LICENCIATURA EM BIOLOGIA

DISCIPLINA

BIOQUÍMICA

Ano Lectivo de 2012/2013

Aula nº 14

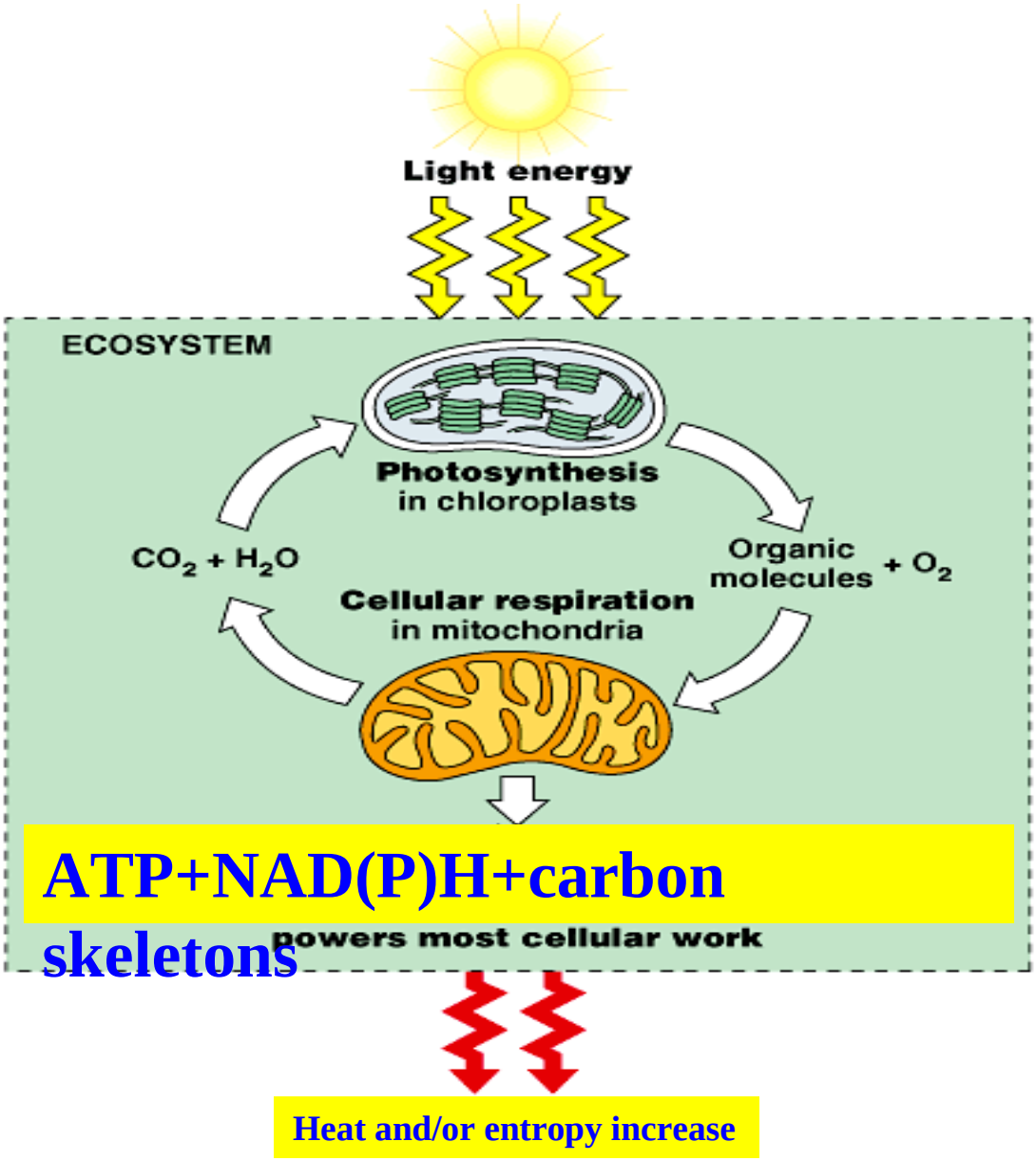
04 ABR 2014 **Ricardo Boavida Ferreira** **Laboratório 46**

Metabolismo dos glúcidos

A respiração e os substratos respiratórios. As três funções básicas da respiração.
Vias de entrada de diferentes glúcidos, de reserva e alimentares, no metabolismo da glucose. Glicólise. Reoxidação do NADH em condições de anaerobiose ou de aerobiose. Fermentações láctica e alcoólica. O ciclo dos Cori. Metabolismo do piruvato em condições de aerobiose. Mecanismos de transferência (*shuttle*) do potencial redutor do citoplasma para o mitocôndrio. Balanço energético. Regulação.
Material de estudo: diapositivos das aulas e bibliografia recomendada.

Glycolysis

Flow of energy in eukaryotes



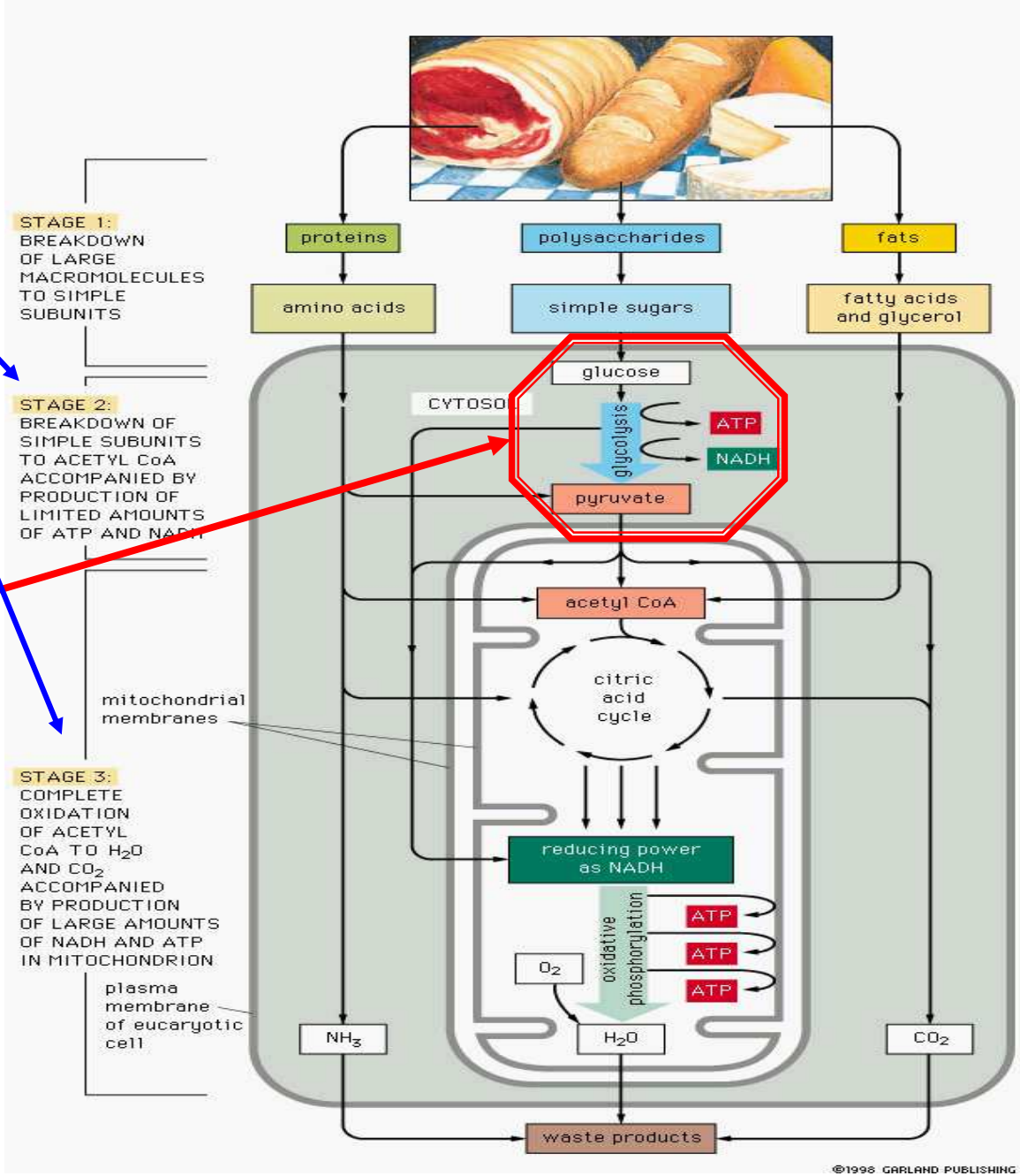
Cells require a constant supply of energy to generate and maintain the biological order that keeps them alive.

Plants make their organic molecules by photosynthesis, whereas animal cells obtain them by eating other organisms.

During respiration of carbohydrates, lipids and/or proteins, useful energy is derived from chemical bond energy as the organic molecules are broken down and oxidized to CO_2 and H_2O.

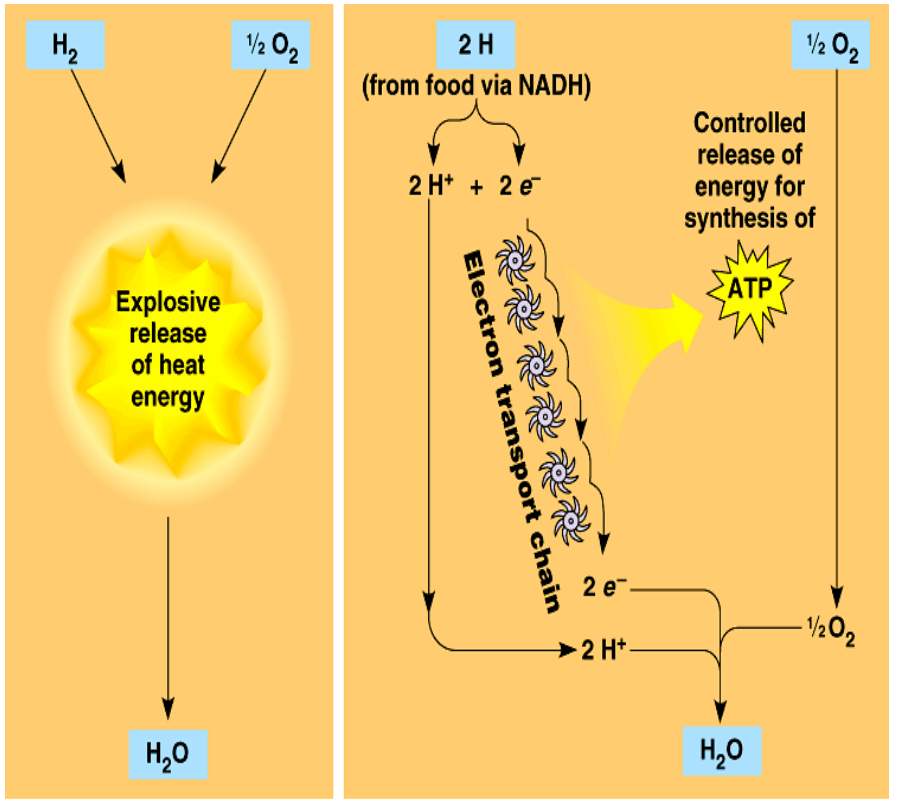
Food molecules are broken down in three stages, to produce **energy** (ATP), **reducing power** (NAD(P)H) and **carbon skeletons**.

A chain of reactions called ***glycolysis*** converts one molecule of D-glucose into two of **pyruvate**; meanwhile 2 **ATP** and 2 **NADH** are formed. In addition, glycolysis also **supplies** (and **accepts**) **intermediates** to (and from) a variety of biosynthetic reactions.

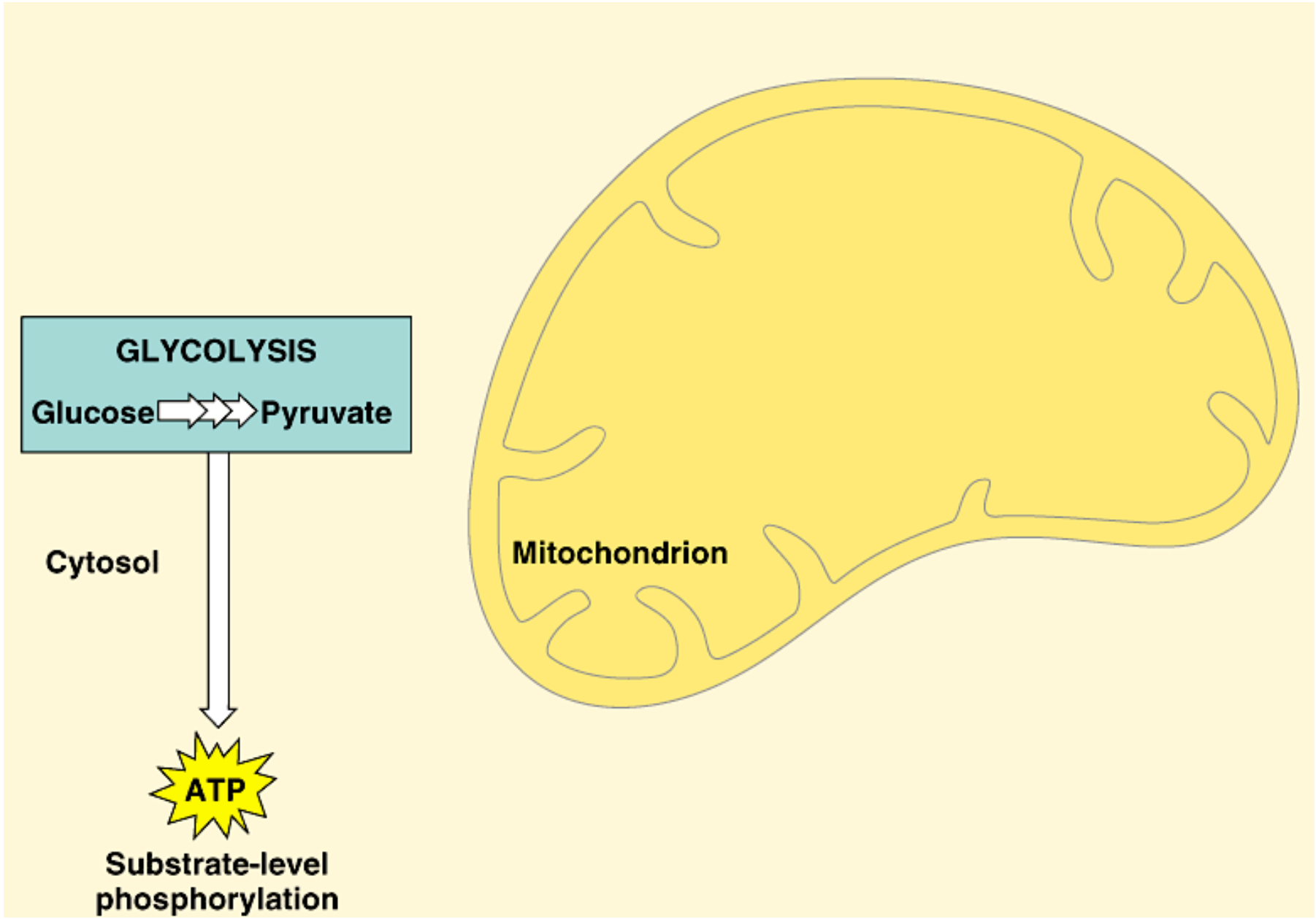


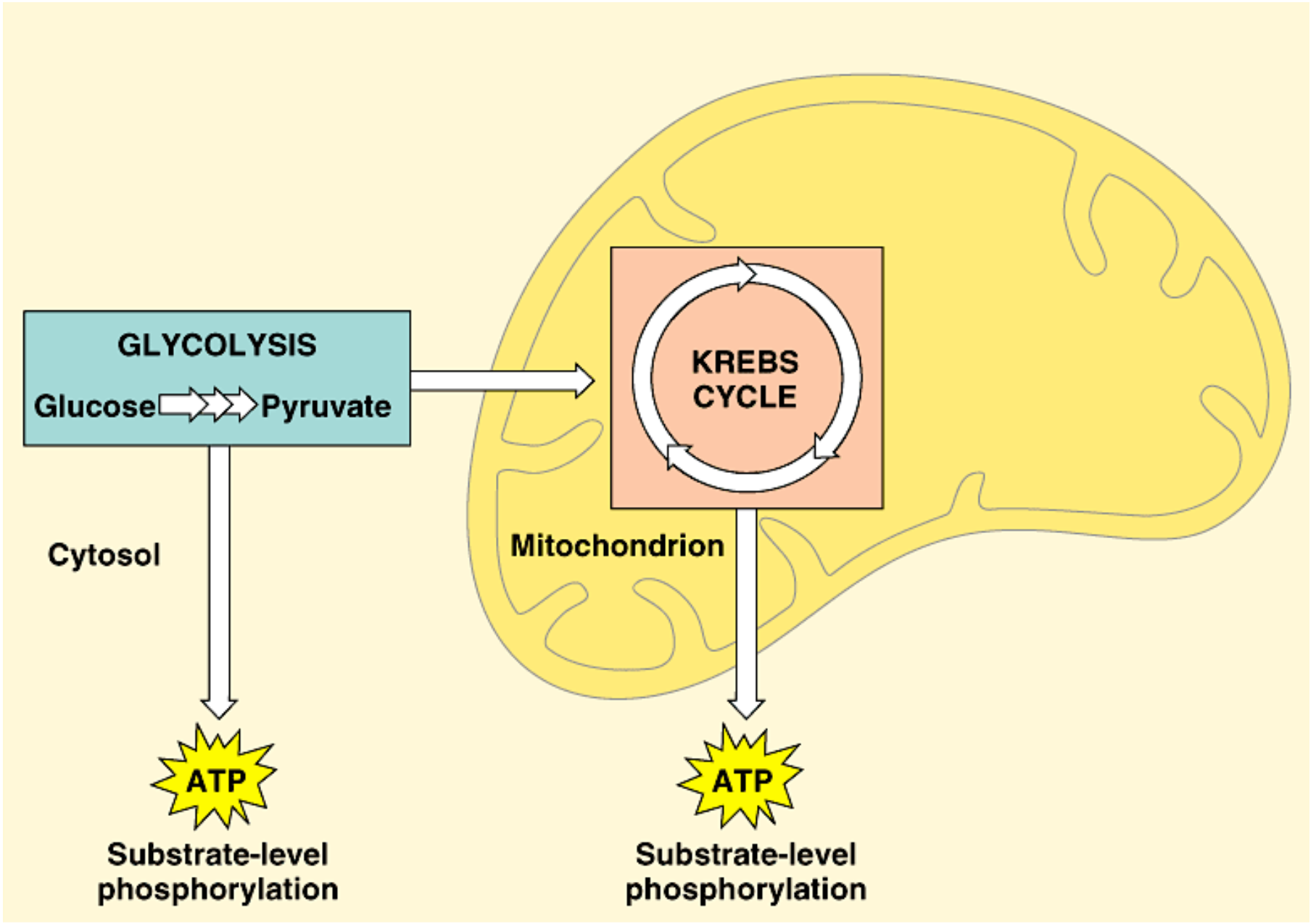
Burning versus stepwise oxidation

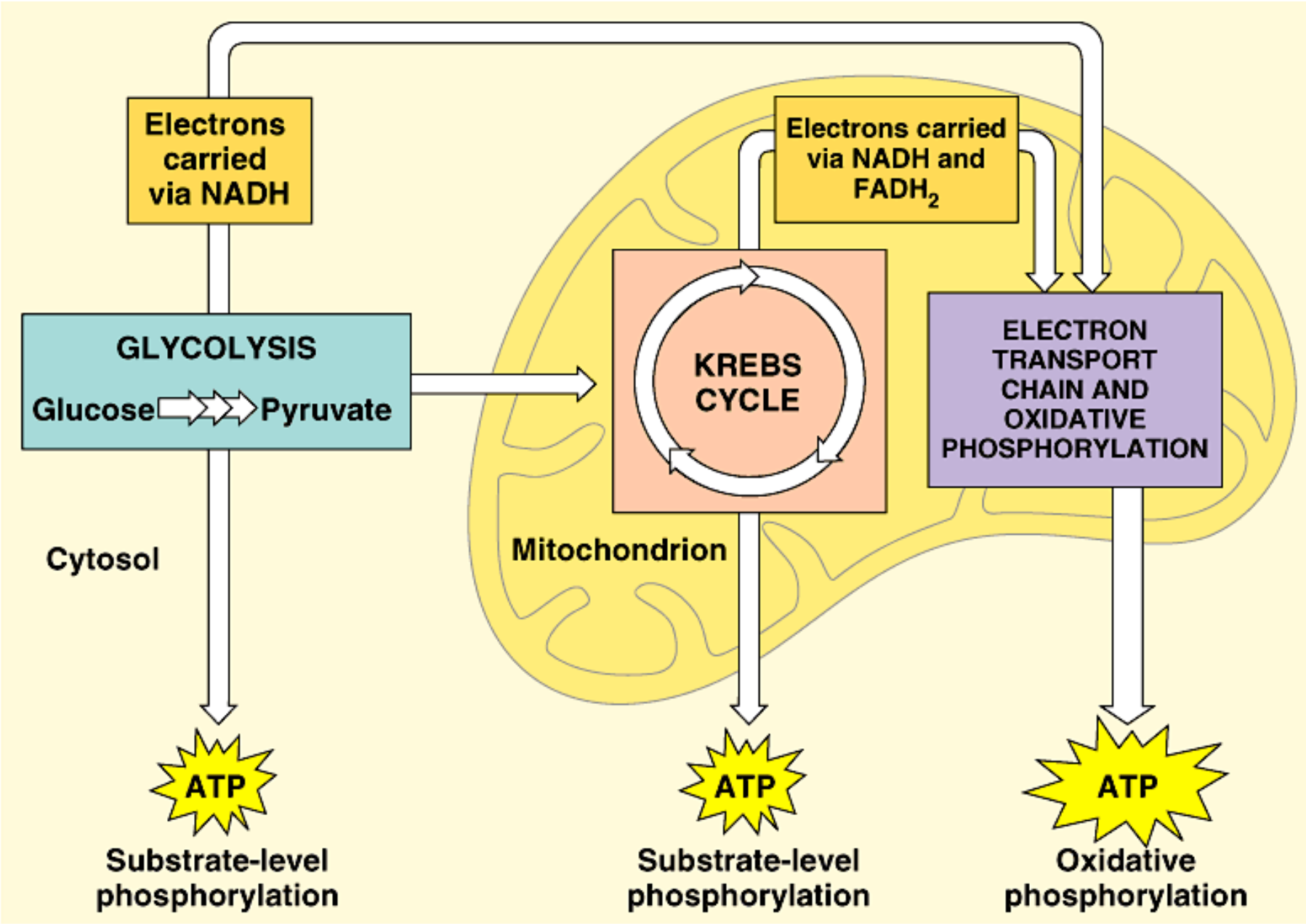
- Burning (oxidizing) a respiratory substrate such as a carbohydrate, lipid or protein molecule releases energy in the form of heat.
- In the cell, enzymes catalyze the oxidation of respiratory substrates via a series of small steps in which energy is transferred to carrier molecules – ATP and NADH.
- The total free energy released is exactly the same in (a) and (b).



