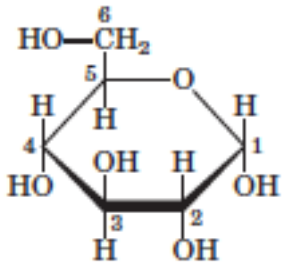
Glicólise, fermentação e ciclo de Krebs

A glicose é uma boa "escolha" como substrato metabólico:



1. Sua oxidação completa libera uma quantidade de energia equivalente a 2.870 kJ/mol;
2. Polímeros de glicose, que armazenam grandes quantidades de energia, podem ser estocados no interior das células sem mudanças significativas na osmolaridade celular;
3. É um intermediário metabólico muito versátil, capaz de suprir esqueletos carbônicos para biossíntese de aminoácidos, nucleotídeos, coenzimas e ácidos graxos (Ex: vários microrganismos conseguem sintetizar todos os 20 aminoácidos à partir de glicose e uma fonte de NH_4^+;
4. O metabolismo de glicose converge as vias catabólicas de outros substratos energéticos, como aminoácidos;
5. A glicose foi uma "escolha" primária na evolução de vias metabólicas, pois a maioria absoluta de organismos celulares metaboliza glicose para a obtenção de energia;
6. Organismos que não têm acesso a glicose de outras fontes evoluíram vias metabólicas para sintetiza-la, como a fotossíntese em plantas (glicose a partir de CO2) ou a gliconeogênese (glicose a partir de precursores de 3 e 4 carbonos) em células não fotossintéticas.

Os principais destinos metabólicos da glicose são vias oxidativas

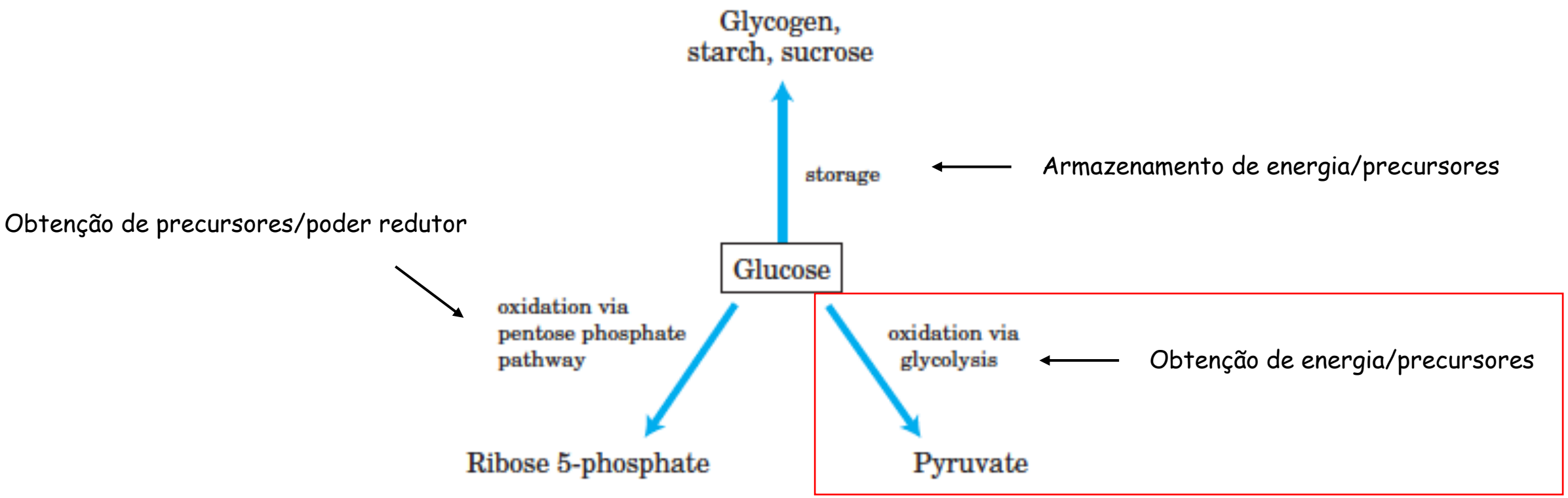


FIGURE 14–1 Major pathways of glucose utilization. Although not the only possible fates for glucose, these three pathways are the most significant in terms of the amount of glucose that flows through them in most cells.

