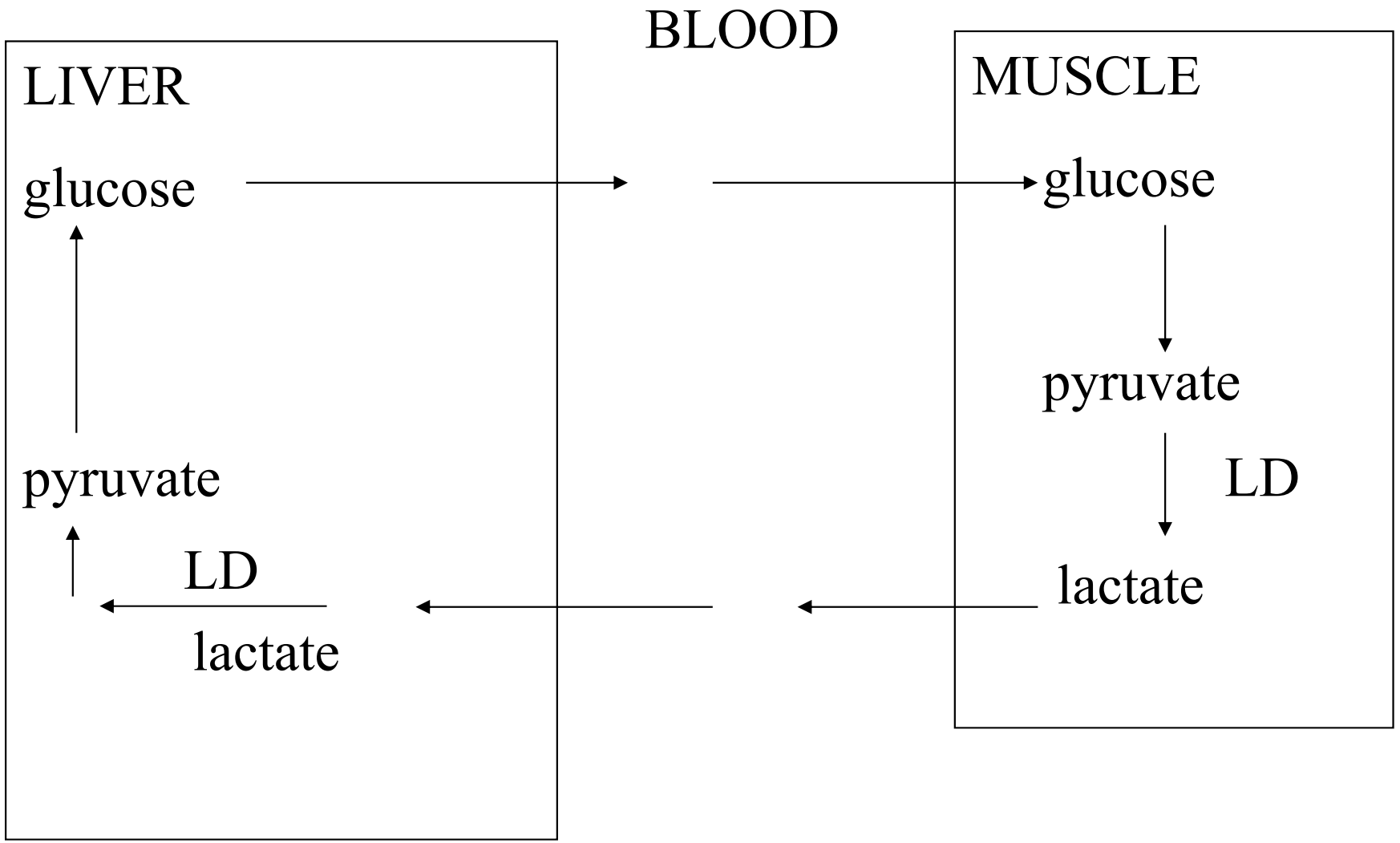
In average 1,3 mol/day (a man, 70 kg)

- short-term inexercising mucle ≈ 14 %
- in erythrocytes (lacking mitochondria) ≈ 25 %
- skin ≈ 25 %
- brain ≈ 14 %
- mucose cells of small intestine ≈ 8 %
- kidney medulla, testes, leukocytes, lens

Concentration of lactate in blood: ≈ 1 mmol/l

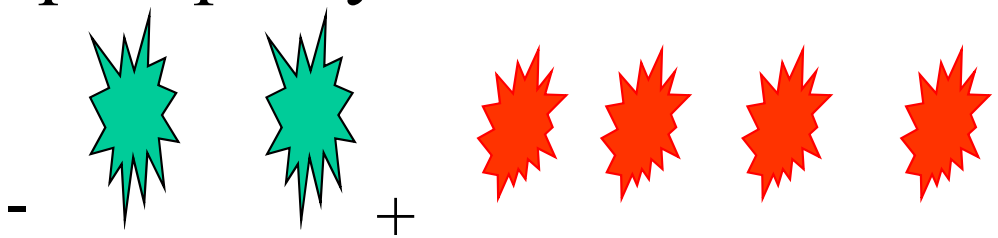
Changes at intensive muscle work (up to 30 mmol/l)

Cori cycle – tranport of lactate from tissues into the liver, utilization for gluconeogenesis



Energetic yield of glycolysis

1. Direct gain of ATP by substrate-level phosphorylation



Consumption of ATP

Gain of ATP $\Rightarrow$

2 ATP per one glucose

This yield is the same for both, anaerobic and aerobic glycolysis.

This is the only yield in anaerobic glycolysis

2. Further yield of ATP at aerobic glycolysis:

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

Transfer by „shuttles“ into the respiratory chain – a yield	2x 2-3 ATP
- Conversion of pyruvate to acetylCoA (2 NADH)	2x3 ATP
- Conversion of acetylCoA in citric acid cycle	2x12 ATP

Total energy yield of aerobic glycolysis

Aerobic glycolysis till pyruvate:

Reaction	ATP yield
glucose →2 pyruvate (substrate level phosporylation)	2
2 NADH → 2NAD$^+$	4-6*

** Depending on shuttle type (see lecture Respiratory chain)*

Further conversions of pyruvate:

Reakce	ATP yield
2 pyruvate → 2 acetylCoA + 2 NADH	6*
2 acetyl CoA → 2 CO_2 + 6 NADH + 2 $FADH_2$	2x 12
Total maximal energy yield	36-38 ATP

* (2x NADH to the resp. chain)

Energy yield of anaerobic glycolysis

Anaerobic glycolysis till pyruvate:

Reaction	ATP yield
glucose →2 x pyruvate (substrate-level phosphorylation)	2
2 NADH → 2NAD+	0

Formation and consumption of NADH at **anaerobic** glycolysis

Reaction	Yield/loss NADH
2 glyceraldehyde-3-P → 2 1,3-bisP-glycerát	+2
2 pyruvate → 2 lactate	-2
In sum	0

•The energy yield of anaerobic glycolysis is only 2 ATP from substrate level phosphorylation

- it is only small portion of the total energy conserved in molecule of glucose
- it has high significance at situations when
  - supply of oxygen is limited
  - tissue do not dispose of mitochondrias (ercs, leukocytes, ..)
  - it is necessary to spare lactate for gluconeogenesis

Oxidative decarboxylation of pyruvate

- pyruvate dehydrogenase complex
- conversion of pyruvate to acetylCoA

mitochondrial

matrix

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

acetylCoA

Cofactors necessary for this reaction: : thiamindiphosphate, lipoamide, CoA, FAD, NAD^+

Mechanismus is in more details discussed in the lecture Citric acid cycle