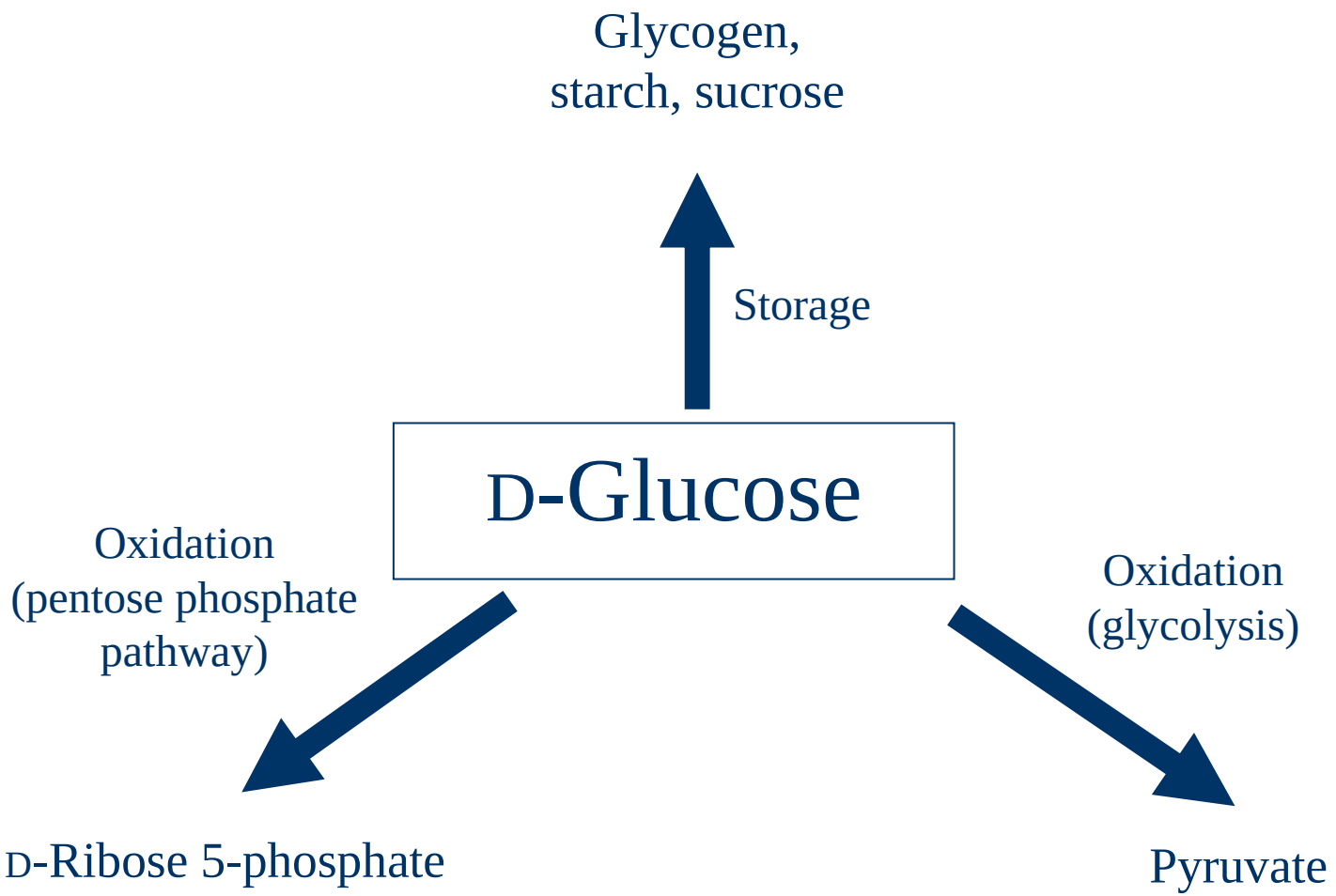
ATP and glucose are both energy-rich molecules. However, while it is relatively easy to understand why is ATP rich in energy, the same is not true for glucose.


GLYCOLYSIS
Glucose
Pyruvate
Cytosol
ATP
Substrate-level phosphorylation
Mitochondrion

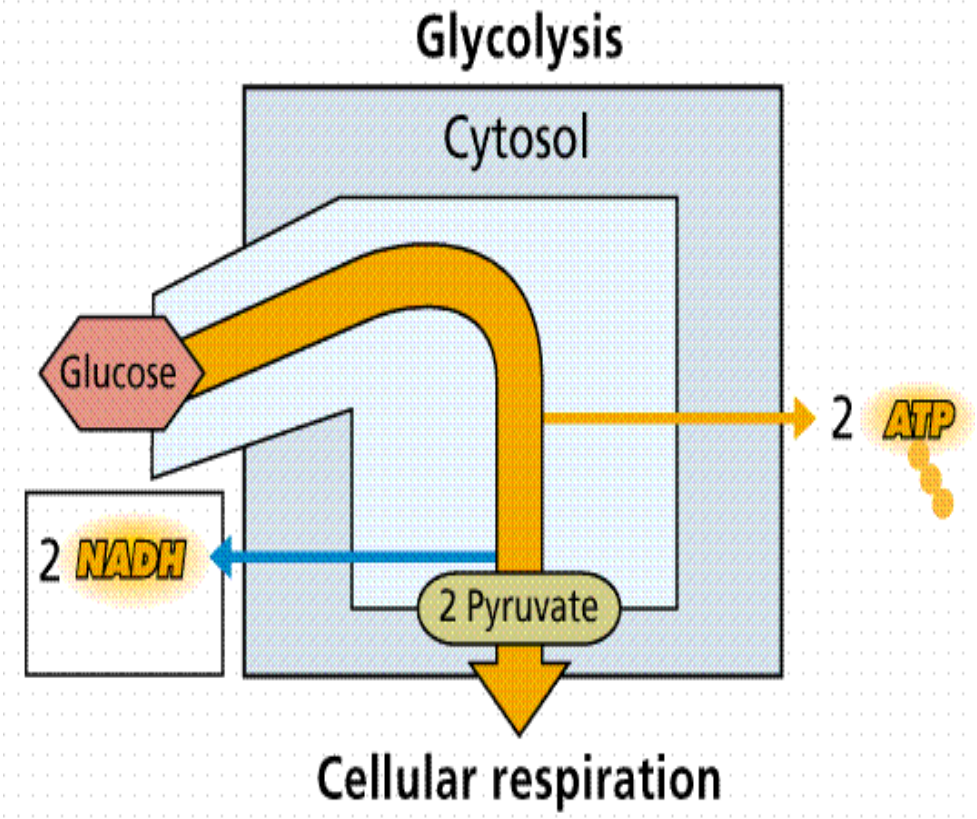

GLYCOLYSIS
Glucose
Pyruvate
KREBS CYCLE
Cytosol
Mitochondrion
ATP
ATP
Substrate-level phosphorylation
Substrate-level phosphorylation


Electrons carried via NADH
Electrons carried via NADH and $FADH_2$
GLYCOLYSIS
Glucose
Pyruvate
KREBS CYCLE
ELECTRON TRANSPORT CHAIN AND OXIDATIVE PHOSPHORYLATION
Cytosol
Mitochondrion
ATP
ATP
ATP
Substrate-level phosphorylation
Substrate-level phosphorylation
Oxidative phosphorylation

Major pathways of glucose utilization



What is glycolysis ?



- Glycolysis (from the Greek *glykys* = sweet, *lysis* = splitting) is a catabolic pathway in which one molecule of D-glucose (6-C) or a glucosyl unit of glycogen is degraded to yield two molecules of pyruvate (3-C), two molecules of NADH and two molecules of ATP.
- Each reaction in the pathway is catalyzed by an enzyme.

- A **glicólise** é uma via catabólica de degradação da glucose de outros açúcares simples.

- Tem uma ocorrência quase universal nos seres vivos.

- Consiste num conjunto linear de 10 reacções químicas, através das quais uma molécula de glucose é convertida em duas moléculas de piruvato, com formação de duas moléculas de ATP.

- Ocorre no citoplasma das células e pode ser considerada um processo anaeróbio, na medida em que as suas reacções ocorrem na ausência de oxigénio.

- Desempenha um papel fundamental no metabolismo energético das células:
  - Em condições de aerobiose, prepara a glucose e outros hidratos de carbono para degradação oxidativa, no ciclo do ácido cítrico e na cadeia mitocondrial de transporte de electrões.
  - Em condições de anaerobiose, o piruvato é convertido em lactato ou etanol+CO_2, por acção das fermentações láctica ou alcoólica, respectivamente.

Overview of glycolysis

The glycolytic pathway (Embden-Meyerhof pathway) breaks down D-glucose through ten sequential reactions to form pyruvate.

None of the reactions requires oxygen, therefore glycolysis can occur both aerobically and anaerobically.

Under anaerobic conditions, pyruvate cannot enter mitochondria, instead it is converted in the cytosol to lactate or ethanol plus CO_2, via lactic or alcoholic fermentations, respectively.

Overview of glycolysis (cont.)

Breakdown of glucose into two molecules of pyruvate occurs in ten cytosolic reactions.

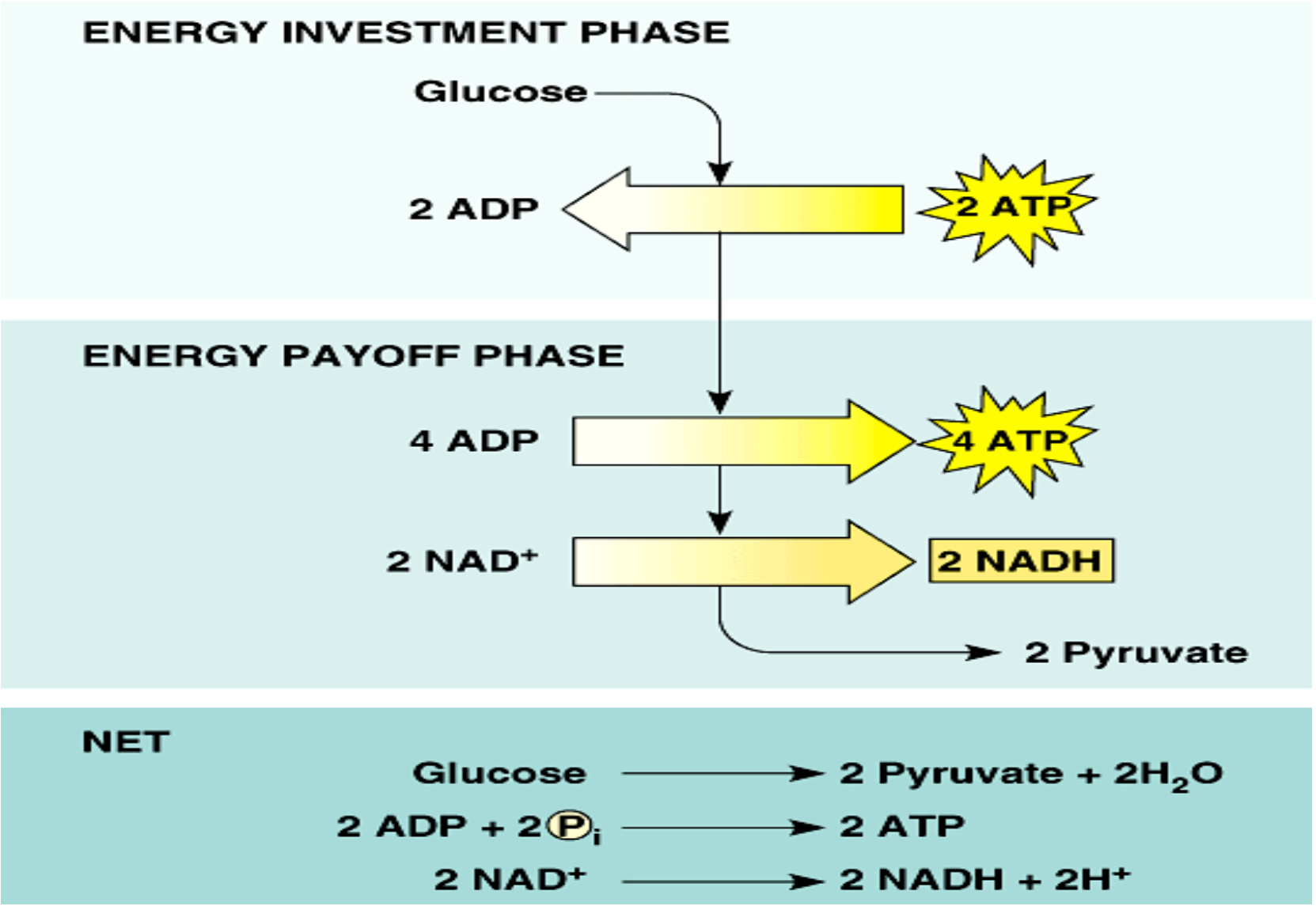
All intermediates between glucose and pyruvate are phosphorylated molecules.

The first five reactions constitute the *preparatory (energy investment) phase*.

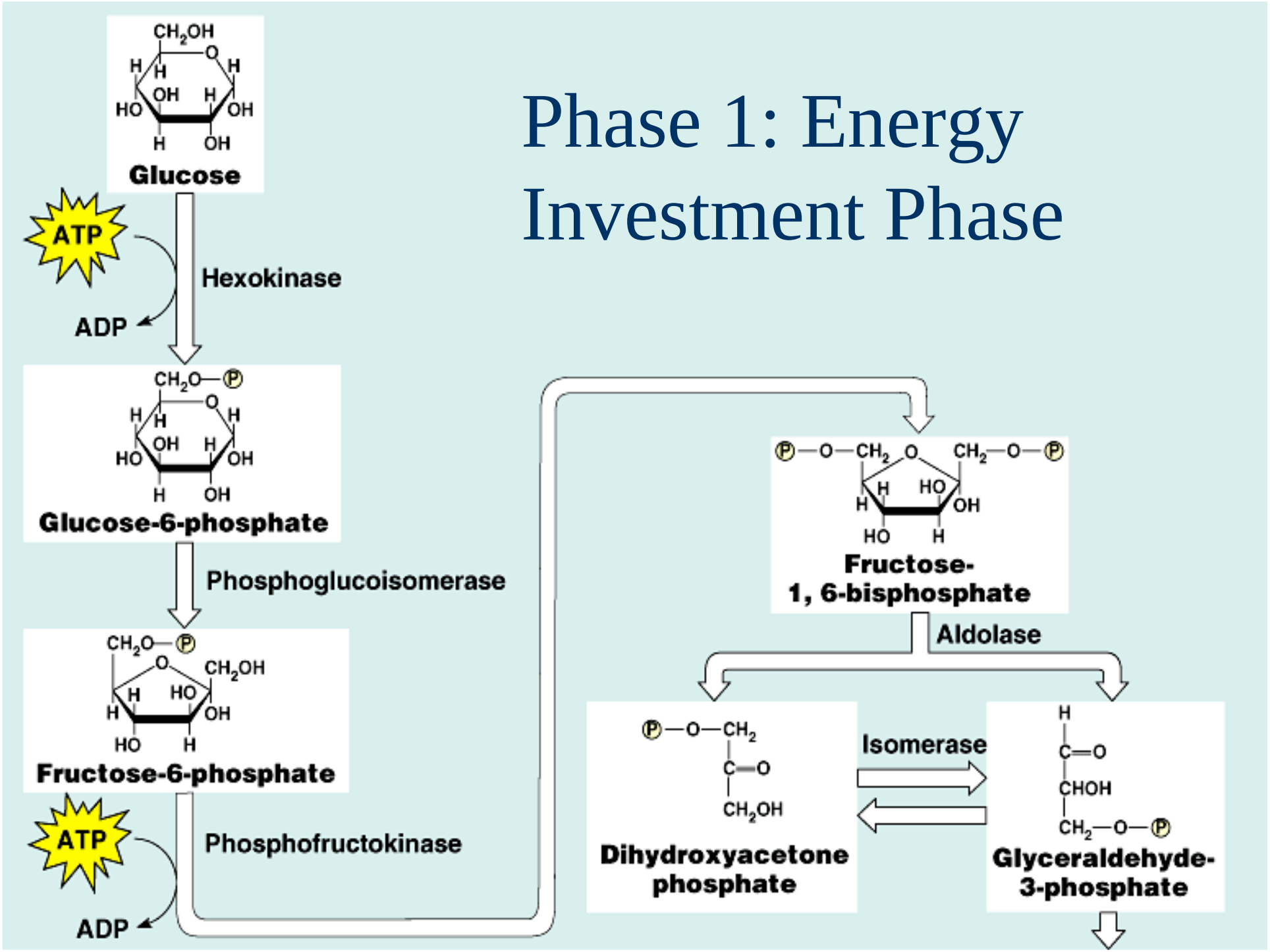
Energy gain comes from the remaining five reactions, the *payoff (energy generation) phase*.

Glycolysis is exquisitely controlled at key points.

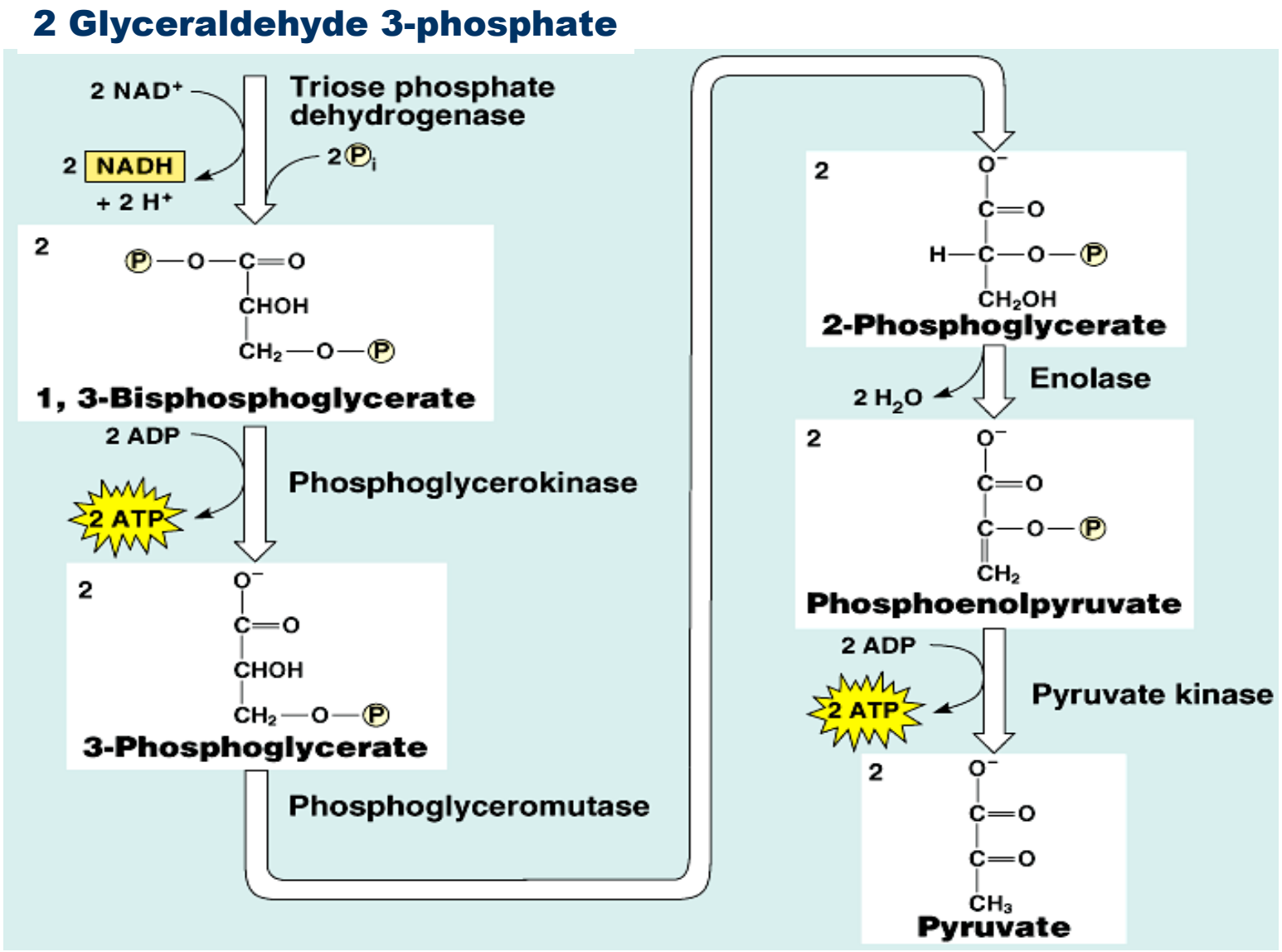
The two phases of glycolysis



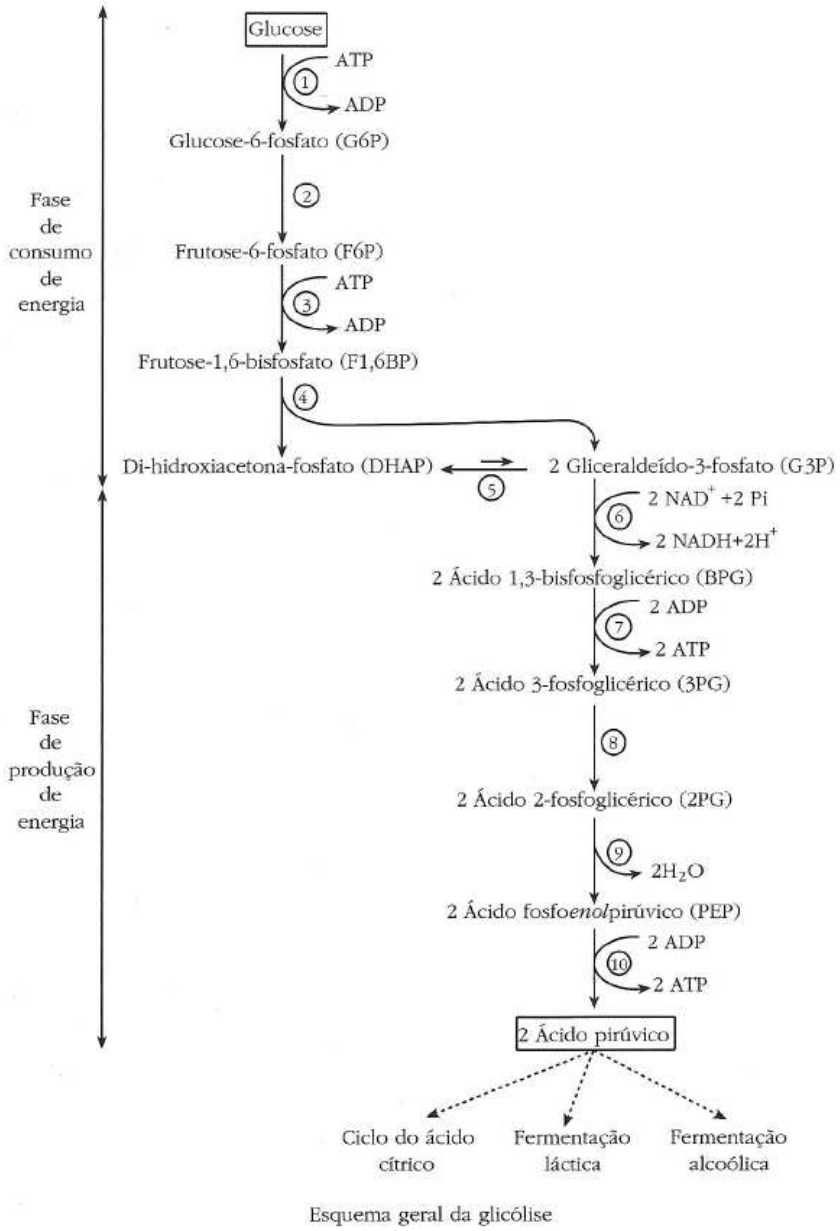
Phase 1: Energy Investment Phase



Phase 2. Energy Generation Phase



Esquema geral da glicólise



Esquema geral da glicólise

Notar que as vias metabólicas não funcionam de um modo estanque. Deste modo, podem considerar-se **cinco funções principais para a glicólise**:

1 – Produção de ácido pirúvico

2 – Formação de ATP

3 – Formação de NADH

4 – Fornecimento de metabolitos intermediários para uma variedade de reações biossintéticas

5 – Via de entrada para uma variedade de metabolitos resultantes do catabolismo celular

Equação global da glicólise:

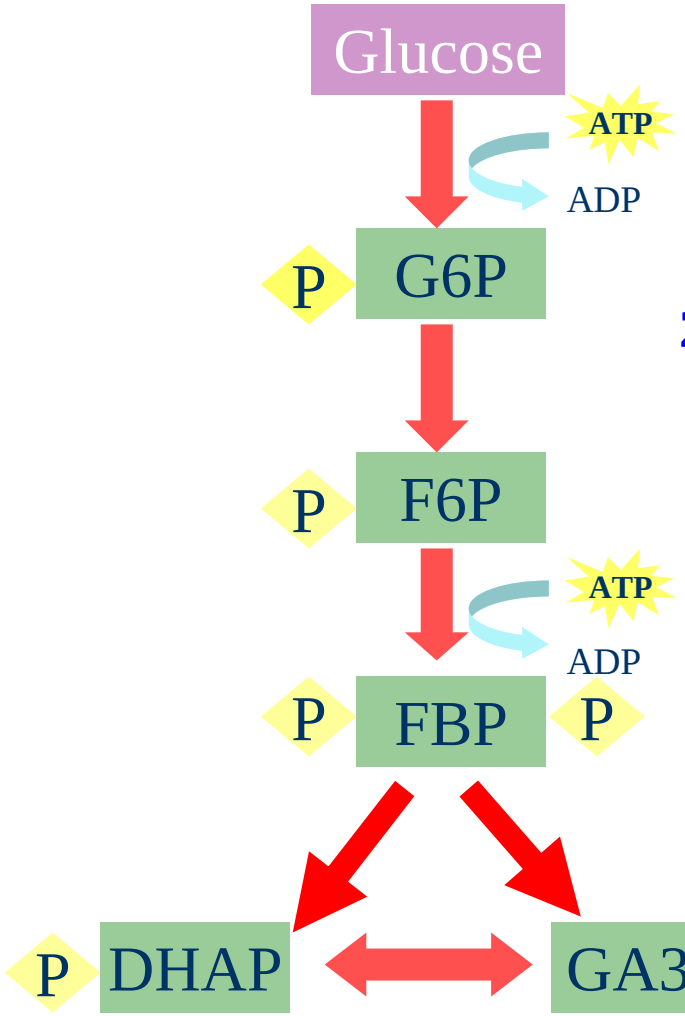
D-Glucose + 2ADP + $2H_3PO_4$ + $2NAD^+$ → 2ácido pirúvico + 2ATP + 2NADH + $2H^+$ + $2H_2O$