O destino metabólico do piruvato depende da presença de O_2 e do tipo celular

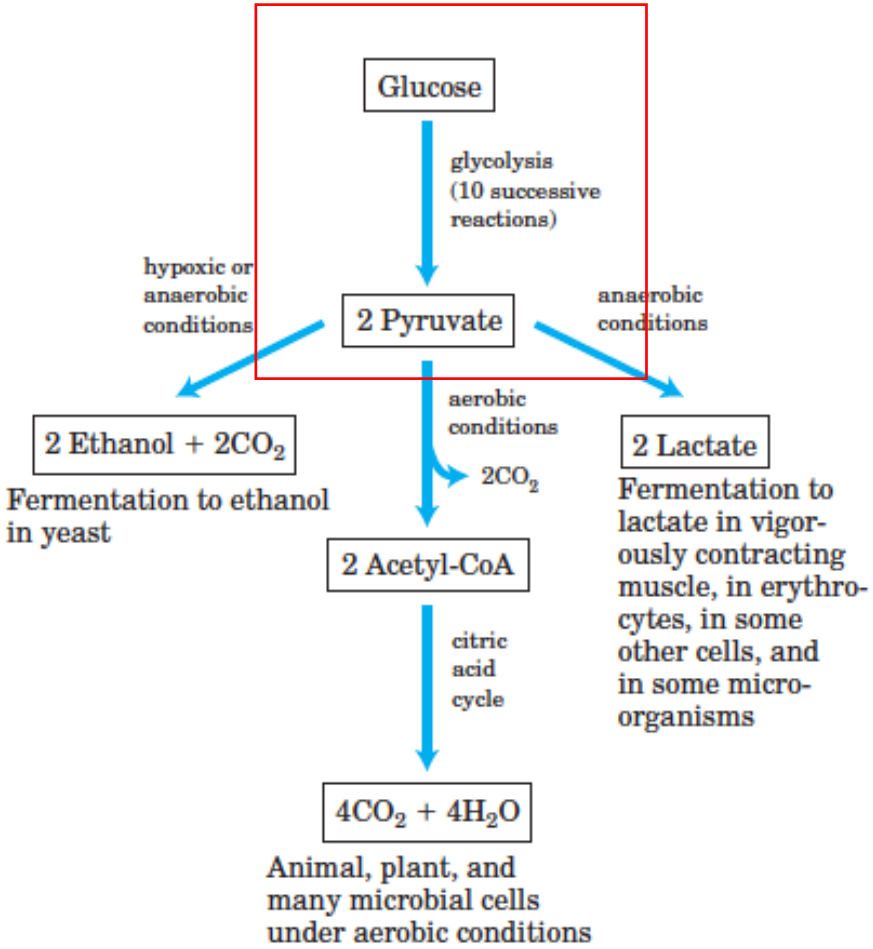
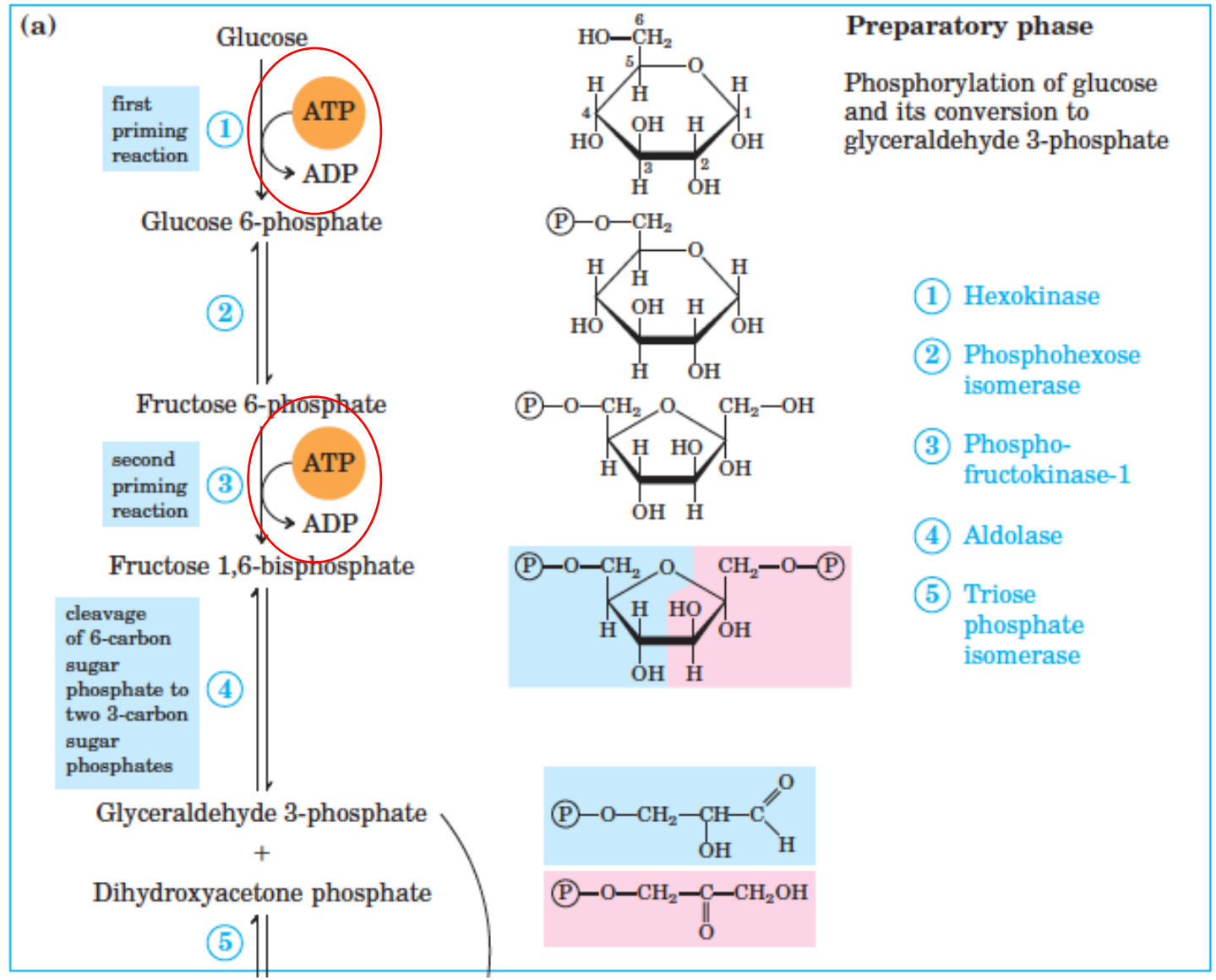
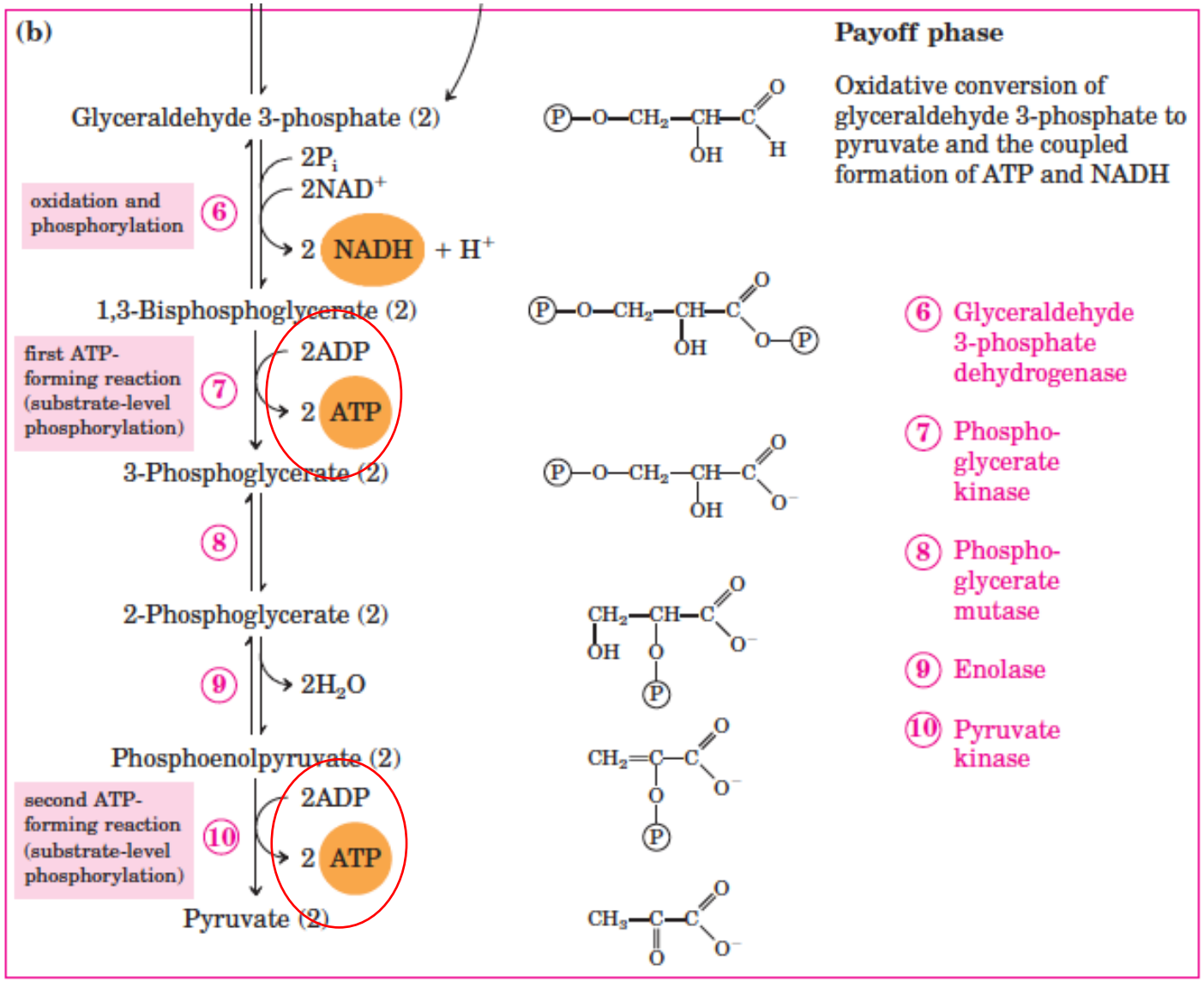