Animations of glycolytic reactions

www.johnkyrk.com

www.tcd.ie/Biochemistry/IUBMB-nicholson/swf/glycolysis.swf

Reactions of Glycolysis: Phase 1



1. Phosphorylation - **Hexokinase** (EC 2.7.1.1)
ΔG°' = +16.7 kJ.mol-1

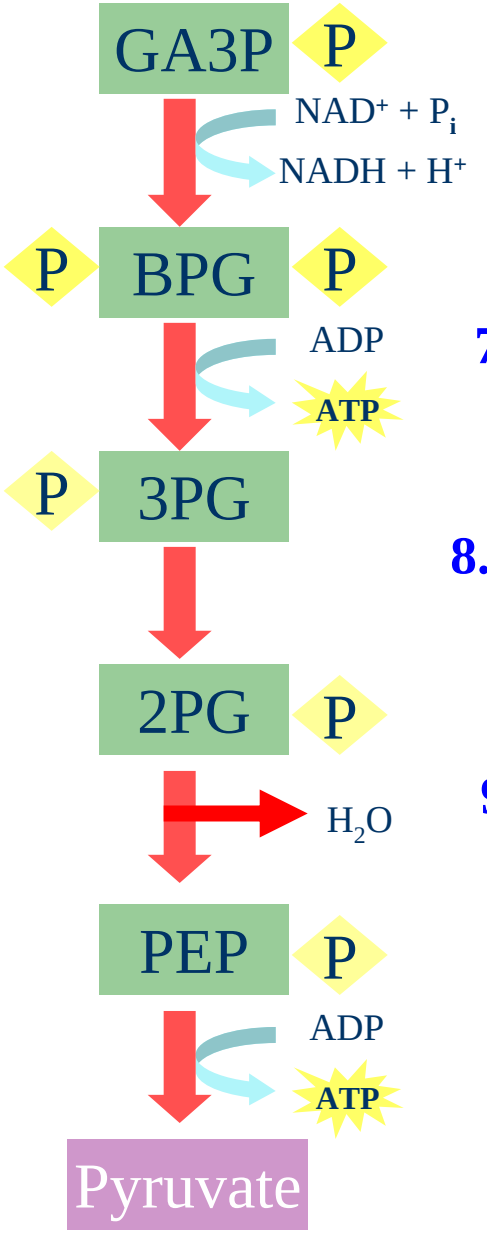
2. Isomerization - **Phosphoglucoisomerase** or **glucose-6-phosphate isomerase** (EC 5.3.1.9)
ΔG°' = +1.67 kJ.mol^{-1}

3. Phosphorylation - **Phosphofructokinase-1** or **6-phosphofructokinase** (EC 2.7.1.11)
ΔG°' = -14.2 kJ.mol^{-1}

4. Cleavage – **Fructose bisphosphate aldolase** or **aldolase** (EC 4.1.2.13)
ΔG°' = +23.9 kJ.mol^{-1}

5. Isomerization - **Triosephosphate isomerase** (EC 5.3.1.1)
ΔG°' = +7.56 kJ.mol^{-1}

Reactions: Phase 2



6. Oxidation and phosphorylation - **Glyceraldehyde-3-phosphate dehydrogenase** (EC 1.2.1.12)

$\Delta G^{o'} = +6.30$ kJ.mol^{-1}

7. Phosphorylation - **Phosphoglycerate kinase** (EC 2.7.2.3)

$\Delta G^{o'} = -18.9$ kJ.mol^{-1}

8. Isomerization - **Phosphoglycerate mutase** (EC 5.4.2.1)

$\Delta G^{o'} = +4.4$ kJ.mol^{-1}

9. Dehydration – **Enolase** (EC 4.2.1.11)

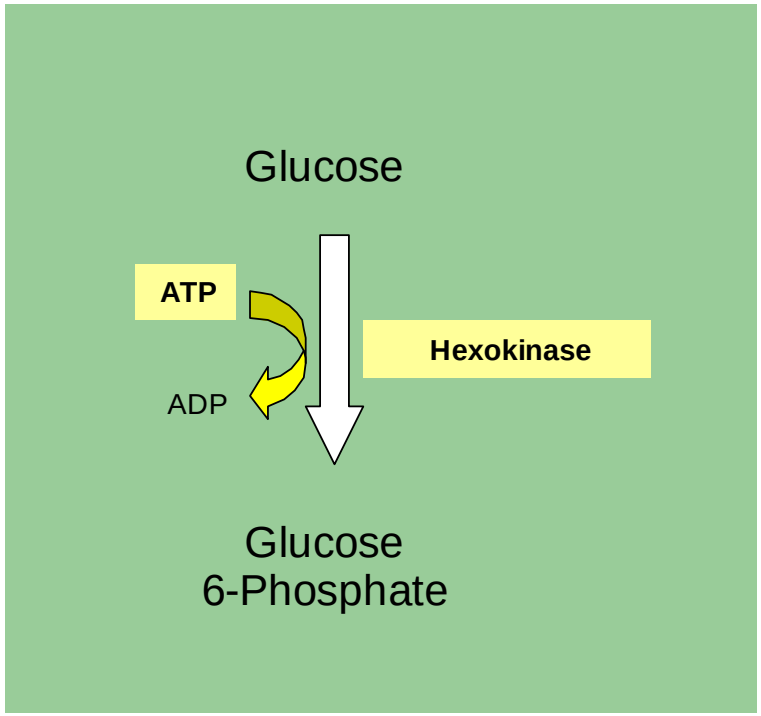
$\Delta G^{o'} = +1.8$ kJ.mol^{-1}

10. Phosphorylation - **Pyruvate kinase** (EC 2.7.1.40)

$\Delta G^{o'} = -31.7$ kJ.mol^{-1}

Reaction 1: The first ATP investment: phosphorylation of glucose

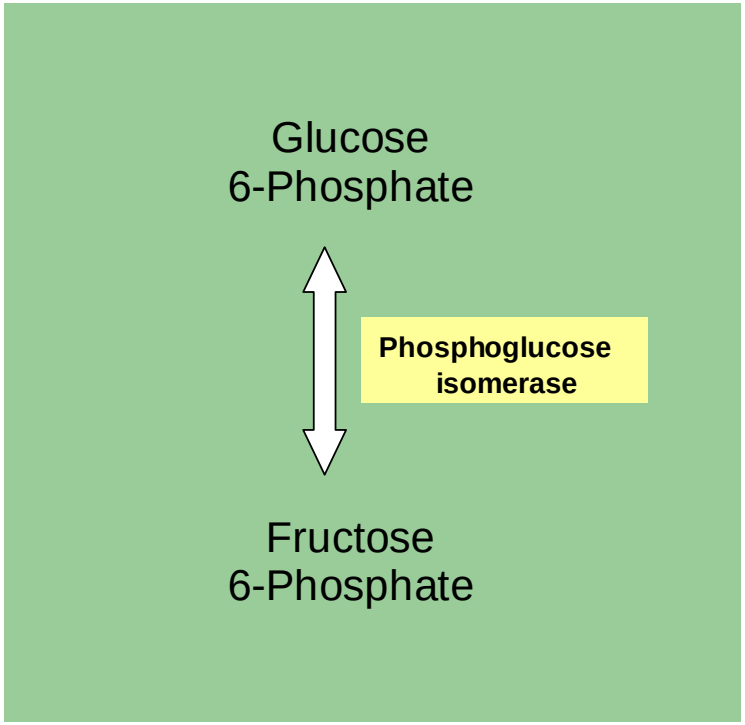
- Phosphorylation of glucose yields glucose 6-phosphate.
- Glucose 6-phosphate is a charged molecule therefore it is entrapped in the cell.
- ATP is the source of the phosphate group.
- The reaction is catalyzed by hexokinase (EC 2.7.1.1) or by glucokinase (in the liver; EC 2.7.1.2).



$\Delta G^{o'} = +16.7\ kJ.mol^{-1}$

Reaction 2: Isomerization of glucose 6-phosphate

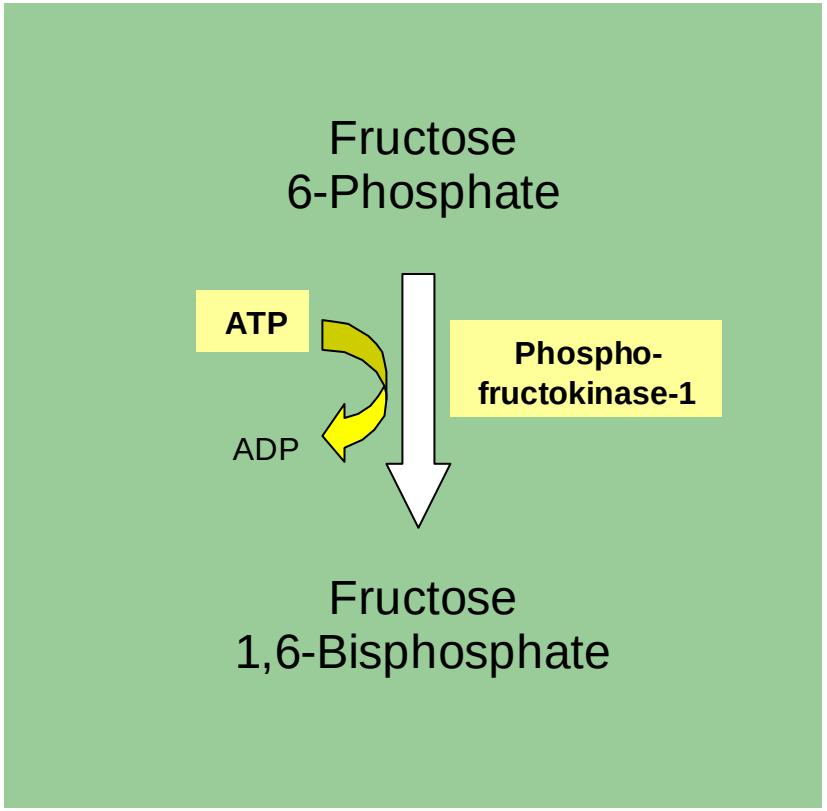
- Glucose 6-phosphate (aldohexose) isomerizes to give fructose 6-phosphate (ketohexose).
- Phosphoglucoisomerase or glucose-6-phosphate isomerase (EC 5.3.1.9) catalyzes the reaction.
- There is no net oxidation or reduction.



$\Delta G^{o\prime} = +1.67\ kJ.mol^{-1}$

Reaction 3: The second ATP investment: phosphorylation of fructose 6-phosphate

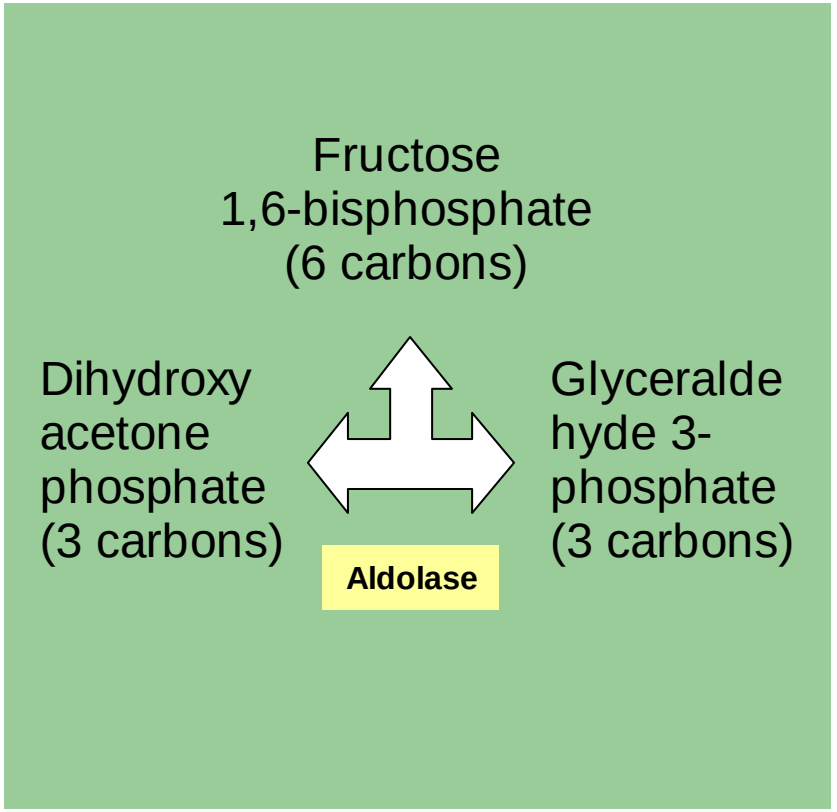
- Phosphorylation of fructose 6-phosphate gives fructose 1,6-bisphosphate.
- ATP is the source of the phosphate group.
- The reaction is irreversible and is catalyzed phosphofructokinase-1 or 6-phosphofructokinase (PFK-1; EC 2.7.1.11), an allosteric enzyme.



$\Delta G^{o\prime} = -14.2\ kJ.mol^{-1}$

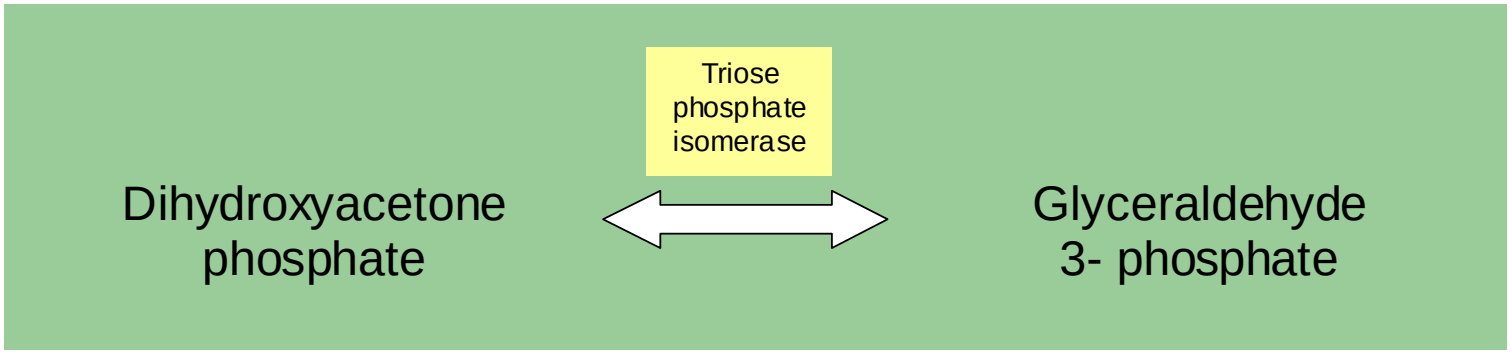
Reaction 4: Cleavage of fructose 1,6-bis-phosphate to two triose phosphates

- This reaction is the reversal of an aldol condensation.
- Fructose bisphosphate aldolase or aldolase (EC 4.1.2.13) is the enzyme catalyzing this reversible reaction.
- A Schiff base is formed as the key intermediate.



$\Delta G^{o\prime} = +23.9\ kJ.mol^{-1}$

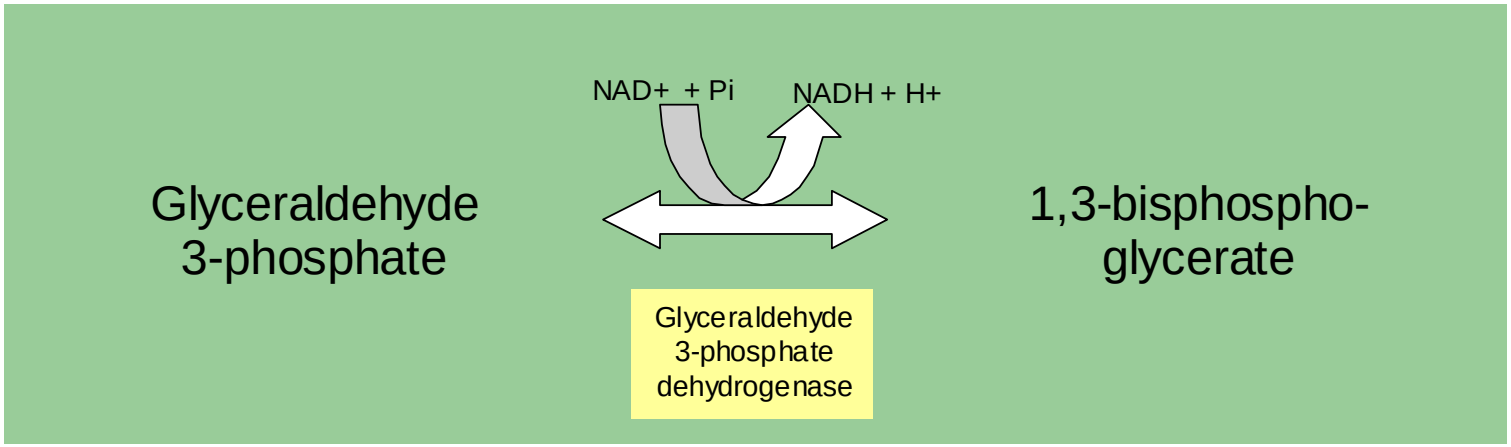
Reaction 5: Isomerization of dihydroxyacetone phosphate



$$\Delta G^{o\prime} = +7.56\ kJ.mol^{-1}$$

- Dihydroxyacetone phosphate is converted to glyceraldehyde 3-phosphate, another triose phosphate.
- Triosephosphate isomerase (EC 5.3.1.1) catalyzes the reaction.

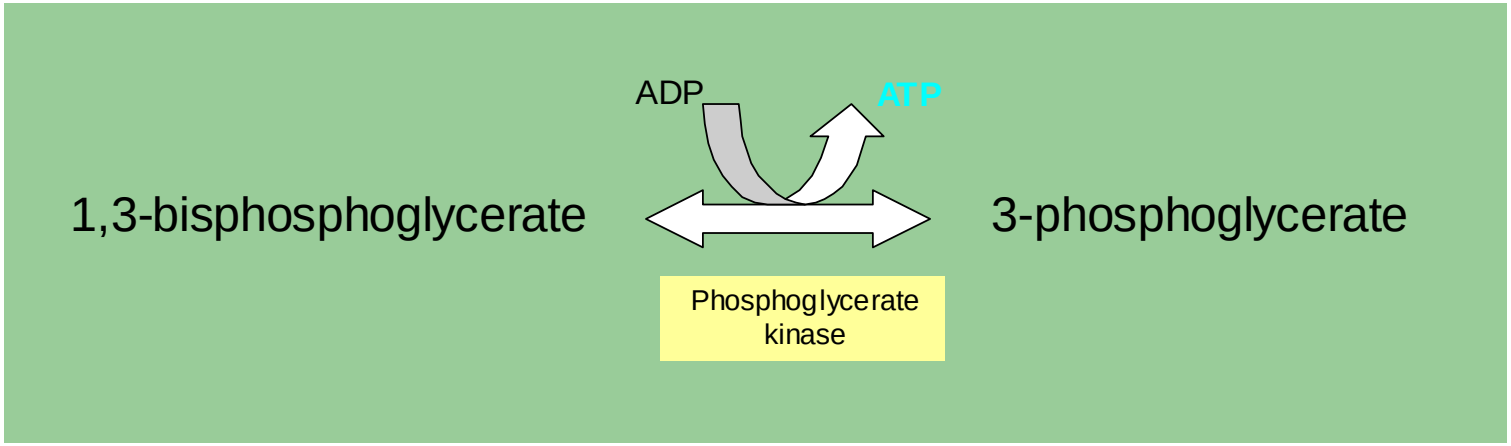
Reaction 6: Oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate



$$\Delta G^{o\prime} = +6.30\ kJ.mol^{-1}$$

- A phosphate group is added to glyceraldehyde 3-phosphate along with oxidation of the aldehyde to carboxylic acid.
- Glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12) catalyzes the reaction.

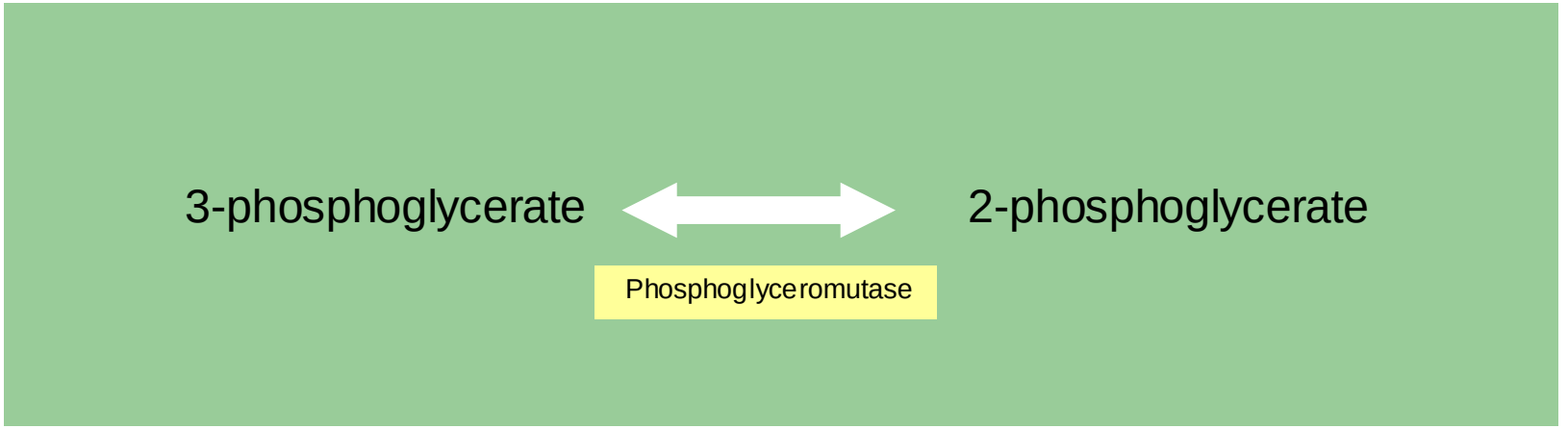
Reaction 7: Transfer of a phosphate group from 1,3-bisphosphoglycerate to ADP



$$\Delta G^{o\prime} = -18.9\ kJ.mol^{-1}$$

- A phosphate group is transferred to ADP and the first ATP generation occurs.
- Phosphoglycerate kinase (EC 2.7.2.3) catalyzes the reaction.

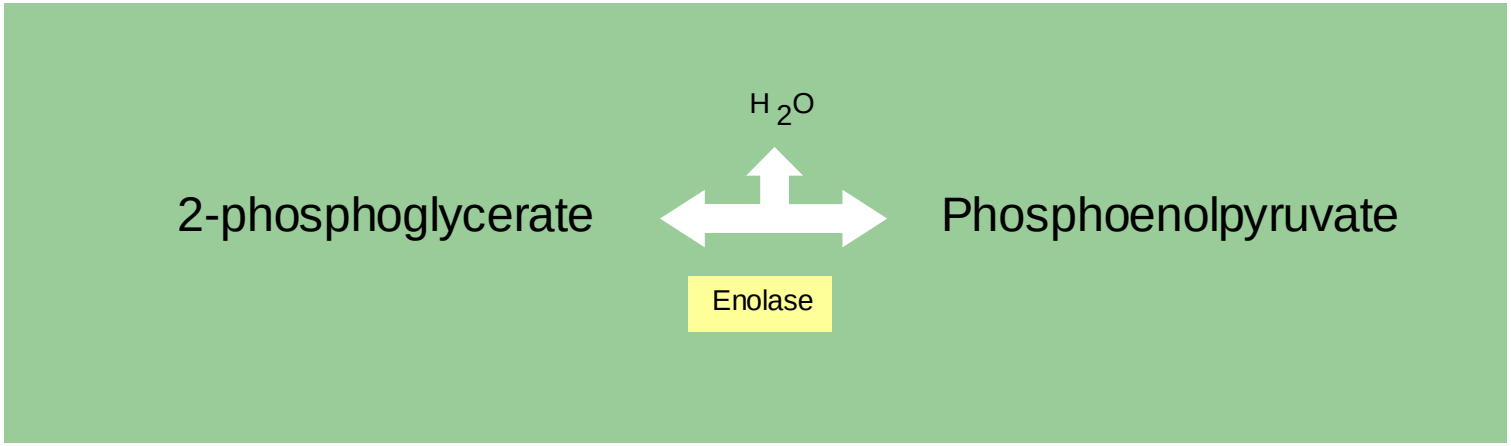
Reaction 8: Isomerization of 3-phosphoglycerate to 2-phoshoglycerate



$$\Delta G^{o\prime} = +4.4\ kJ.mol^{-1}$$

- The phosphate group is transferred from carbon 3 to carbon 2 to form 2-phosphoglycerate.
- Phosphoglycerate mutase (EC 5.4.2.1) catalyzes the reaction.