FIGURE 14–3 Three possible catabolic fates of the pyruvate formed in glycolysis. Pyruvate also serves as a precursor in many anabolic reactions, not shown here.

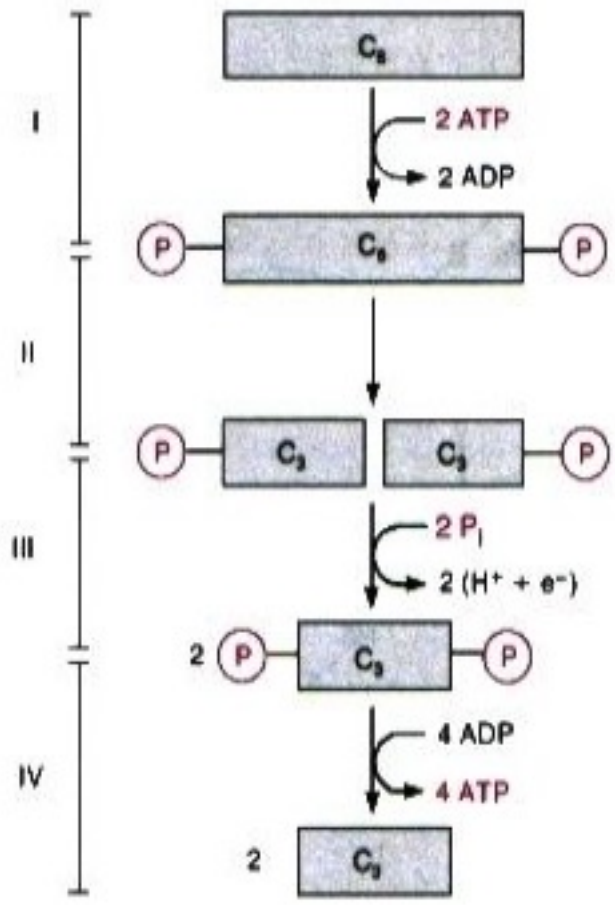
A glicólise é dividida em duas fases: uma preparatória, que consome ATP



E uma segunda fase, que produz ATP



Esquema geral:



$$\text{Glicose} + 2ATP + 2P_i + 2NAD^+ + 2ADP \longrightarrow 2NADH + 4ATP + 2H_2O + 2\ \text{piruvato}$$

Rendimento líquido: 2 moles de ATP/mol de glicose

Note que além da glicose, todos os outros intermediários são fosfatados:

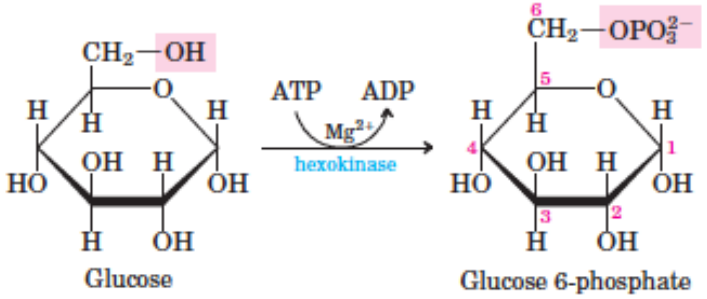
-a membrana plasmática não tem transportadores para açucares fosfatados; assim a retenção dessas moléculas dentro da célula é facilitada

-grupos fosfato são componentes essenciais dos mecanismos enzimáticos de conservação de energia, via reações acopladas de transferência de grupos fosfato

-a energia de ligação resultante da ligação de grupos fosfato aos sítios ativos das enzimas diminui a energia de ativação e aumenta a especificidade da reação enzimática.

Fase preparativa:

1. Fosforilação da glicose



$\Delta G'^{\circ} = -16.7$ kJ/mol

- Hexoquinase é expressa em todas as células de todos os organismos
- Em mamíferos, o fígado expressa uma forma alternativa dessa enzima, chamada glicoquinase, que tem propriedades cinéticas e de regulação distintas

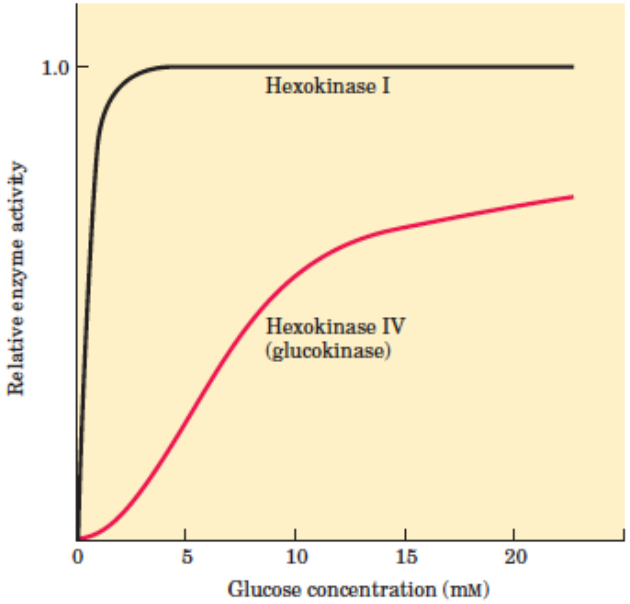
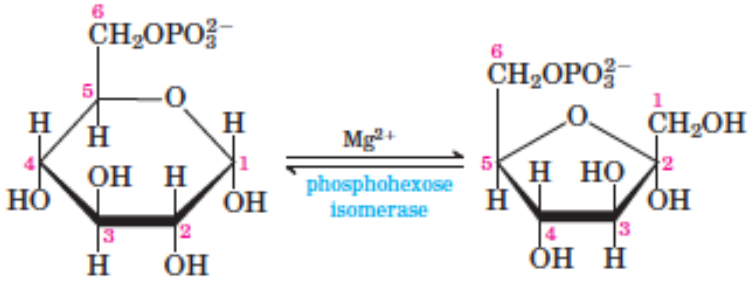


FIGURE 15-16 Comparison of the kinetic properties of hexokinase IV (glucokinase) and hexokinase I. Note the sigmoidicity for hexokinase IV and the much lower K_m for hexokinase I. When blood glucose rises above 5 mM, hexokinase IV activity increases, but hexokinase I is already operating near V_{max} at 5 mM glucose and cannot respond to an increase in glucose concentration. Hexokinase I, II, and III have similar kinetic properties.