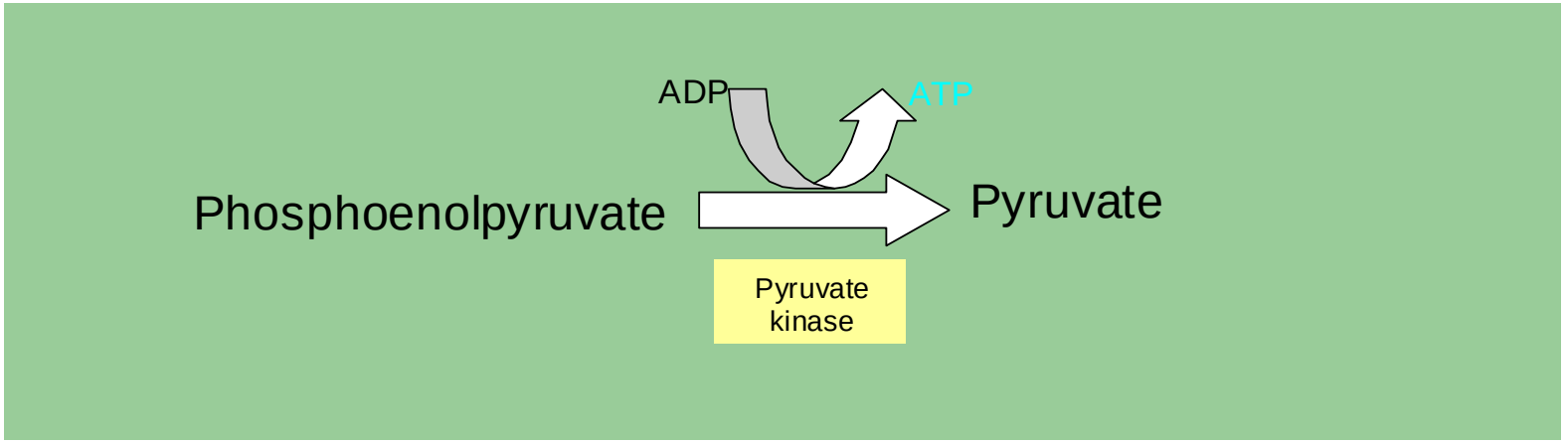
Reaction 9: Dehydration of 2-phosphoglycerate to phospho*enol*pyruvate



$$\Delta G^{o\prime} = +1.8\ kJ.mol^{-1}$$

- The removal of water from 2-phosphoglycerate creates a high-energy enol phosphate linkage.
- Enolase (EC 4.2.1.11) catalyzes the reaction.

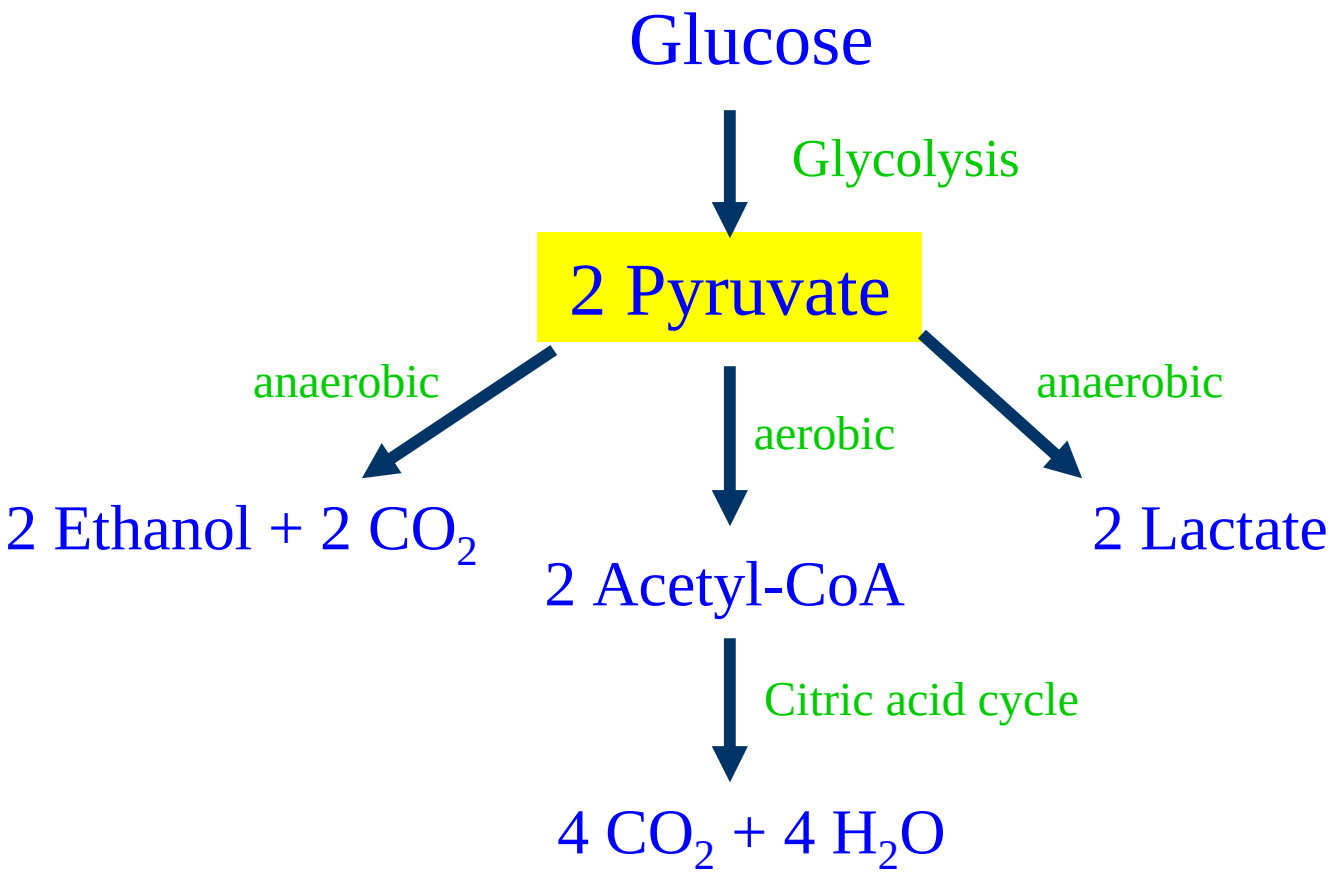
Reaction 10: Transfer of a phosphate group from phospho*enol*pyruvate to ADP



$$\Delta G^{o\prime} = -31.7\ kJ.mol^{-1}$$

- This is the second ATP formation in glycolysis; a phosphate group is transferred to ADP.
- Pyruvate kinase (EC 2.7.1.40) catalyzes the reaction.

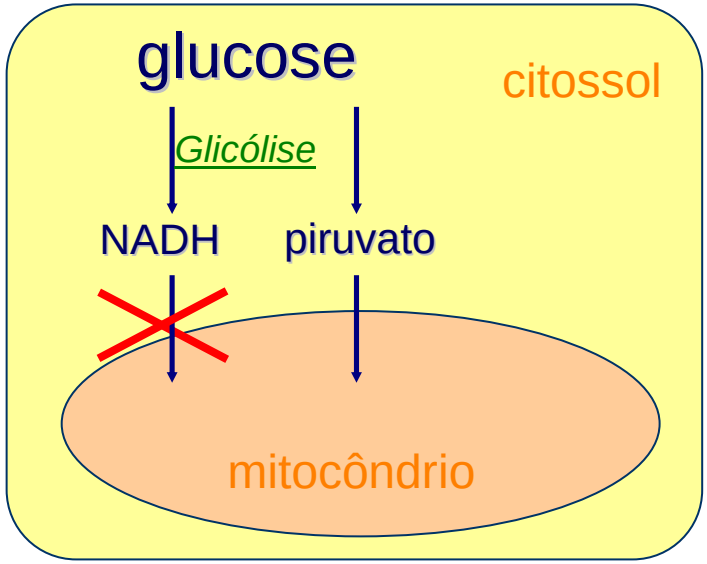
Catabolic fates of pyruvate



As células posssuem, tipicamente, pequenas quantidades de coenzimas, como acontece, por exemplo, com o NAD. Assim, o NADH formado na glicólise tem que ser prontamente oxidado de volta a NAD^+, sob pena de se esgotar o conteúdo citossólico em NAD^+ e a glicólise parar na reacção 6 por falta deste coenzima.

O destino metabólico do piruvato produzido na glicólise, determinado pela presença ou ausência de oxigénio, condiciona o destino e a função do NADH formado na reacção nº 6 da glicólise, catalisada pela enzima gliceraldeído-3-fosfato desidrogenase.

Em condições de aerobiose, o piruvato entra no mitocôndrio, onde é descarboxilado oxidativamente pelo complexo multienzimático piruvato desidrogenase. O acetil-CoA assim formado entra no ciclo do ácido cítrico.



Nestas condições, os electrões do NADH formado na glicólise são transferidos para o interior do mitocôndrio por um mecanismo de *shuttle* (o *shuttle* do malato-aspartato ou o *shuttle* do glicerol-fosfato), uma vez que a membrana interna do mitocôndrio é impermeável ao NADH. Dependendo do mecanismo de *shuttle* que funciona, assim cada NADH da glicólise origina 2 ou 3 moléculas de ATP na cadeia mitocondrial de transporte de electrões.

Em condições de aerobiose, o NADH formado na glicólise é, por isso, considerado um composto rico em energia.

Em condições de anaerobiose, o ciclo do ácido cítrico e a cadeia mitocondrial de transporte de electrões encontram-se parados e o piruvato produzido pela glicólise não entra no mitocôndrio. Pelo mesmo motivo, o NADH formado na glicólise acumula-se.

Nestas condições, as células têm uma necessidade absoluta de re-oxidarem este NADH, sob pena de verem a sua glicólise parar por falta do NAD^+ necessário ao funcionamento continuado da reacção 6. Se a glicólise parar, as células não conseguem produzir ATP, o que significa, em termos práticos, que “estão mortas”.

As células recorrem, então, à redução do piruvato a lactato (fermentação láctica) ou a etanol + CO_2 (fermentação alcoólica) com o objectivo de re-oxidarem o NADH produzido pela gliceraldeído-3-fosfato desidrogenase, permitindo assim o funcionbamento continuado da glicólise.

A oxidação do NADH glicolítico é, pois, a função fisiológica das fermentações láctica e alcoólica.

Em condições de anaerobiose, não se pode, por isso, considerar que o NADH formado na glicólise seja um composto rico em energia, já que a energia libertada aquando da sua oxidação é dissipada sob a forma de calor e/ou de um aumento de entropia do sistema.

The NADH dehydrogenase of the inner mitochondrial membrane of animal cells can accept electrons only from NADH in the matrix. Given that the inner membrane is not permeable to cytosolic NADH, how can the NADH generated by glycolysis outside mitochondria be reoxidized to NAD+ by O2 via the respiratory chain? Special shuttle systems carry reducing equivalents from cytosolic NADH into mitochondria by an indirect route.

The most active NADH shuttle, which functions in liver, kidney, and heart mitochondria, is the **malate-aspartate shuttle**. The reducing equivalents of cytosolic NADH are first transferred to cytosolic oxaloacetate to yield malate by the action of cytosolic malate dehydrogenase. The malate thus formed passes through the inner membrane into the matrix via the malatea-ketoglutarate transport system. Within the matrix the reducing equivalents are passed by the action of matrix malate dehydrogenase to matrix NAD+, forming NADH; this NADH can then pass electrons directly to the respiratory chain in the inner membrane. Three molecules of ATP are generated as this pair of electrons passes to 02. Cytosolic oxaloacetate must be regenerated via transamination reactions and the activity of membrane transporters to start another cycle of the shuttle.

In skeletal muscle and brain, another type of NADH shuttle, the **glycerol-3-phosphate shuttle**, occurs. It differs from the malate-aspartate shuttle in that it delivers the reducing equivalents from NADH into Complex III, not Complex I, providing only enough energy to synthesize two ATP molecules per pair of electrons.

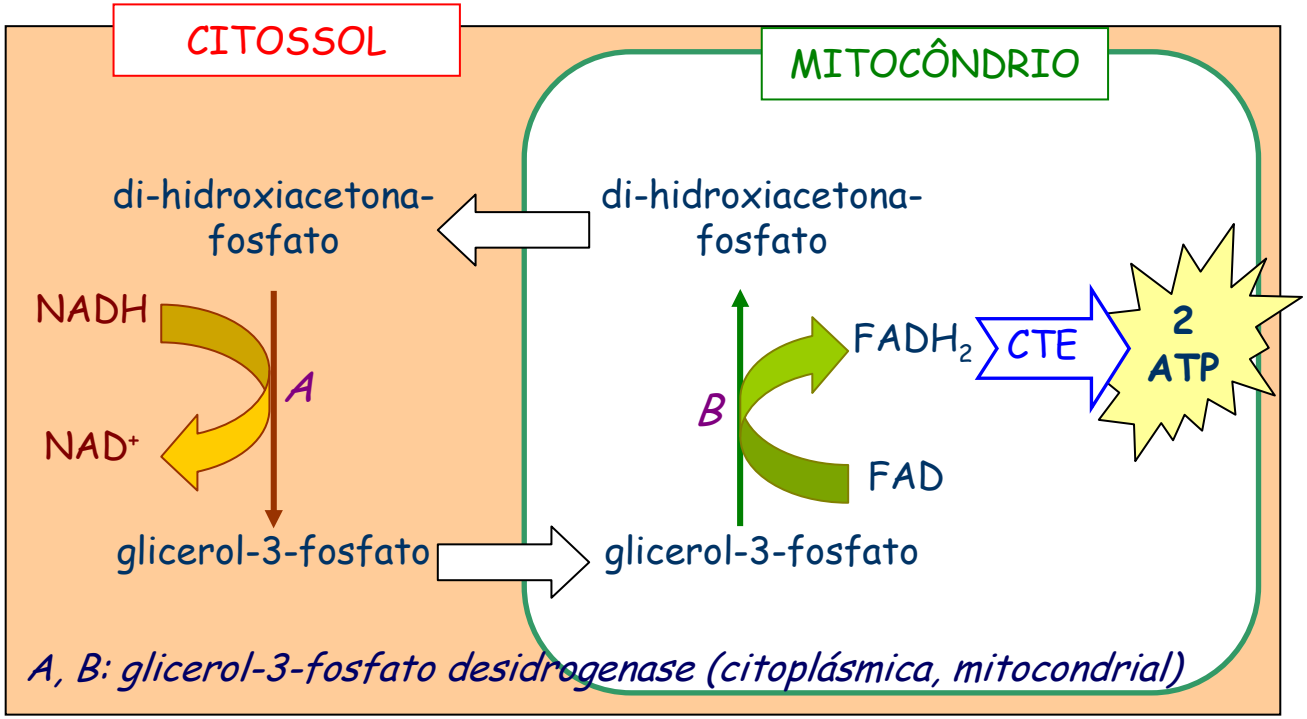
The mitochondria of higher plants have an externally oriented NADH dehydrogenase that is able to transfer electrons directly from cytosolic NADH into the respiratory chain.

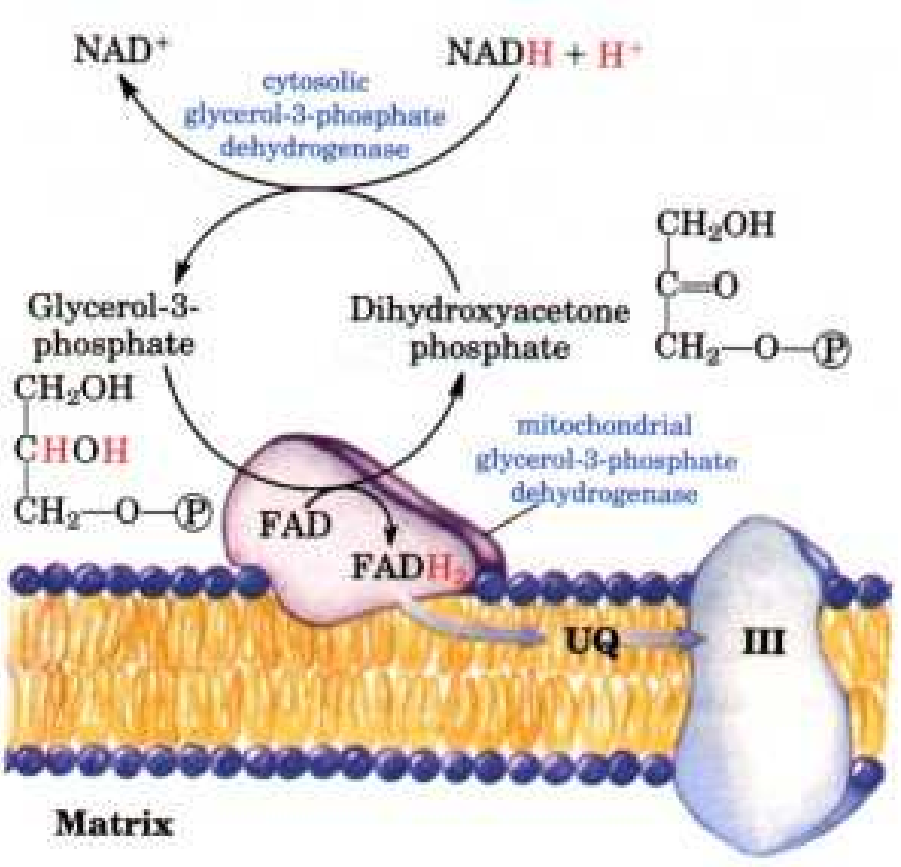
Condições de aerobiose

Como passam os electrões do NADH formado na glicólise para o interior do mitocôndrio?

Mecanismo de *shuttle* do glicerol-3-fosfato

Funciona no cérebro e no músculo esquelético e nos músculos de vôo dos insectos.
Sendo irreversível, funciona bem mesmo contra um gradiente de concentração de NADH.
Como os electrões são transferidos para o FAD, cada NADH citoplasmático conduz à formação de apenas 2 moléculas de ATP.
É, por isso, mais simples e energeticamente menos eficiente que o *shuttle* do malato-aspartato.

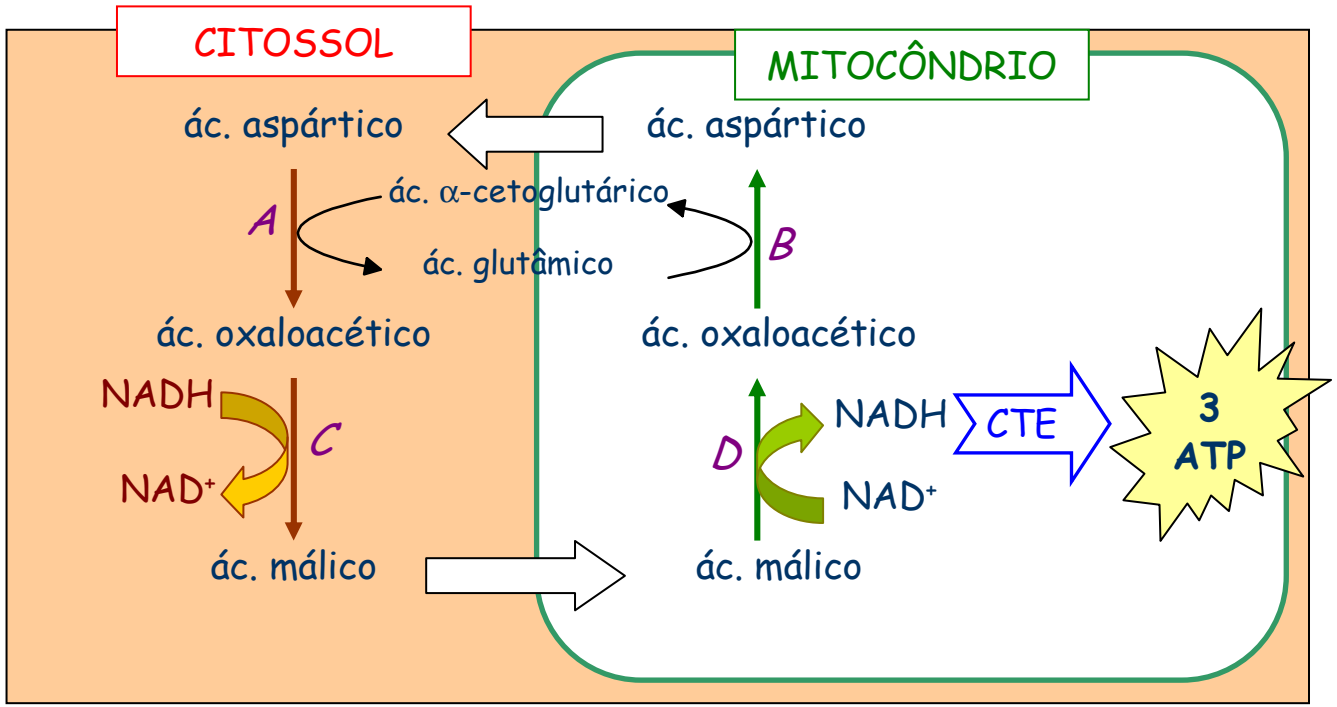




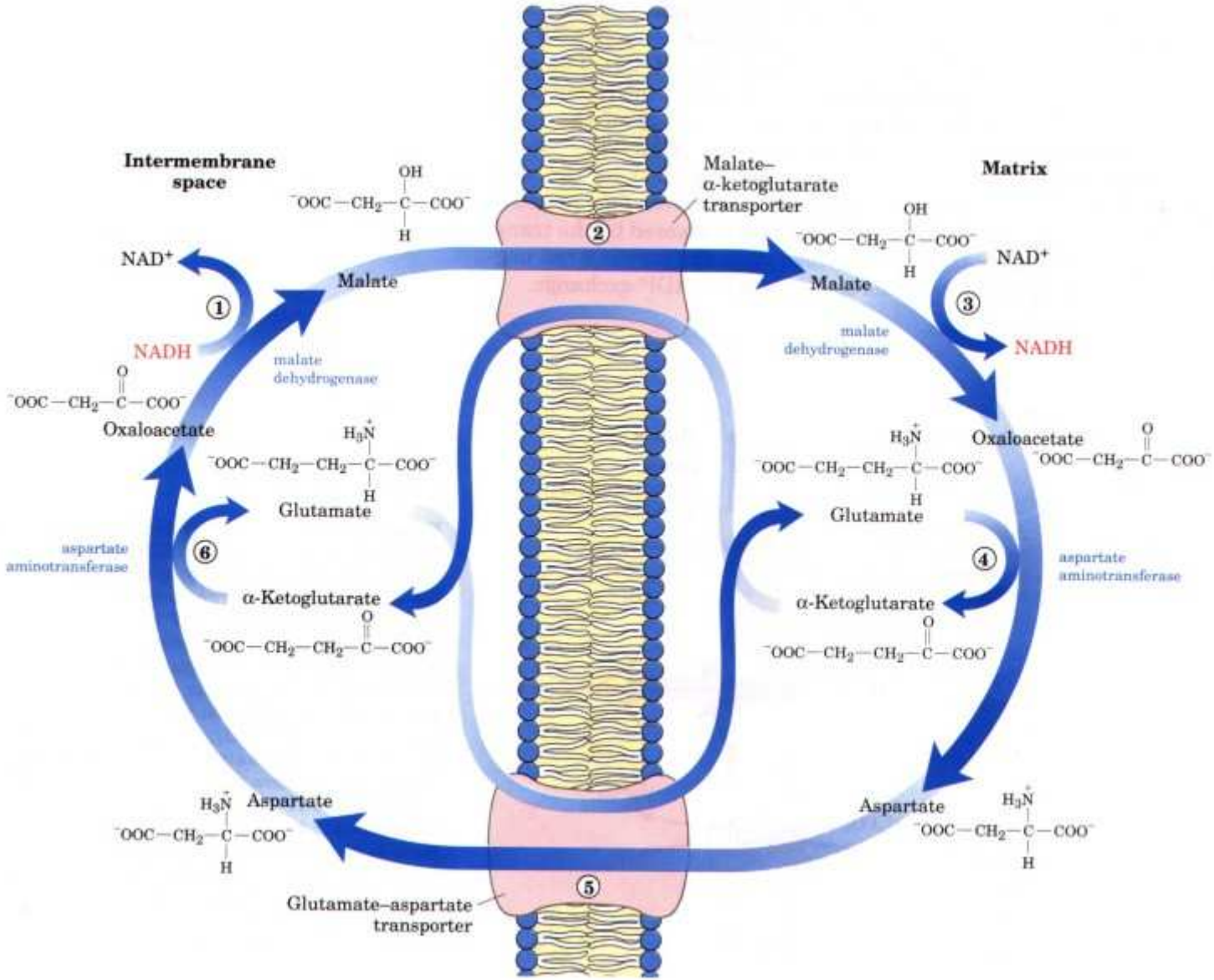
The glycerol-3-phosphate shuttle, an alternative means of moving reducing equivalents from the cytosol to the mitochondrial matrix. Dihydroxyacetone phosphate in the cytosol accepts two reducing equivalents from cytosolic NADH in a reaction catalyzed by cytosolic glycerol-3-phosphate dehydrogenase. A membrane-bound isozyme of glycerol-3-phosphate dehydrogenase, located on the outer face of the inner membrane, transfers two reducing equivalents from glycerol-3-phosphate in the intermembrane space to ubiquinone. Note that this shuttle does not involve membrane transport systems.

Mecanismo de *shuttle* do malato-aspartato

Funciona no coração, fígado e rins.
É reversível e funciona em resposta a gradientes de concentração.



A, B: aspartato aminotransferase (citoplásmica, mitocondrial)
C, D: malato desidrogenase (citoplásmica, mitocondrial)



The malate-aspartate shuttle for transporting reducing equivalents from cytosolic NADH into the mitochondrial matrix. 1. NADH in the cytosol (intermembrane space) passes two reducing equivalants to oxaloacetate, producing malate. 2. Malate is transported across the inner membrane by the malate-a-ketoglutarate transporter. 3. In the matrix, malate passes two reducing equivalants to NAD+; the resulting matrix NADH is oxidized by the mitochondrial respiratory chain. The oxaloacetate formed from malate cannot pass directly into the cytosol. It is first transaminated to form aspartate 4. which can leave via the glutamate-aspartate transporter 5. Oxaloacetate is regenerated in the cytosol 6, completing the cycle.

Condições de anaerobiose

FERMENTATION

Fermentation can have a variety of meanings, ranging from informal to more scientific definitions. The various meanings of fermentation may be summarized as follows:

- Any spoilage of food by microbes. For example, the spoilage of wine to vinegar. This is a very general usage of fermentation;
- Any process that produces alcoholic beverages or acidic dairy products (again general use);
- Any large scale microbial process occurring with or without air (industrial use);
- Any energy-releasing process that occurs only under anaerobic conditions (more scientific).

Any metabolic process that releases energy from a sugar or other organic molecule, does not need oxygen or an electron transport system, and uses an organic molecule as the final electron acceptor. It is this last definition that we will use.

Some other key points that we need to keep in mind are:

- A complete fermentation pathway begins with a substrate, includes glycolysis and results in various end-products. The different fermentation pathways typically are named for the end products that are formed;.
- As far as an energy is concerned, fermentation does not generate ATP directly but recycles a limited amount of NAD^+ back into glycolysis to keep glycolysis going. Recall that each pass through glycolysis generates 2 ATP molecules by substrate level phosphorylation;
- All fermentation pathways are anaerobic;
- Cells that are capable of both respiration and fermentation will typically use respiration when possible. Respiration yields more energy from a lot less substrate.

TYPES OF FERMENTATION PATHWAYS		
PATHWAY	**END PRODUCTS**	**EXAMPLES**
Lactic acid (Homolactic)	lactic acid (2 molecules)	*Lactobacillus*, *Enterococcus*, *Streptococcus* spp. Pathway can result in food spoilage.
Heterolactic	lactic acid, ethanol and CO_2	*Leuconostoc.* Used in sauerkraut production.
Alcohol	ethanol and CO_2	*Saccharomyces* (yeast). Important in production of alcoholic beverages, bread and gasohol.
Proprionic acid	proprionic acid and CO_2	*Proprionibacterium acnes*: metabolizes fatty acids in oil glands to proprionic acid. *Proprionibacterium freudenreichii* gives flavor to and produces holes in Swiss cheese.
Butyric acid	Butyric acid, butanol, acetone, isopropyl alcohol and CO_2	*Clostridium* spp. produce butyric acid that causes butter and cheese spoilage. Butanol and acetone are important organic solvents.
Butanediol	Butanediol and CO_2	Butanediol produced by *Enterobacter*, *Serratia*, *Erwinia* and *Klebsiella*. The intermediate, acetoin, is detected by the VP test. This test is used together with the MR test often to distinguish *Enterobacter* from *Escherichia coli* (VP-). *E.coli* is an important indicator organism of fecal contamination.
	ethanol, acetic acid, lactic	Variety of acid products. Typically carried out by members of the Enterobacteriaceae including

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Mixed acid	ethanol, acetic acid, lactic acid, succinic acid, formic acid and CO_2	Variety of acid products. Typically carried out by members of the Enterobacteriaceae including *E. coli*, *Salmonella* and *Shigella* pathogens. Products detected by reaction with methyl red pH indicator.
Methanogenesis	methane and CO_2	Certain Archaea. Majority of earth's methane production.

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Alcohol	ethanol and CO_2	*Saccharomyces* (yeast). Important in production of alcoholic beverages, bread and gasohol.
Propionic acid	propionic acid and CO_2	*Propionibacterium acnes*: metabolizes fatty acids in oil glands to propionic acid. *Propionibacterium freudenreichii* gives flavor to and produces holes in Swiss cheese.
Butyric acid	Butyric acid, butanol, acetone, isopropyl alcohol and CO_2	*Clostridium* spp. produce butyric acid that causes butter and cheese spoilage. Butanol and acetone are important organic solvents.
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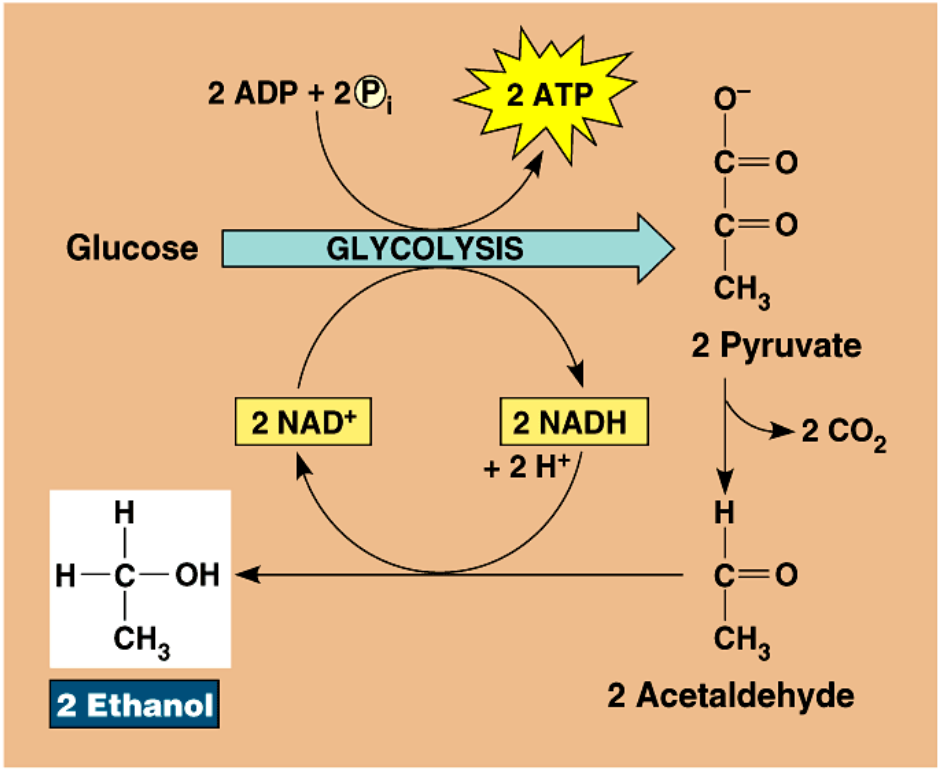
Mixed acid	ethanol, acetic acid, lactic acid, succinic acid, formic	Variety of acid products. Typically carried out by members of the Enterobacteriaceae including *E. coli*, *Salmonella* and *Shigella* pathogens. Products detected by reaction with **methyl red** pH indicator.

Condições de anaerobiose

Alcoholic fermentation



(a) Alcohol fermentation

- Under anaerobic conditions, glycolysis can be maintained by alcoholic fermentation, where pyruvate produced by glycolytic reactions is first converted to acetaldehyde plus CO_2. The former is then converted to ethanol.

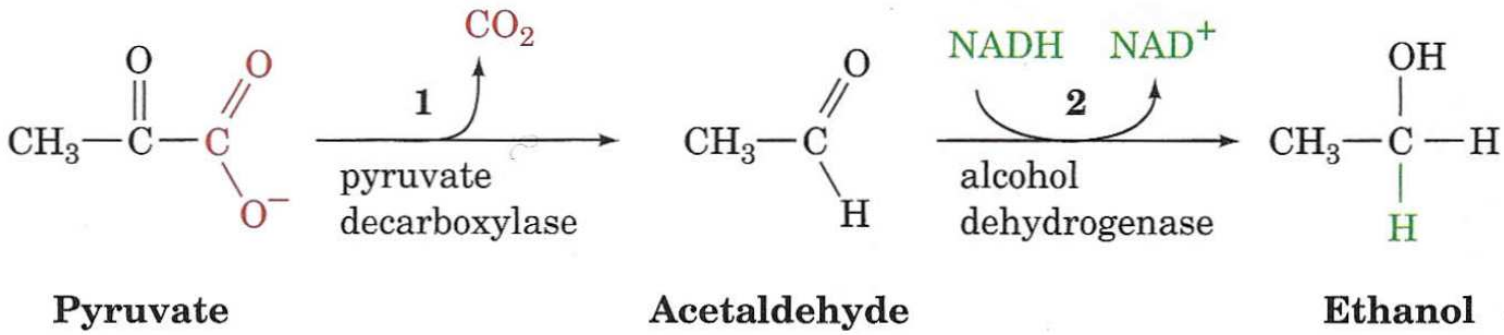
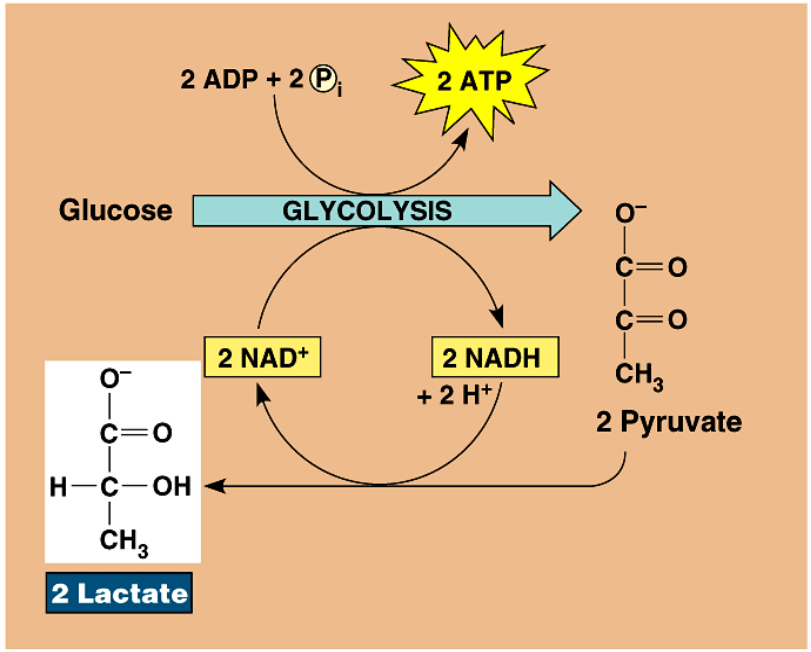


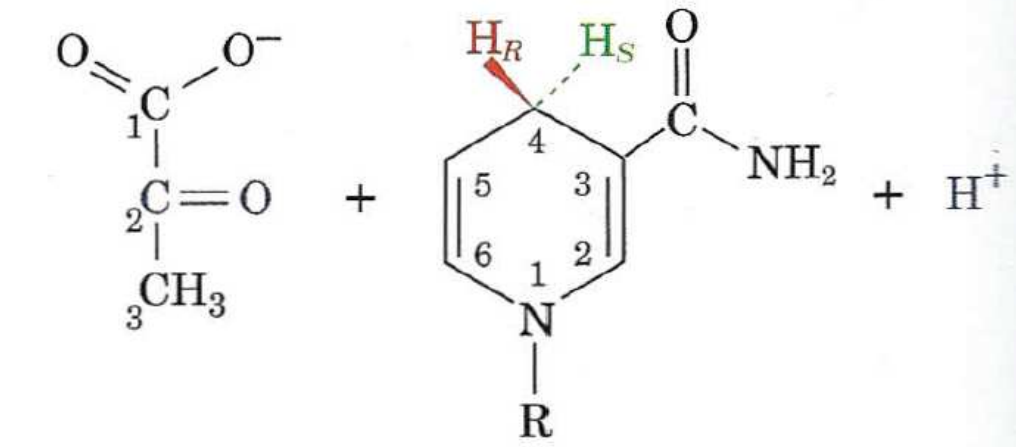
FIGURE 17-25 The two reactions of alcoholic fermentation. (1) Decarboxylation of pyruvate to form acetaldehyde is followed by **(2)** reduction of the acetaldehyde to ethanol by NADH.

Homolactic fermentation



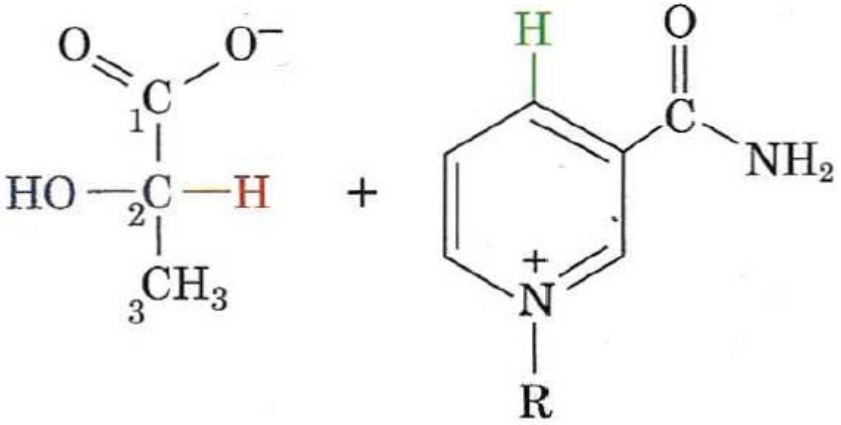
(b) Lactic acid fermentation

- Under anaerobic conditions, glycolysis can be alternatively maintained by lactic acid formation in a reaction catalyzed by lactate dehydrogenase.



Pyruvate **NADH**

lactate
dehydrogenase (LDH)

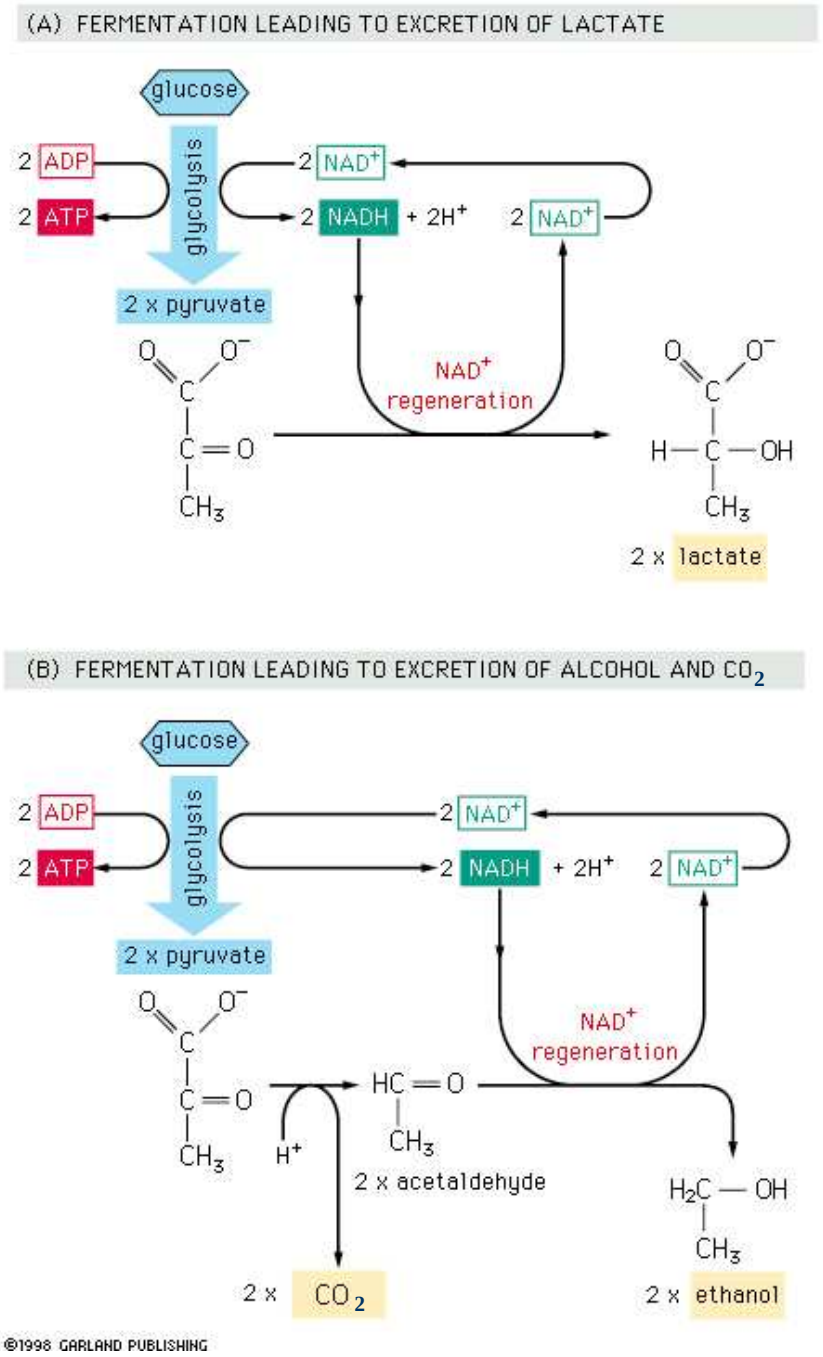


L-Lactate **NAD^+**

When oxygen is not available, mitochondria cannot regenerate NAD^+ and use carbons from glycolysis and the system “backs up”.

Pyruvate → lactic acid (in animals, bacteria)

→ ethanol and CO_2 (in plants, fungi, microbes)



Bioenergetics of glycolysis

TABLE 17-1 Standard Free Energy Changes ($\Delta G^{\circ\prime}$)

Reaction	Enzyme	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})
1	HK	−20.9
2	PGI	+2.2
3	PFK	−17.2
4	Aldolase	+22.8
5	TIM	+7.9
6 + 7	GAPDH + PGK	−16.7
8	PGM	+4.7
9	Enolase	−3.2
10	PK	−23.0

Bioenergetics of glycolysis

TABLE 17-1 Standard Free Energy Changes ($\Delta G^{\circ\prime}$), and Physiological Free Energy Changes (ΔG) in Heart Muscle, of the Reactions of Glycolysis[a]

Reaction	Enzyme	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})	ΔG (kJ · mol^{-1})
1	HK	−20.9	−27.2
2	PGI	+2.2	−1.4
3	PFK	−17.2	−25.9
4	Aldolase	+22.8	−5.9
5	TIM	+7.9	Negative
6 + 7	GAPDH + PGK	−16.7	−1.1
8	PGM	+4.7	−0.6
9	Enolase	−3.2	−2.4
10	PK	−23.0	−13.9

[a]Calculated from data in Newsholme, E.A. and Start, C., *Regulation in Metabolism, p.* 97, Wiley (1973).

C. *Energetics of Fermentation*

Thermodynamics permits us to dissect the process of fermentation into its component parts and to account for the free energy changes that occur. This enables us to calculate the efficiency with which the free energy of degradation of glucose is utilized in the synthesis of ATP. The overall reaction of homolactic fermentation is

$$\text{Glucose} \rightarrow 2\text{lactate} + 2H^+$$

$$\Delta G^{\circ\prime} = -196 \text{ kJ} \cdot \text{mol}^{-1} \text{ of glucose}$$

($\Delta G^{\circ\prime}$ is calculated from the data in Table 3-4 using Eqs. [3.19] and [3.21] adapted for $2H^+$ ions.) For alcoholic fermentation, the overall reaction is

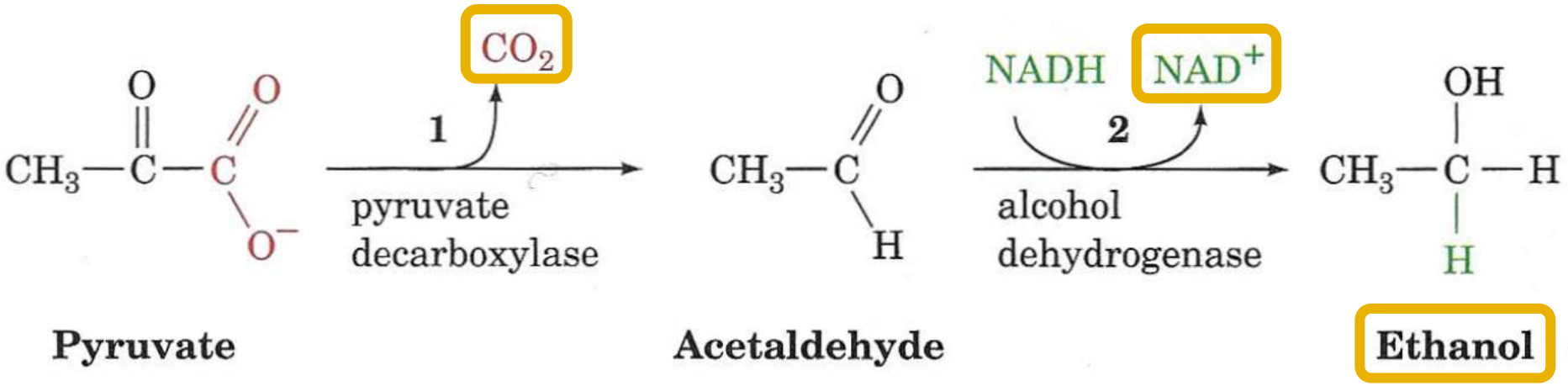
$$\text{Glucose} \rightarrow 2CO_2 + 2\text{ethanol}$$

$$\Delta G^{\circ\prime} = -235 \text{ kJ} \cdot \text{mol}^{-1} \text{ of glucose}$$

Each of these reactions is coupled to the net formation of two ATPs, which requires $\Delta G^{\circ\prime} = +61 \text{ kJ} \cdot \text{mol}^{-1}$ of glucose consumed (Table 16-3). Dividing the $\Delta G^{\circ\prime}$ of ATP formation by that of lactate formation indicates that homolactic fermentation is 31% "efficient"; that is, 31% of the free energy released by this process under standard biochemical conditions is sequestered in the form of ATP. The rest is dissipated as heat, thereby making the process irreversible. Likewise, alcoholic fermentation is 26% efficient under biochemical standard state conditions. Actually, *under physiological conditions, where the concentrations of reactants and products differ from those of the standard state, these reactions have free energy efficiencies of >50%.*