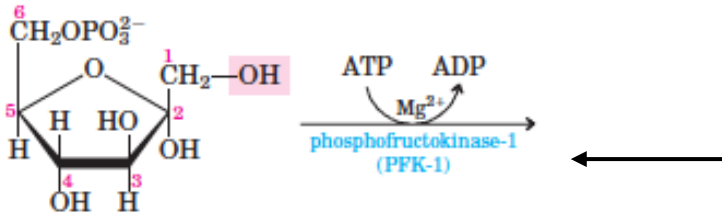
2. Isomerização a frutose 6-fosfato



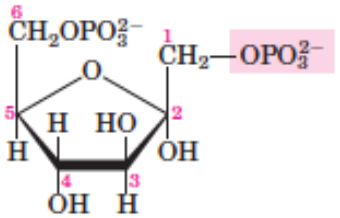
Glucose 6-phosphate — Fructose 6-phosphate

$\Delta G'^{\circ} = 1.7$ kJ/mol

3. Fosforilação de frutose 6-fosfato a frutose 1,6-bisfosfato



Fructose 6-phosphate



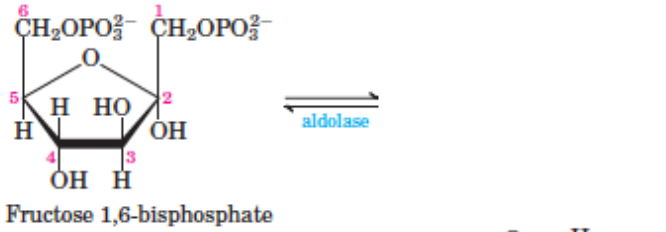
Fructose 1,6-bisphosphate

$\Delta G'^{\circ} = -14.2$ kJ/mol

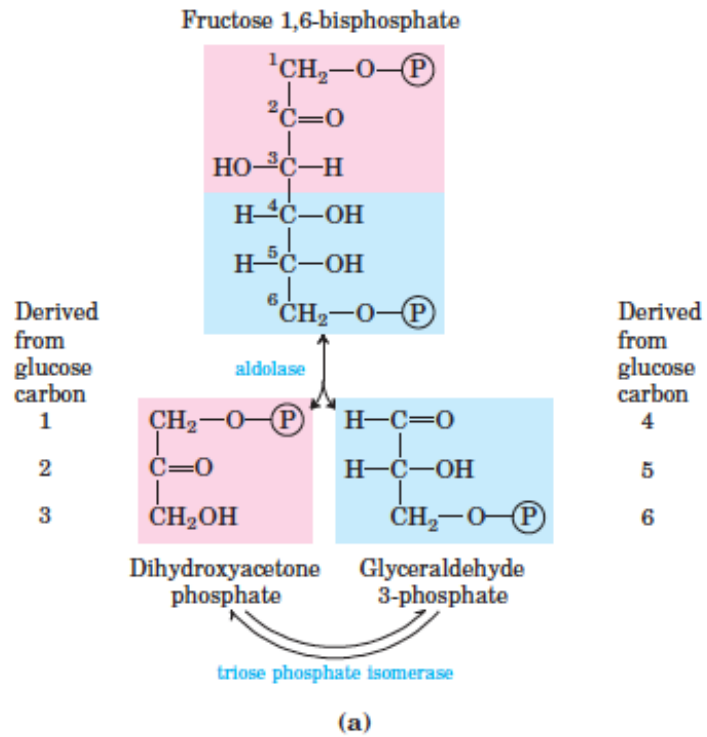
PFK-1 é uma enzima regulatória; principal ponto de regulação da via glicolítica

*Primeira reação de comprometimento

4. Clivagem da frutose 1,6-bisfosfato

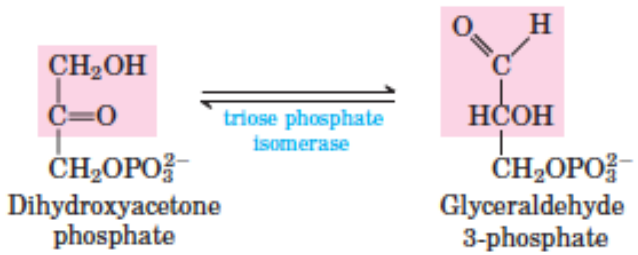


$\Delta G'^{\circ} = 23.8$ kJ/mol



(a)