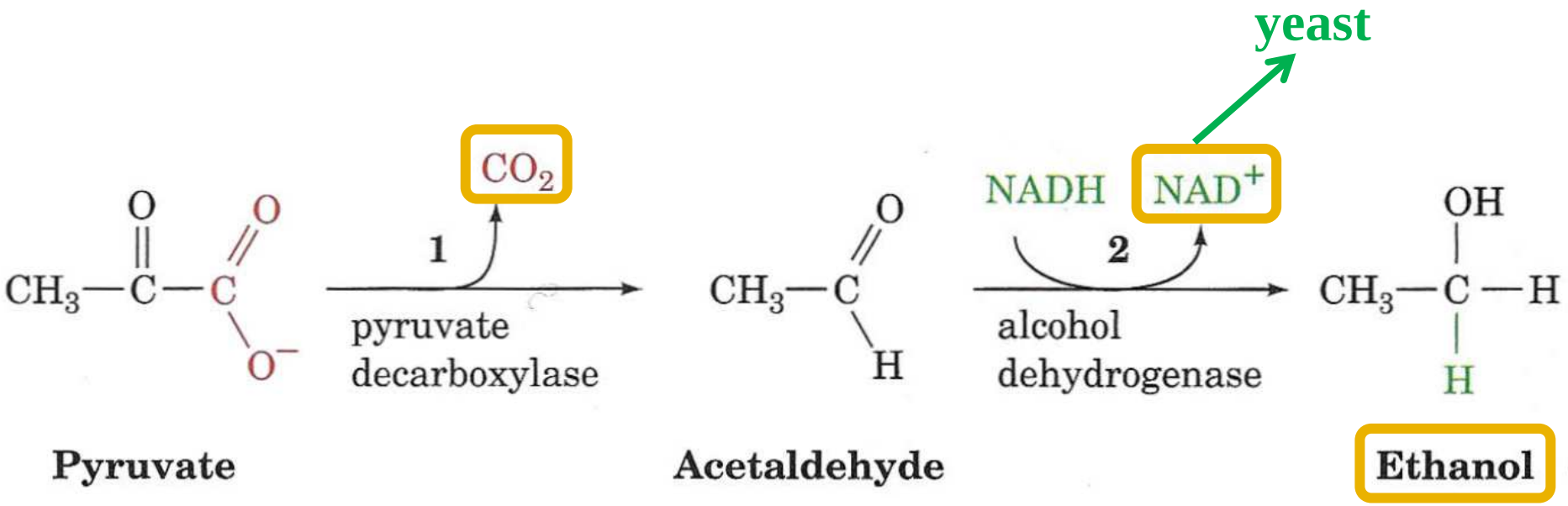
Alcoholic Fermentation

End-Product Applications



Alcoholic Fermentation

End-Product Applications



Alcoholic Fermentation

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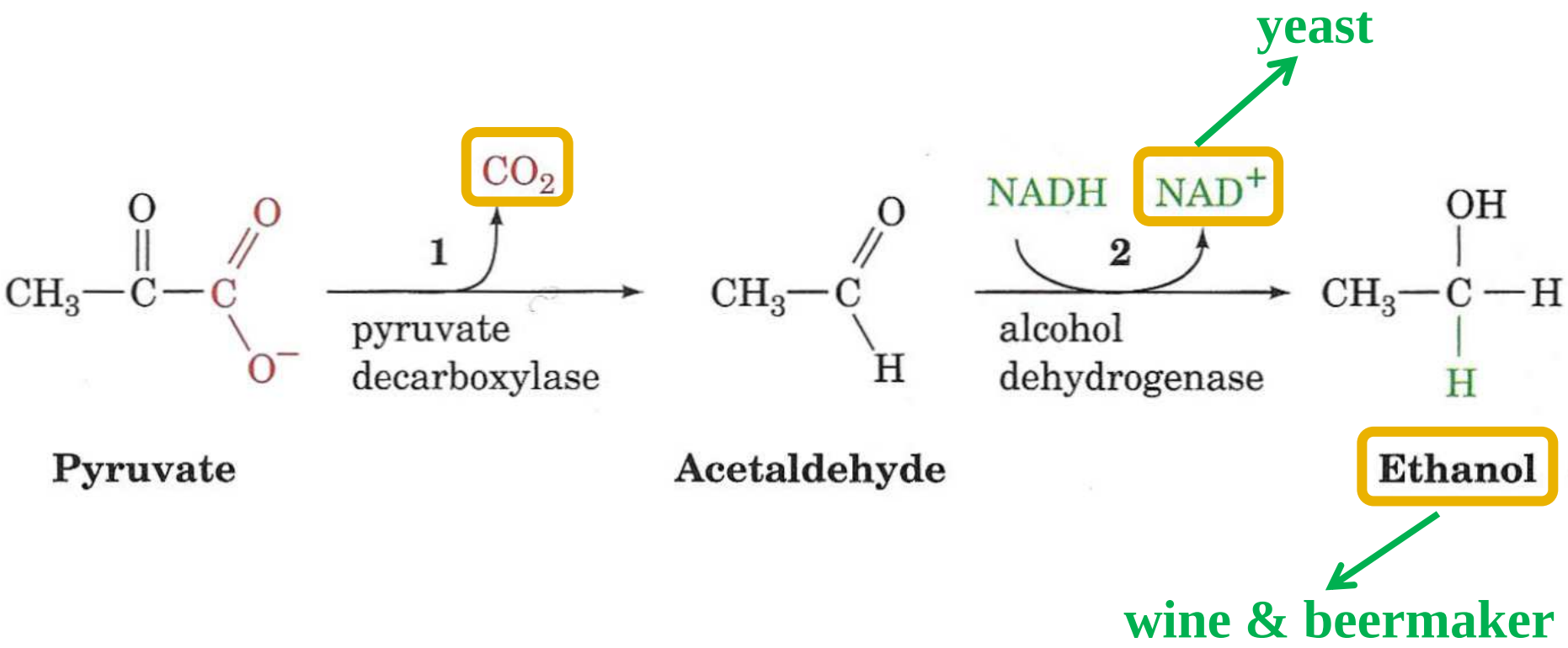

Alcoholic Fermentation

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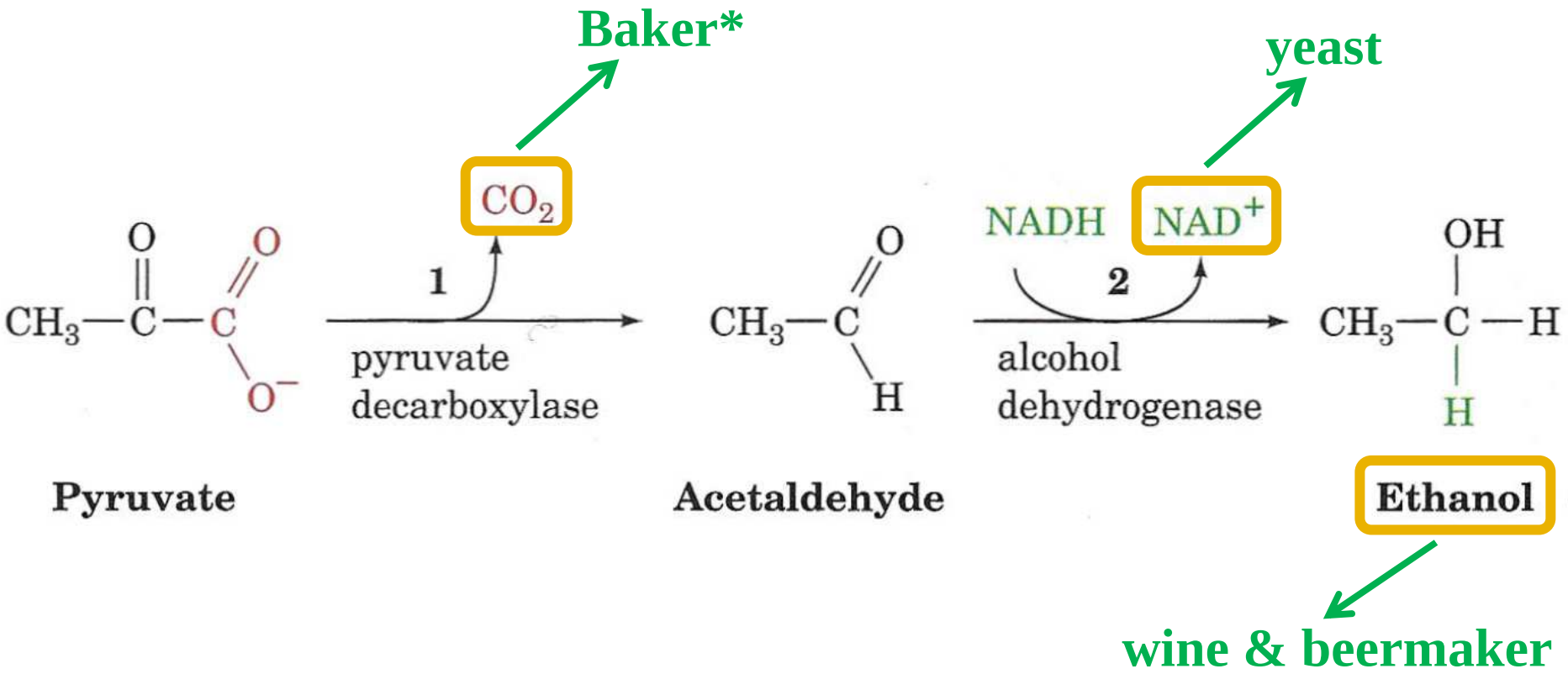


***Gluten** = viscoelastic mixture formed by mixing prolamins, starch and water from wheat and rye.

Levedura da cerveja -- *Saccharomyces cereviseae*

Condições de aerobiose

Condições de anaerobiose

Fermentação alcoólica

Fabrico do vinho e da cerveja

Fabrico do pão (glúten)

Bactérias lácticas do leite

Fabrico do iogurte

Raízes de plântulas de milho em terrenos alagados

Músculo humano em actividade física intensa

Regulation of glycolysis

The rate of glycolysis is controlled by three regulatory enzymes, all catalyzing irreversible reactions

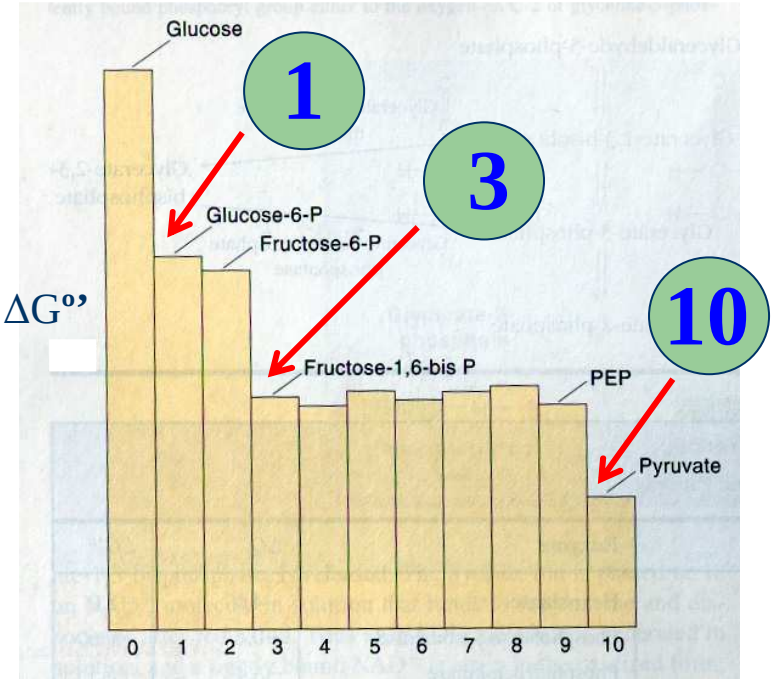
Hexokinase

Phosphofructokinase-1

Pyruvate kinase

Three irreversible reactions catalysed by:

- **Hexokinase**
- **Phosphofructokinase-1**
- **Pyruvate kinase**



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Hexokinase / Glucokinase

Hexokinase

inhibited by glucose 6-phosphate

low K_m for glucose

present in all tissues

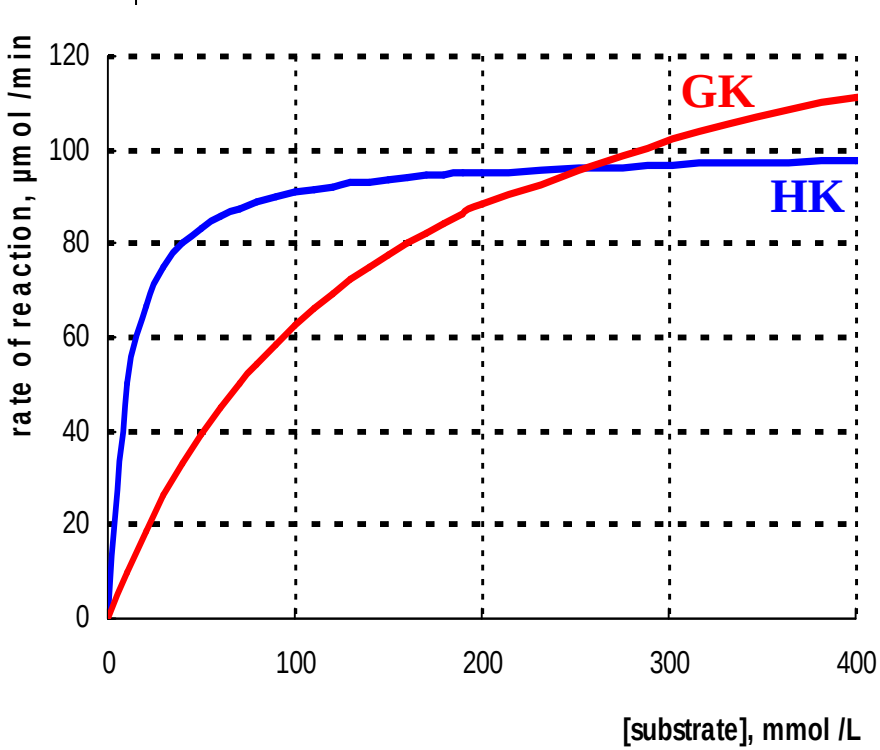
Glucokinase

not inhibited by glucose 6-phosphate

high K_m value for glucose

present only in the liver

induced by insulin



Phosphofructokinase-1

The key enzyme in the control of glycolysis

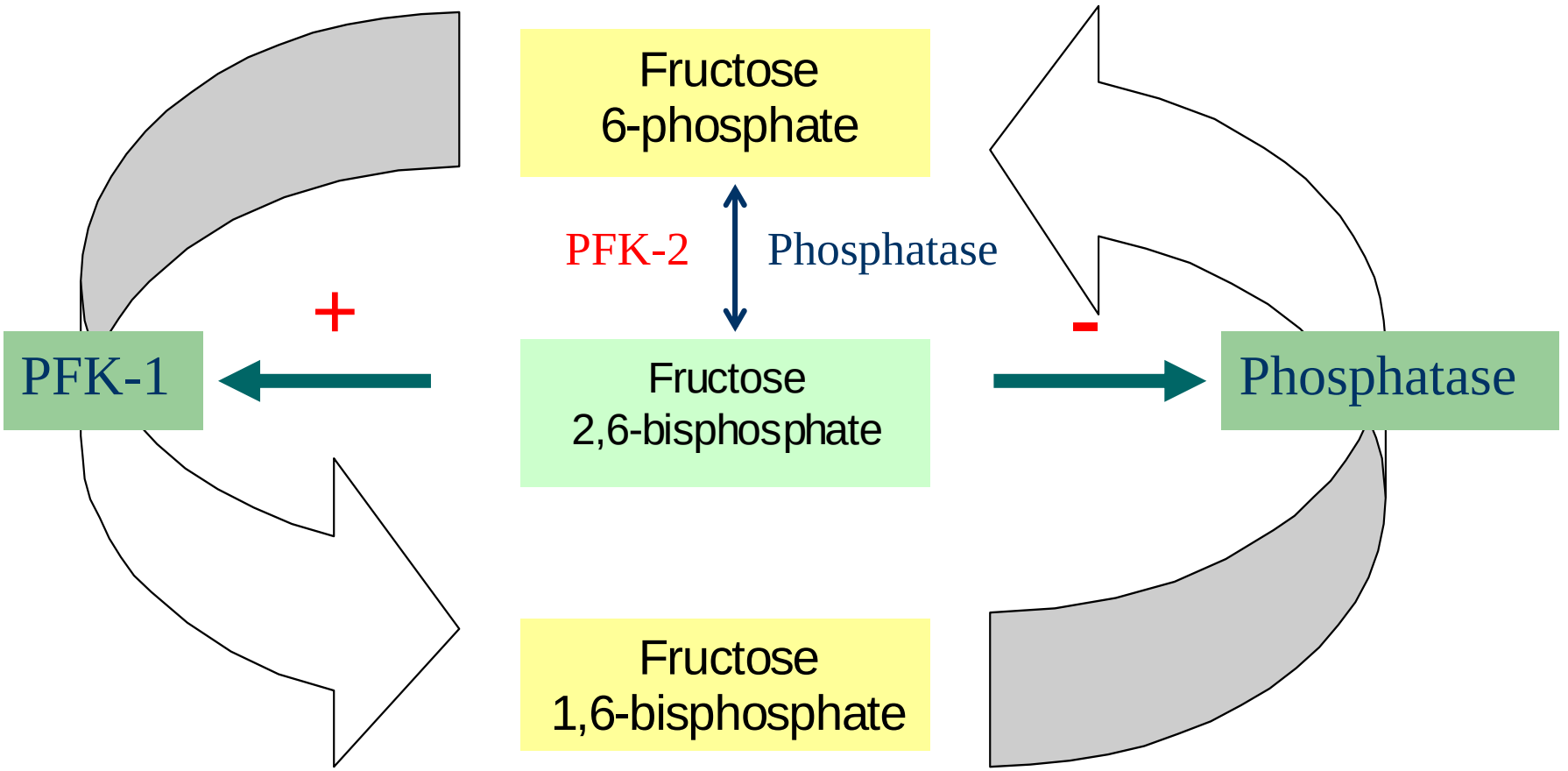
Inhibited by high levels of ATP

Allosteric regulators

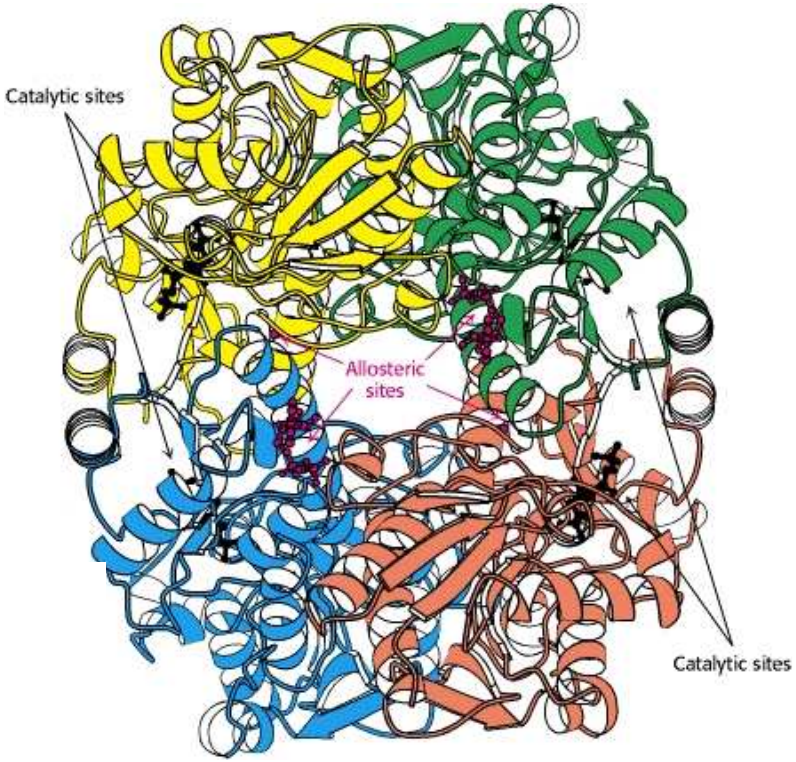
ATP (-), citrate (-), long-chain fatty acids (-)

AMP/ADP (+), fructose 2,6-bisphosphate (+)

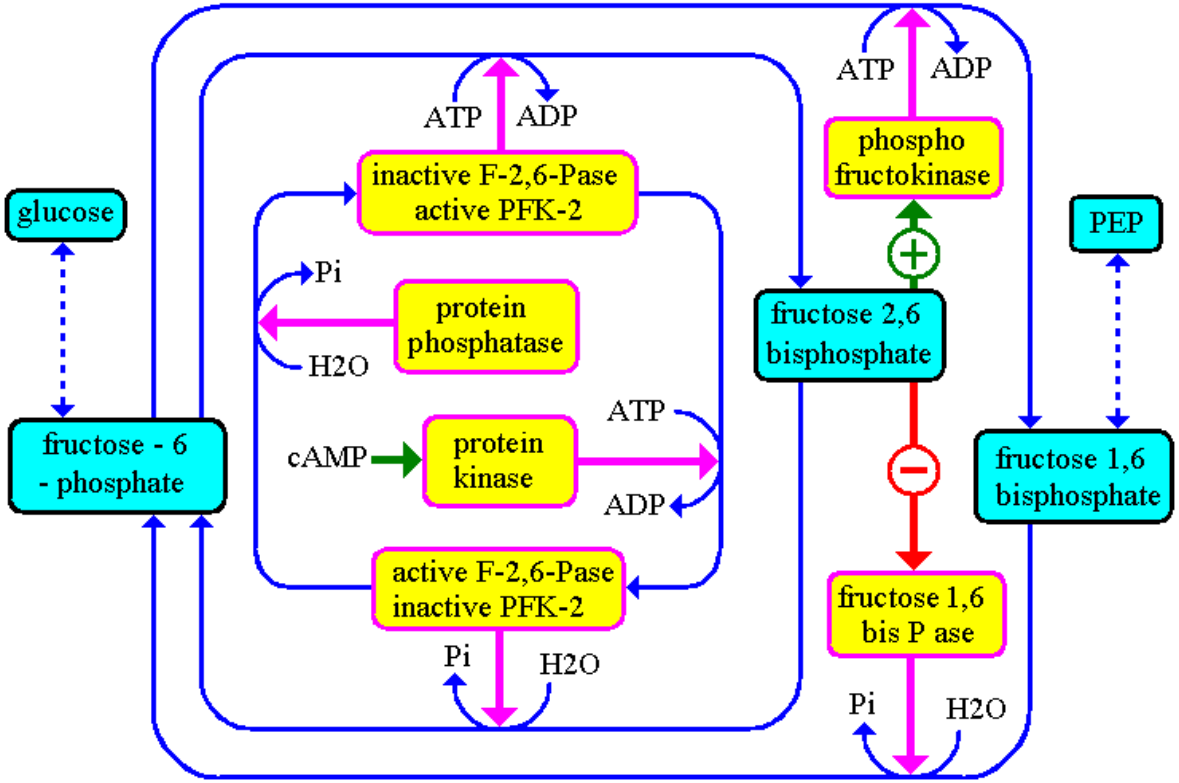
Fructose 2,6-bisphosphate
(formation, degradation and regulatory effects)



Mammalian PFK-1 is a 340 kDa tetramer



Phosphofructokinase regulation



Pyruvate kinase

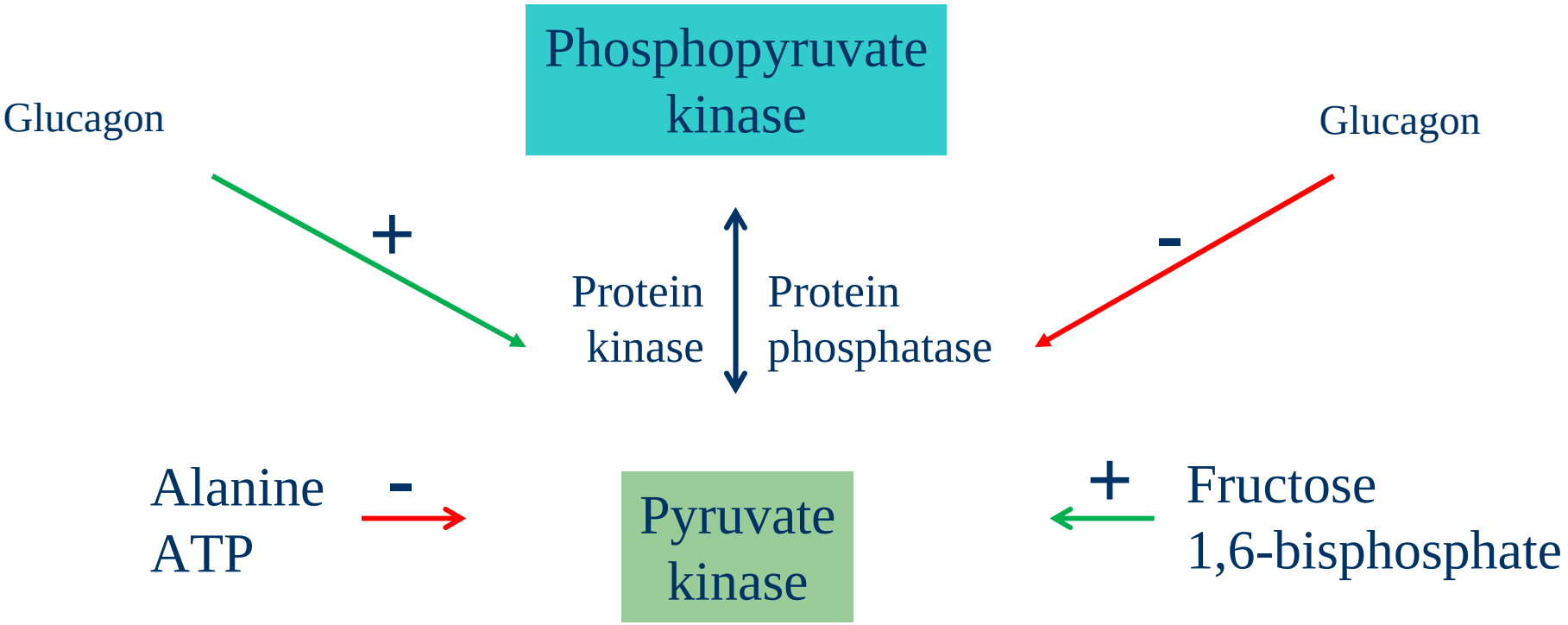
Controlled by both covalent modification (phosphorylation / dephosphorylation) and allosteric regulation

Allosteric regulators

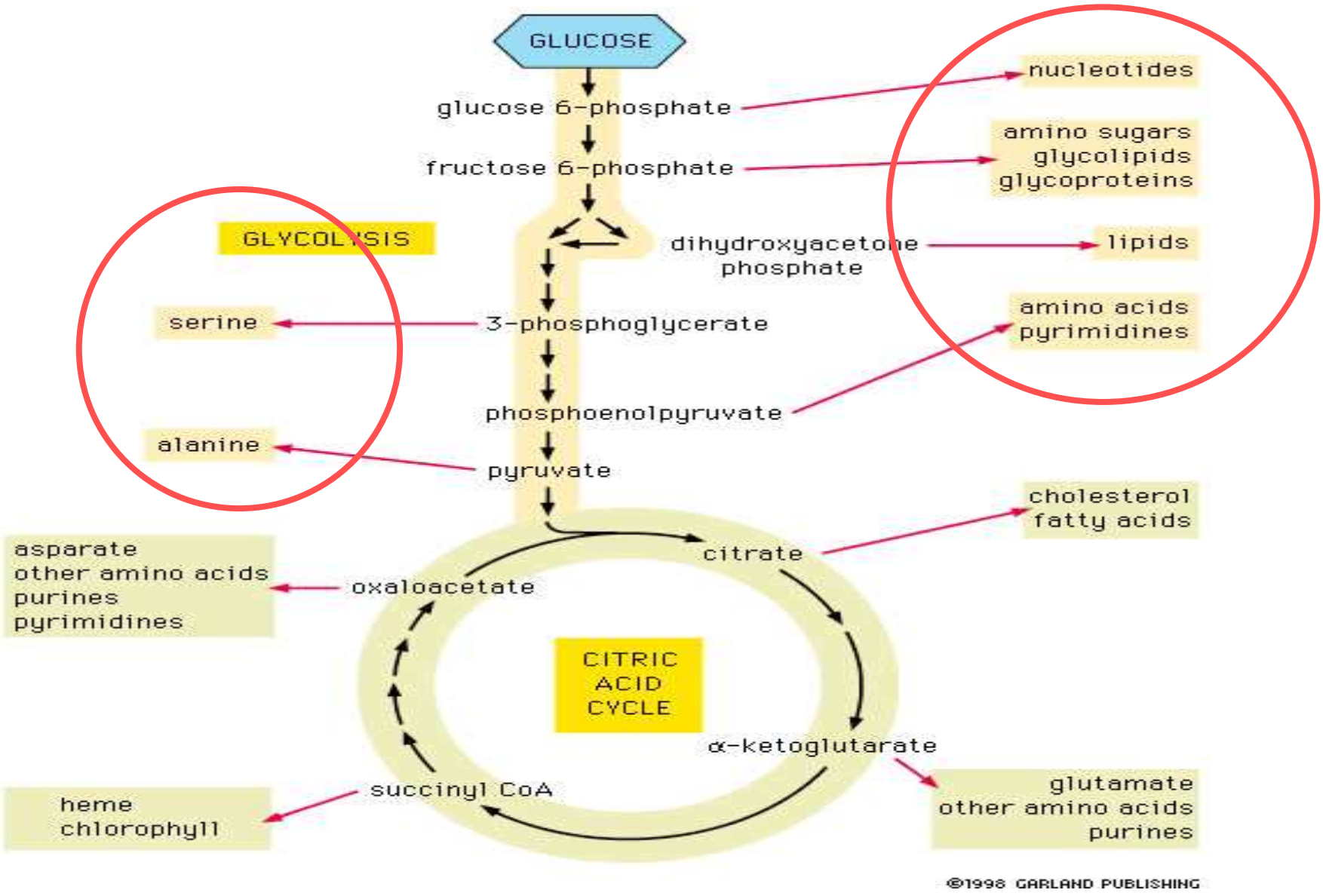
ATP (-), acetyl-CoA (-), alanine (-)

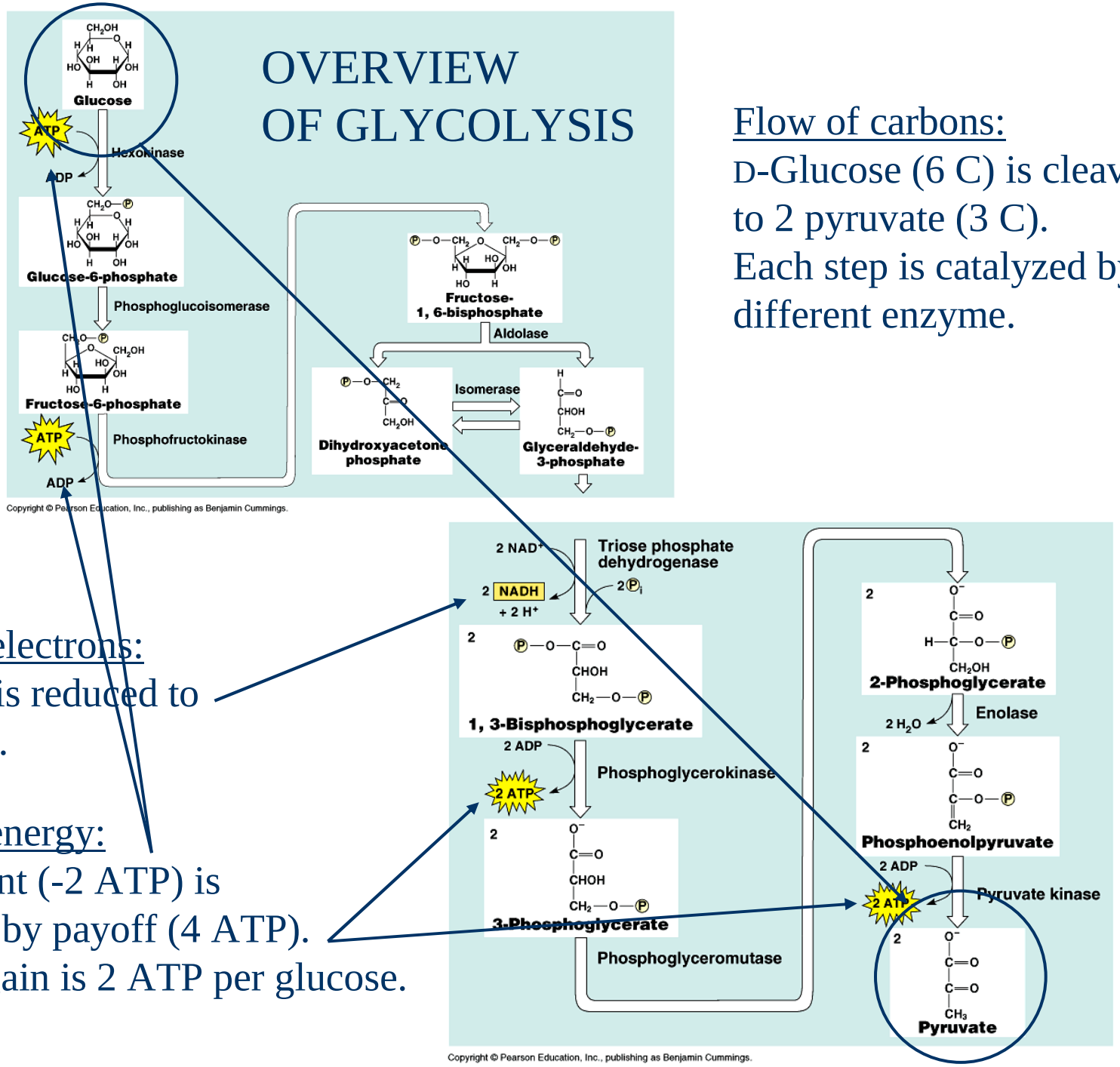
fructose 1,6-bisphosphate (+)

Regulation of pyruvate kinase



Glycolysis produces intermediates for other metabolic pathways





Flow of carbons:

D-Glucose (6 C) is cleaved to 2 pyruvate (3 C).

Each step is catalyzed by a different enzyme.

Flow of electrons:

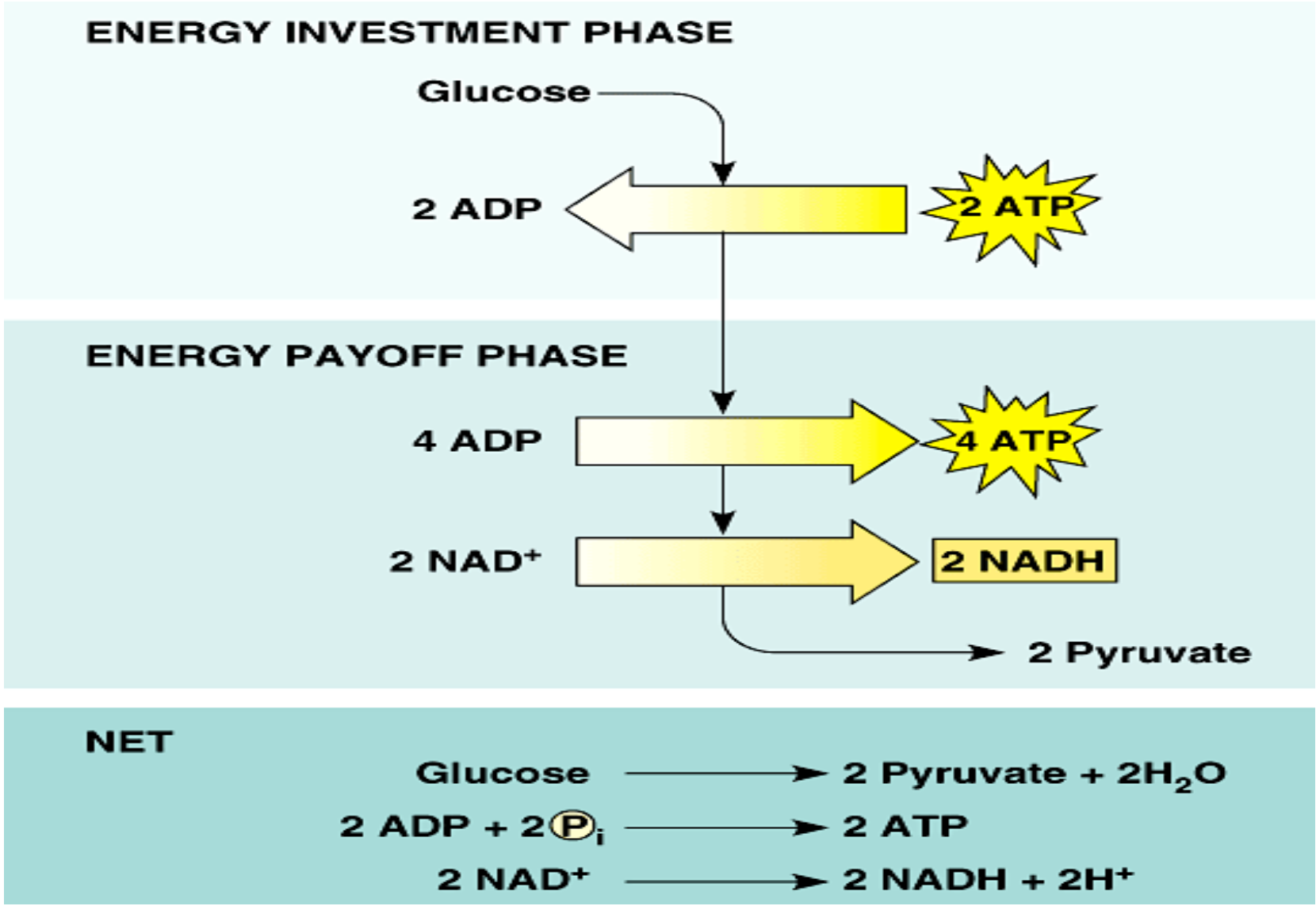
2 NAD^+ is reduced to 2 NADH.

Flow of energy:

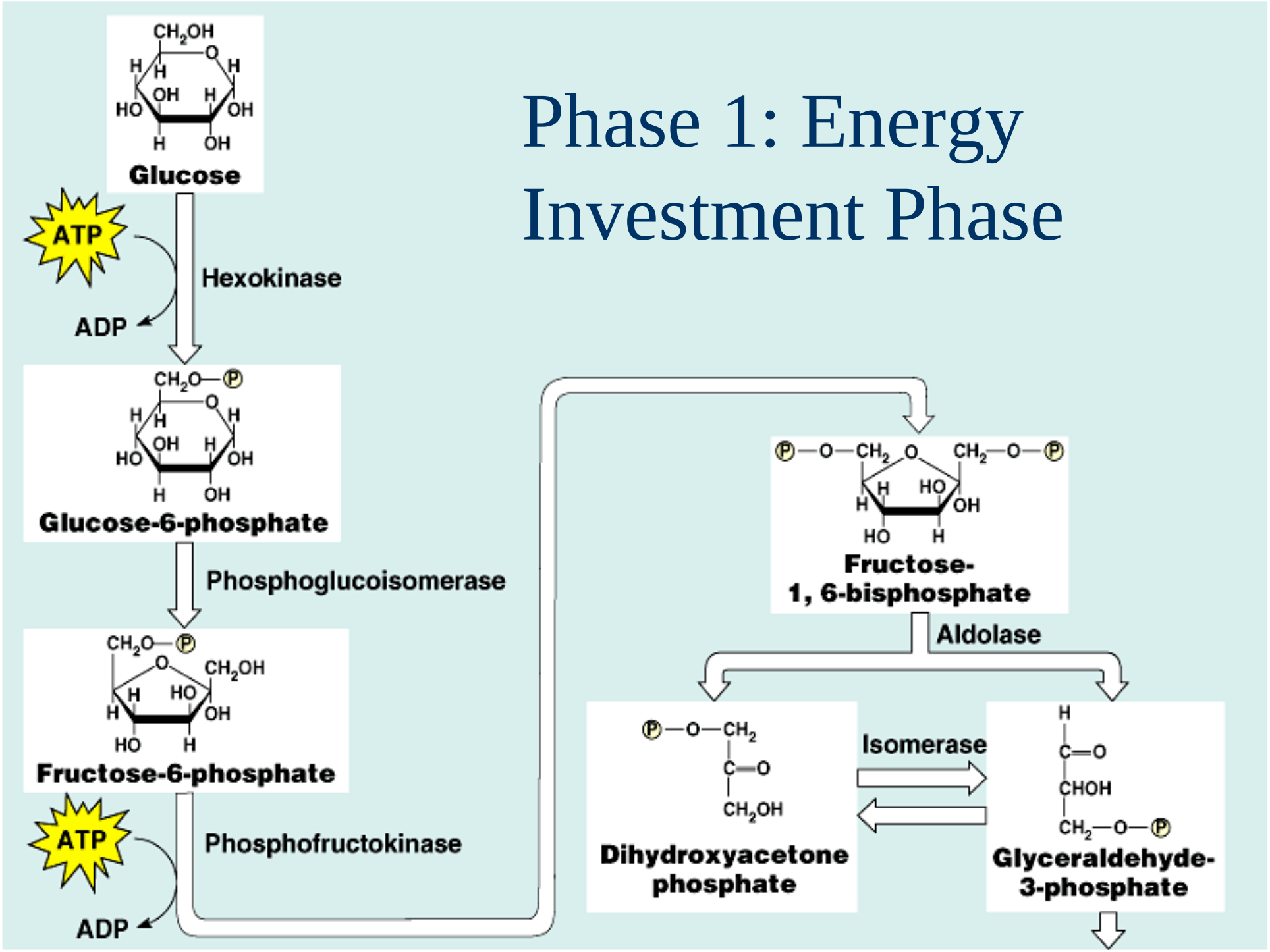
Investment (-2 ATP) is followed by payoff (4 ATP).

The net gain is 2 ATP per glucose.