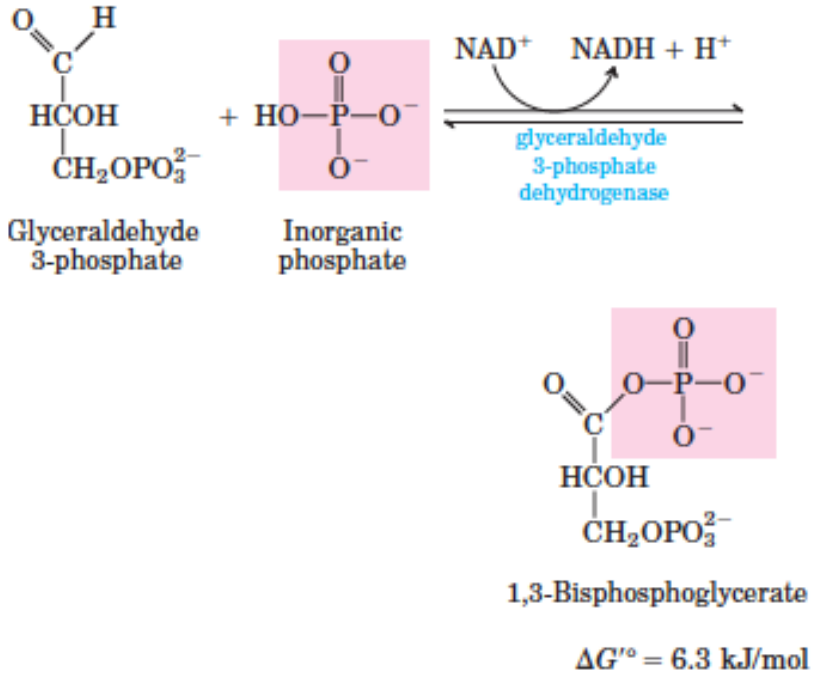
5. Interconversão das triose-fosfatos



$\Delta G'^{\circ} = 7.5$ kJ/mol

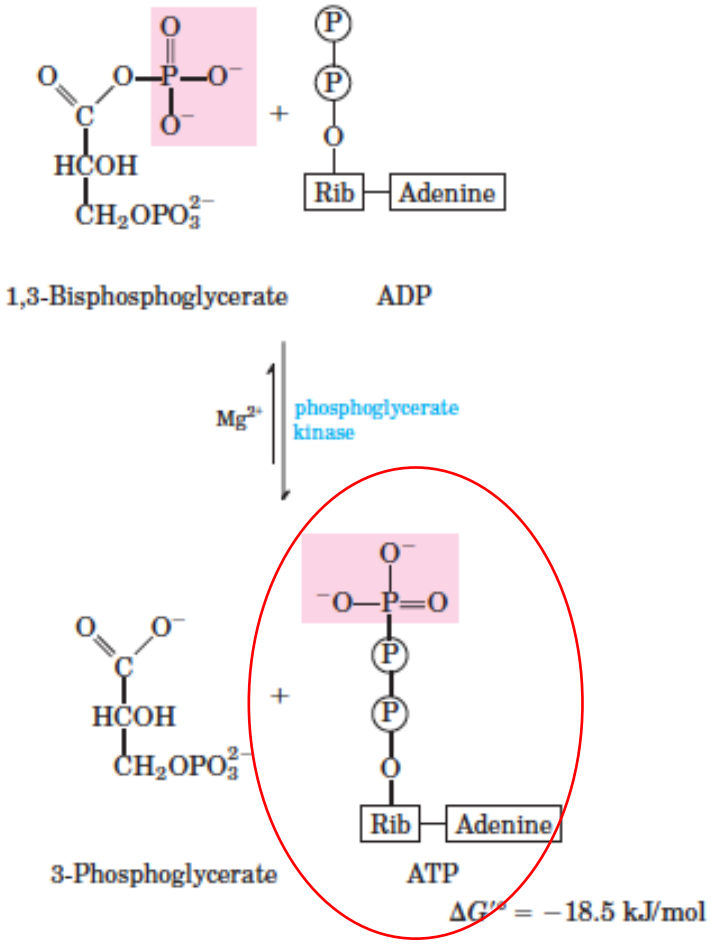
Fase de *payoff:*

6. Oxidação de gliceraldeido 3-fosfato a 1,3-bisfosfoglicerato

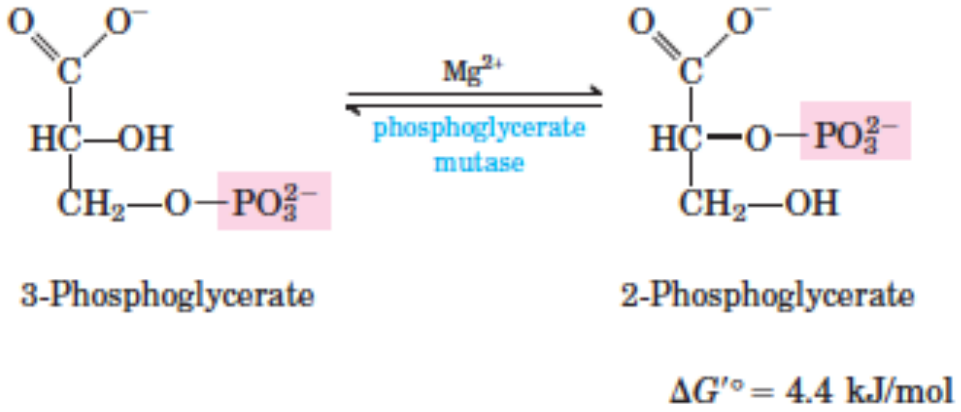


- Primeira reação de conservação de energia, que eventualmente resulta na produção de ATP
- Consome NAD^+

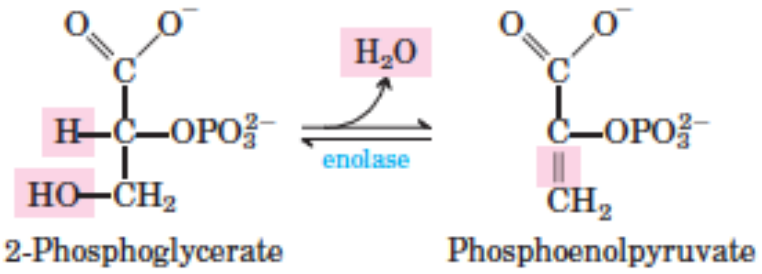