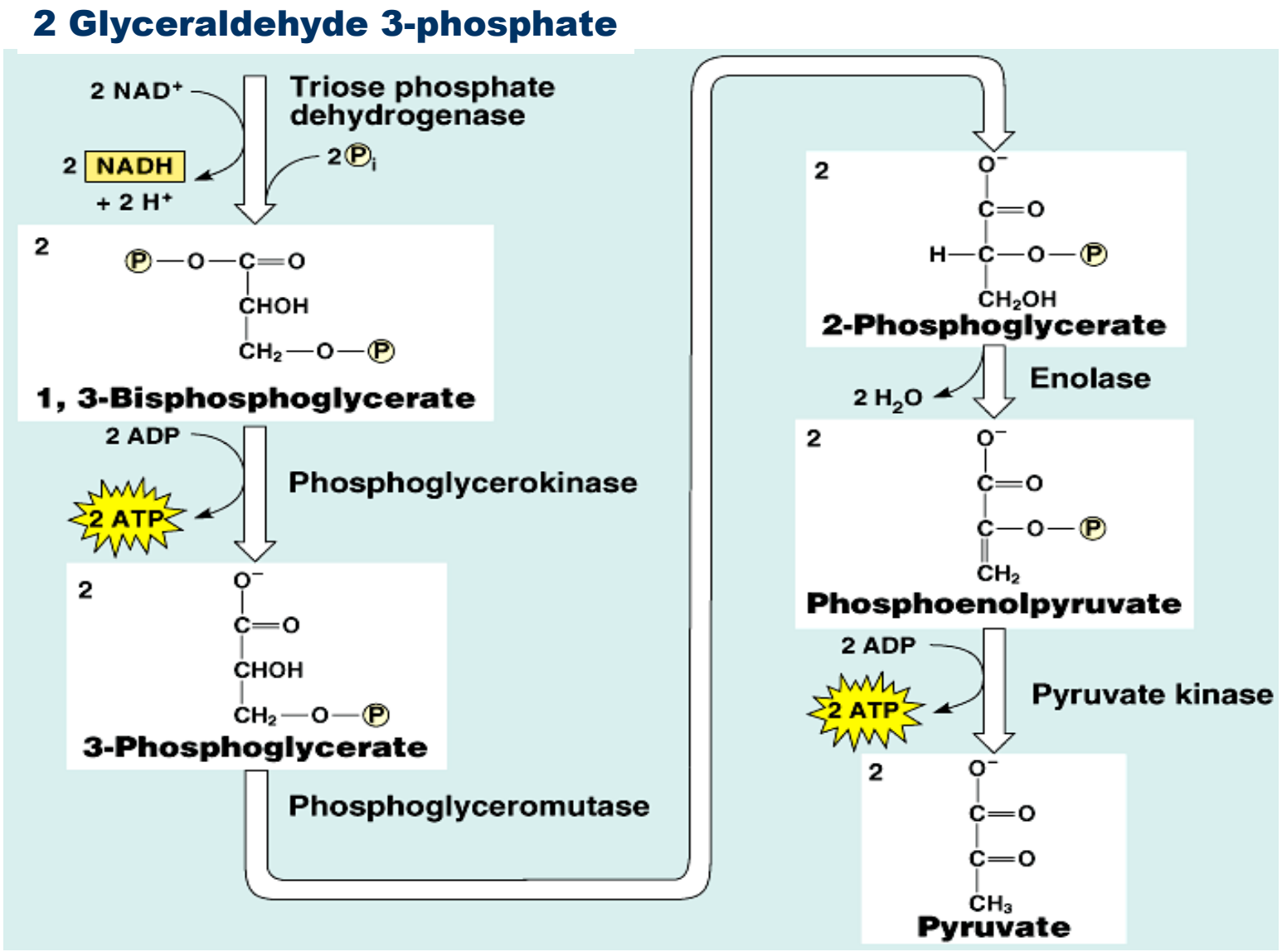
The two phases of glycolysis



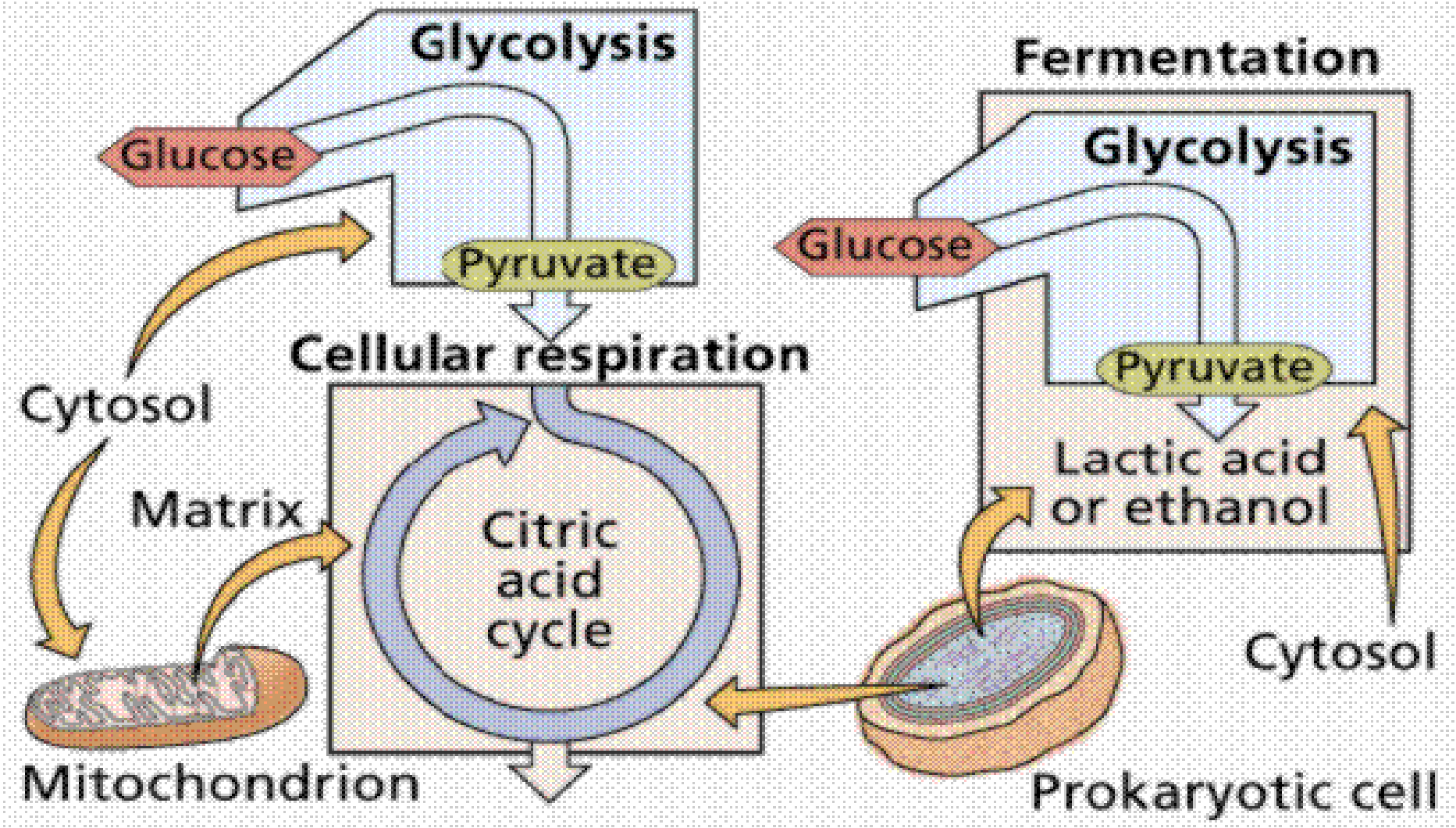
Phase 1: Energy Investment Phase



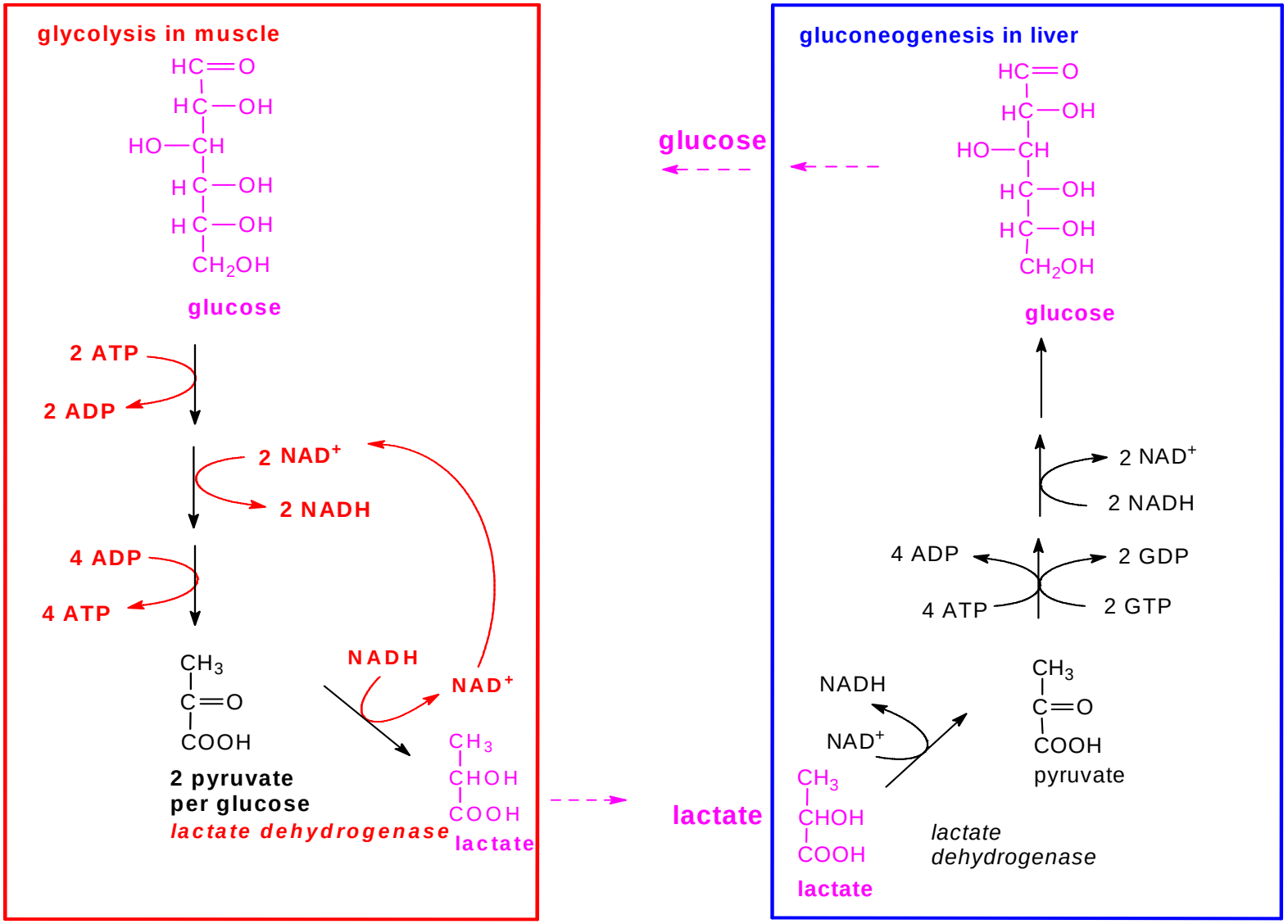
Phase 2. Energy Generation Phase



Aerobic versus anaerobic glycolysis



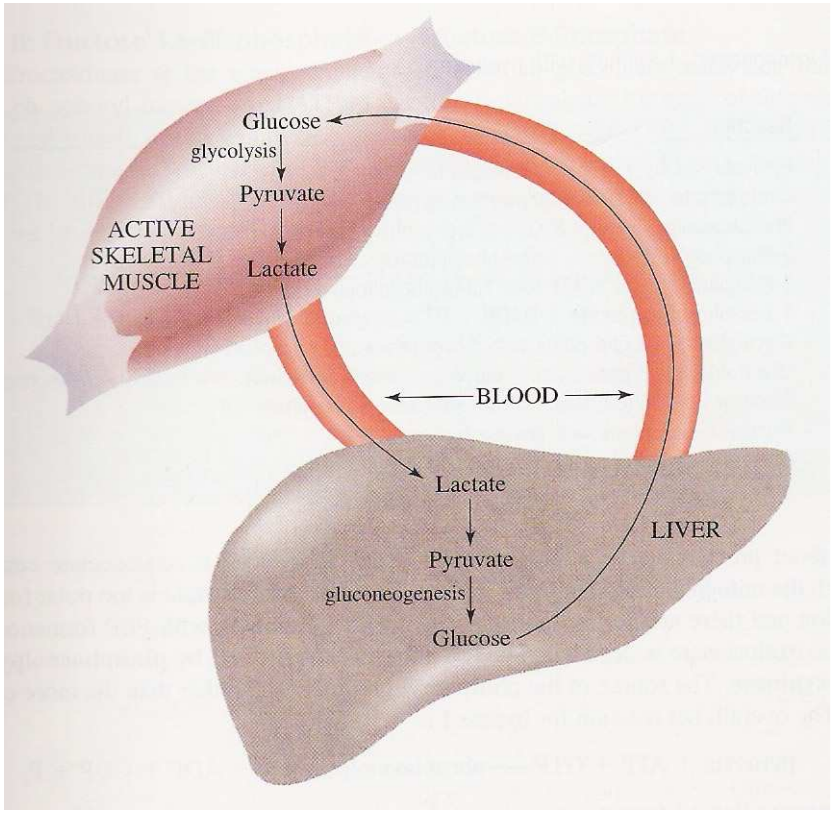
Cori cycle

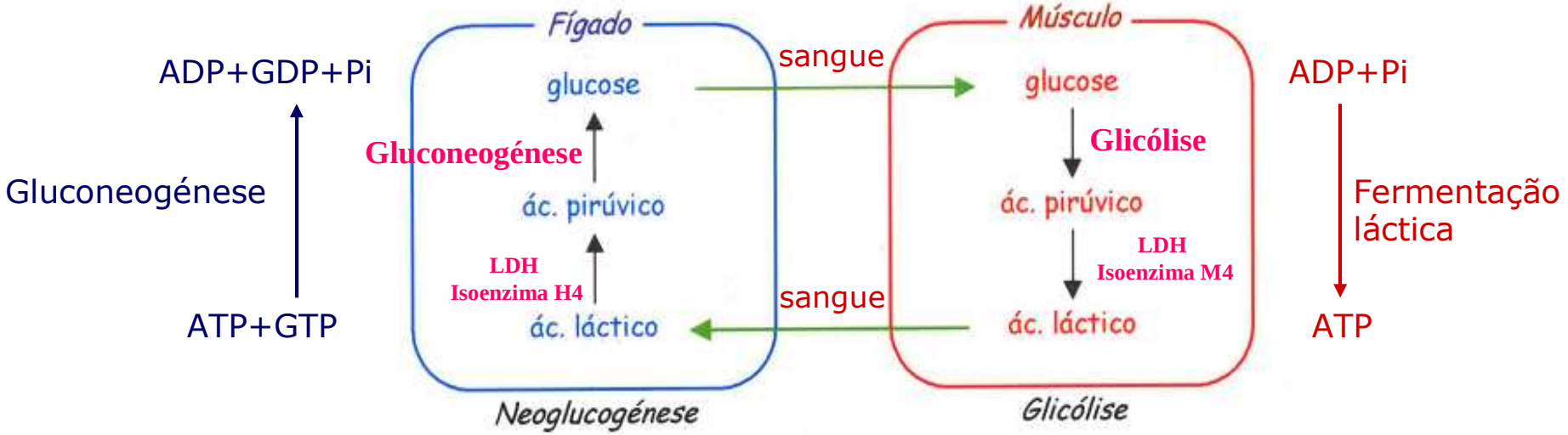

glycolysis in muscle
glucose
2 ATP
2 ADP
2 NAD+
2 NADH
4 ADP
4 ATP
NADH
NAD+
2 pyruvate
per glucose
lactate dehydrogenase
lactate
glucose
lactate
gluconeogenesis in liver
glucose
2 NAD+
2 NADH
4 ADP
2 GDP
4 ATP
2 GTP
NADH
NAD+
pyruvate
lactate dehydrogenase
lactate

Ciclo dos Cori* ou ciclo do ácido láctico

***Em homenagem a Carl e Gerti Cori, que foram os primeiros a descrevê-lo**

Ligação entre a *glicólise* no músculo em actividade e a *gluconeogénese* no fígado.
Em condições de exercício físico violento, o metabolismo da glucose no músculo estriado passa de aeróbio para anaeróbio, devido à escassez de O_2. Nestas condições, a concentração de lactato aumenta de 1,5 para 20 a 50 mM.
A sensação de fadiga muscular que sentimos durante ou após exercício físico violento não é, como se pensou durante muito tempo, devida à acumulação de ácido láctico, mas sim devido ao abaixamento do pH provocado pela acumulação de ácido láctico.





Problemas:

1. A hidrólise da adenosina trifosfato (ATP), pela qual se liberta o grupo fosfato terminal, é uma reacção fornecedora de energia de grande importância bioquímica e muitas determinações têm sido realizadas no sentido de medir os valores de ΔH e de ΔG para as reacções em condições fisiológicas. Numa dessas determinações (a 36 ºC e a pH 7,0) foi calculado que quando ΔH era igual a -4800 cal/mole, ΔG era de -7000 cal/mole. Calcule a variação de entropia que ocorre em tal reacção.

R: $\Delta S = 7{,}12\ cal.mol^{-1}.K^{-1}$

2. A glucose-6-fosfato foi hidrolisada enzimaticamente (a 25 ºC e a pH 7,0) a glucose e a fosfato inorgânico. Se esta reacção for iniciada pela adição de enzima a uma solução 0,1 M de glucose-6-fosfato pode observar-se, por análise química do meio de reacção, que se estabelece o equilíbrio quando a concentração final da glucose-6-fosfato é $0{,}5 \times 10^{-4}$ M.

Calcule:

a) A variação de energia livre padrão a pH 7,0 ($\Delta G^{O'}$) para a reacção de hidrólise;

b) A constante de equilíbrio para a reacção de síntese da glucose-6-fosfato a partir da glucose e do fosfato inorgânico;

c) A variação de energia livre padrão a pH 7,0 ($\Delta G^{O'}$) para a reacção de síntese referida em b).

R: a) $\Delta G^{O'} = 3.137\ cal.mol^{-1}$

b) $K_{eq} = 0{,}005$

c) $\Delta G^{O'} = +3.137\ cal.mol^{-1}$

6. Considere a seguinte reacção de oxidação-redução catalisada pela enzima lactato desidrogenase

$$\text{Piruvato} + NADH + H^+ \rightarrow \text{lactato} + NAD^+$$

Os potenciais redox padrão das meias reacções envolvidas são:

$$\text{Piruvato} + 2H^+ + 2e^- \rightarrow \text{lactato} \qquad E'_O = -0{,}19 \text{ volts}$$

$$NAD^+ + 2H^+ + 2e^- \rightarrow NADH + H^+ \qquad E'_O = -0{,}32 \text{ volts}$$

Calcule a variação de energia livre padrão da reacção.

R: $\Delta G^{O'} = -5.996 \text{ cal.mol}^{-1}$

7. A enzima triose fosfato isomerase catalisa a interconversão de gliceraldeído-3-fosfato (G 3-P) a di-hidroxiacetona-fosfato (DHAP):

$$\text{G 3-P} \rightarrow \text{DHAP}$$

Qual é a variação total de energia livre desta interconversão se a reacção se iniciar com 0,001 M de G 3-P e 0,344 M de DHAP, sabendo que, no equilíbrio, o G 3-P tem uma concentração de 0,015 M e a DHAP de 0,330 M. Considere uma temperatura de 25 ºC.

R: $\Delta G = +1.628 \text{ cal.mol}^{-1}$

8. Considere a reacção:

$$\text{D-Gliceraldeído-3-fosfato} + NAD^+ + H_3PO_4 \rightarrow \text{Ácido 1,3-difosfoglicérico} + NADH + H^+ \qquad \Delta G^{O'} = +1500 \text{ cal.mol}^{-1}$$

In vivo são observadas as seguintes concentrações:

$[\text{D-Gliceraldeído-3-P}] = 10^{-4}\text{ M}$

$[\text{Ácido 1,3-difosfoglicérico }] = 10^{-5}\text{ M}$

$[\text{Fosfato inorgânico}] = [Pi] = 0{,}01\text{ M}$

Qual deverá ser o valor da razão $[NAD^+]/[NADH]$ de modo a que a reacção se processe espontaneamente da esquerda para a direita?

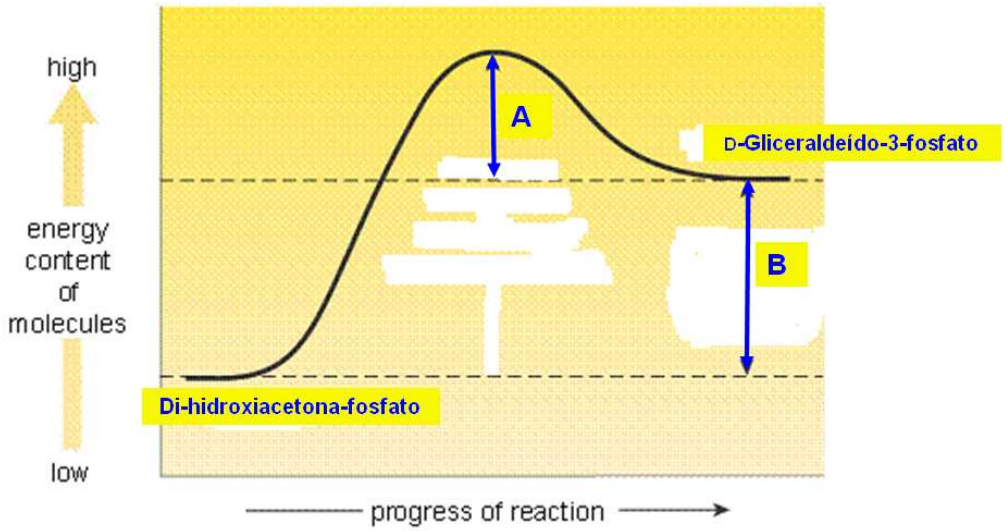
R: $[NAD^+]/[NADH] > 125{,}94$

Questões:

1. a) Defina metabolismo, catabolismo e anabolismo.
b) Indique o nome das três principais enzimas responsáveis pela regulação da glicólise.
c) Explique porque razão o genoma é um conceito estático, enquanto o proteoma é um conceito dinâmico.
d) Dê exemplo de uma via metabólica linear e de uma via metabólica cíclica.

2. a) Defina transcritoma, proteoma e metaboloma.
b) Indique o nome das três principais enzimas responsáveis pela regulação da glicólise.
c) Qual o nome dos dois mecanismos que permitem às células transferir os electrões do NADH citoplasmático para o interior dos mitocôndrios?
d) Dê dois exemplos de metabolitos primários e de dois metabolitos secundários.

3. Considere a seguinte reacção da glicólise:
a) Qual o nome da enzima que catalisa esta reacção?
b) Identifique as grandezas representadas por A e por B. Em que unidades se expressam?
c) Qual dos compostos tem maior nível de energia?
d) Tal como a reacção está escrita, diga, justificando, se $\Delta G^{0'}$ é menor, igual ou maior que zero e se K_{eq} é menor, igual ou superior a 1?
e) Em que sentido funciona, na glicólise?
f) Sabendo que as reacções do metabolismo funcionam espontaneamente, isto é, com $\Delta G' < 0$, explique detalhadamente como é possível a sua resposta à alínea e)?



4. Considere a seguinte reacção da glicólise:

a) Qual o nome da enzima que catalisa esta reacção parcial?

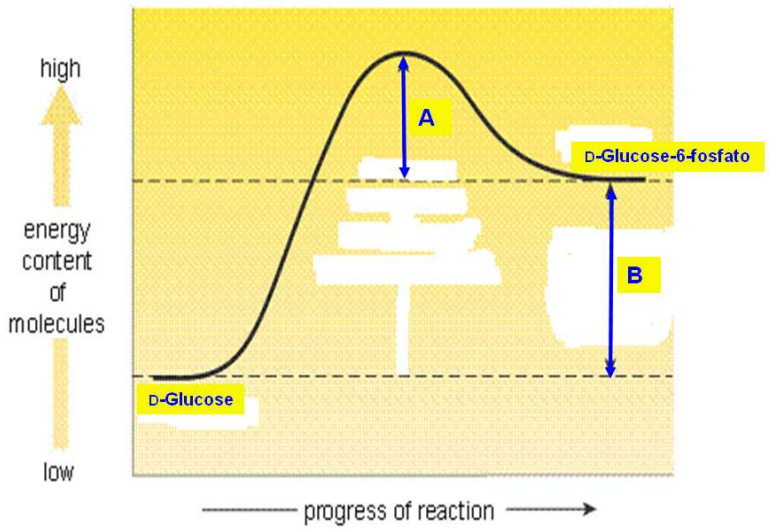
b) Identifique as grandezas representadas por A e por B. Em que unidades se expressam?

c) Qual dos compostos tem maior nível de energia?

d) Tal como a reacção está escrita, diga, justificando, se $\Delta G^{0'}$ é menor, igual ou maior que zero e se K_{eq} é menor, igual ou superior a 1?

e) Em que sentido funciona, na glicólise?

f) Sabendo que as reacções do metabolismo funcionam espontaneamente, isto é, com $\Delta G' < 0$, explique detalhadamente como é possível a sua resposta a e)?



5. Considere a fermentação alcoólica.

a) Escreva as reacções e dê os nomes das enzimas que as catalisam.

b) Qual a função fisiológica da fermentação alcoólica?

c) Descreva uma situação que ocorre frequentemente com as plantas onde decorre a fermentação alcoólica.

d) Descreva uma situação bem conhecida de aproveitamento pelo homem da fermentação alcoólica.

e) Porque razão se deve oxigenar uma suspensão de leveduras quando se pretende fazer um *starter*?

6. Considere a fermentação láctica.

a) Escreva a reacção e dê o nome da enzima que a catalisa.

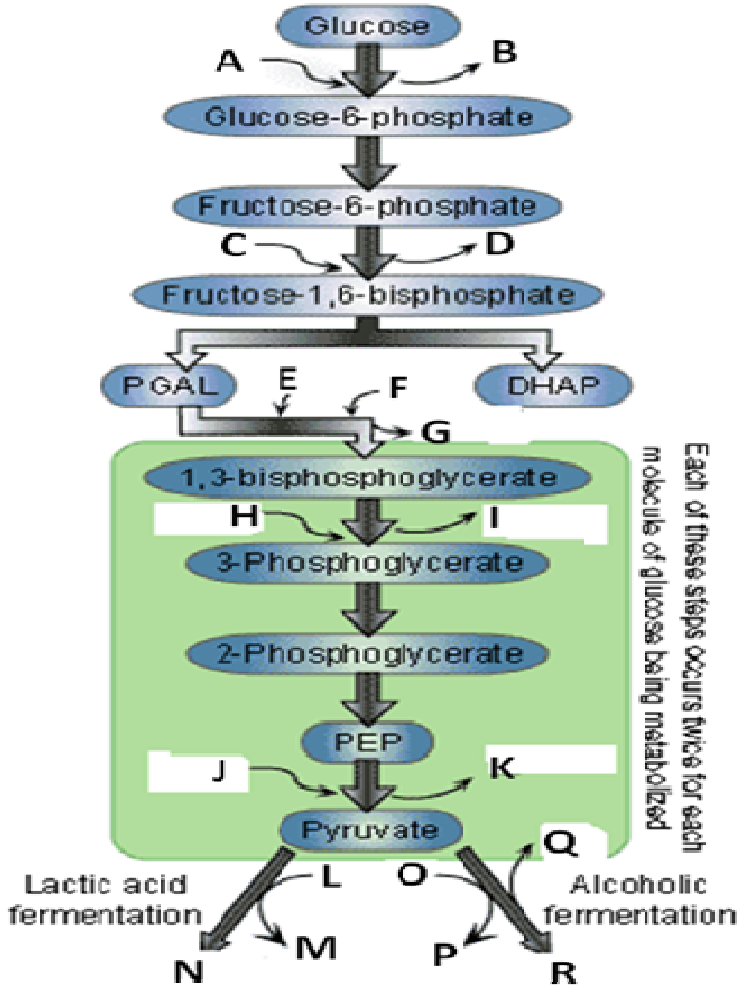
b) Qual a função fisiológica da fermentação láctica?

c) Descreva uma situação no corpo humano onde decorre a fermentação láctica.

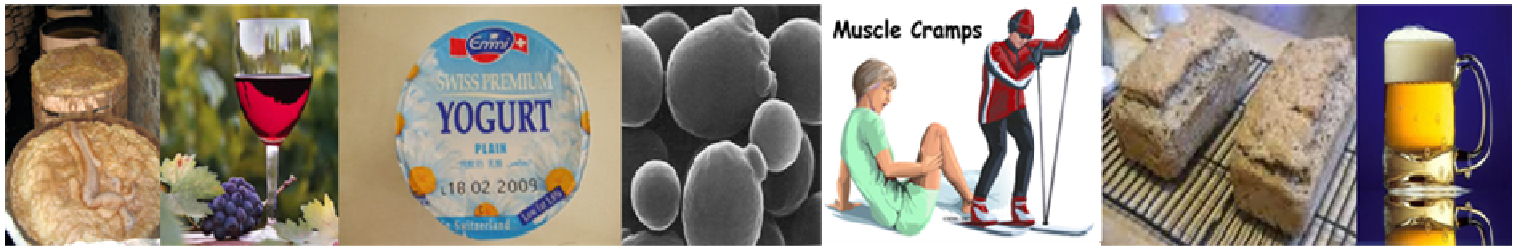
d) Descreva uma situação bem conhecida de aproveitamento pelo homem da fermentação láctica.

e) Indique a razão da alteração da consistência na resposta à alínea anterior.

7. Considere as três vias metabólicas representadas na figura seguinte – a via principal e as duas laterais:



S T U V W Y Z



a) Identifique essas vias metabólicas.
b) Classifique essas vias metabólicas quanto à
    (i) Forma.
    (ii) Função (anabólica, catabólica ou outra).
    (iii) Condições de funcionamento (normoxia, hipoxia ou anoxia).
c) Quais os quatro principais produtos da via metabólica principal?
d) Qual a função fisiológica das duas vias laterais?
e) Qual o significado das letras **A** a **R** na via principal?

A –
B –
C –
D –
E –
F –
G –
H –
I –
J –
K -
L –
M –
N –
O –
P –
Q –
R –

f) Qual o nome da enzima que catalisa a interconversão reversível entre P GAL e DHAP?
g) Espera que esta reacção decorra com uma variação grande de energia livre padrão? Justifique.
h) Sabendo que o valor de $\Delta G^{o'} = +7{,}56$ kJ.mol^{-1}, explique como é possível esta reacção funcionar de um modo espontâneo nas condições existentes nas células.

i) Identifique cada um dos processos representados pelas letras **S** a **Z.**

S –
T –
U –
V –
W –
Y –
Z –

j) Relacione cada um dos processos representados pelas letras **S** a **Z** com uma ou mais das três vias metabólicas referidas.

S –
T –
U –
V –
W –
Y –
Z –

k) Sabendo que uma das vias laterais tem dois produtos (**M** e **N**) e a outra tem três (**P**, **Q** e **R**) , indique quais são os produtos de interesse e os subprodutos (sem interesse) em cada um dos processos representados pelas letras **S** a **Z.**

S –
T –
U –
V –
W –
Y –
Z –

FIM