7. Transferência de fosforil de 1,3-bisfosfoglicerato para ADP



8. Conversão de 3-fosfoglicerato a 2-fosfoglicerato

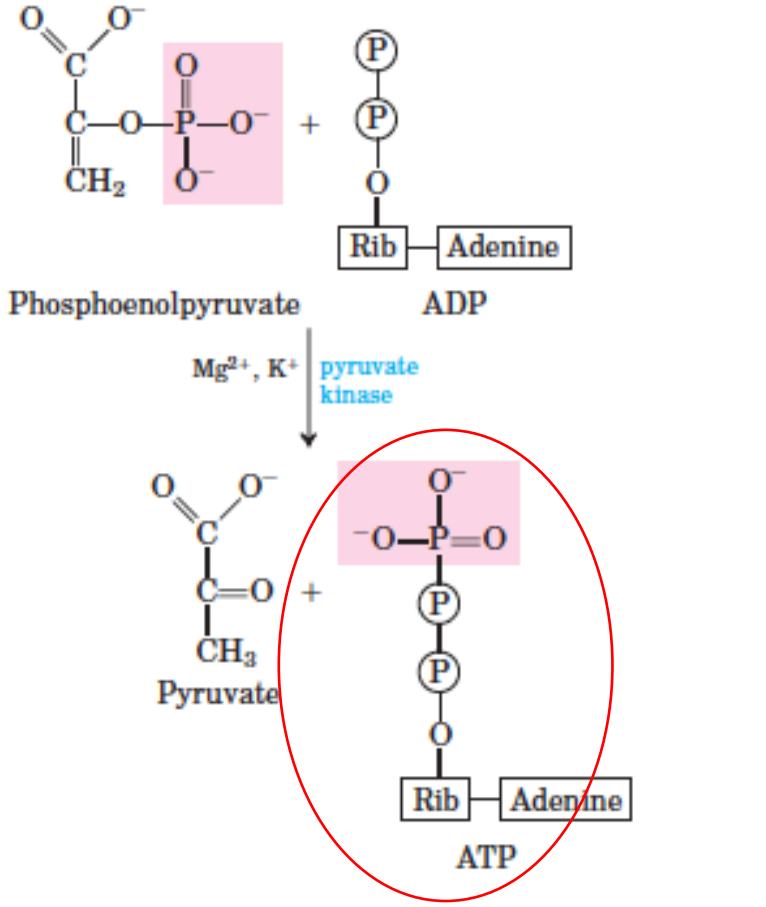


9. Desidratação de 2-fosfoglicerato a fosfoenolpiruvato



$\Delta G'^{\circ} = 7.5$ kJ/mol

10. Conversão de fosfoenolpiruvato em piruvato



$\Delta G'^{\circ} = -31.4$ kJ/mol

Outros açucares também entram na via glicolítica

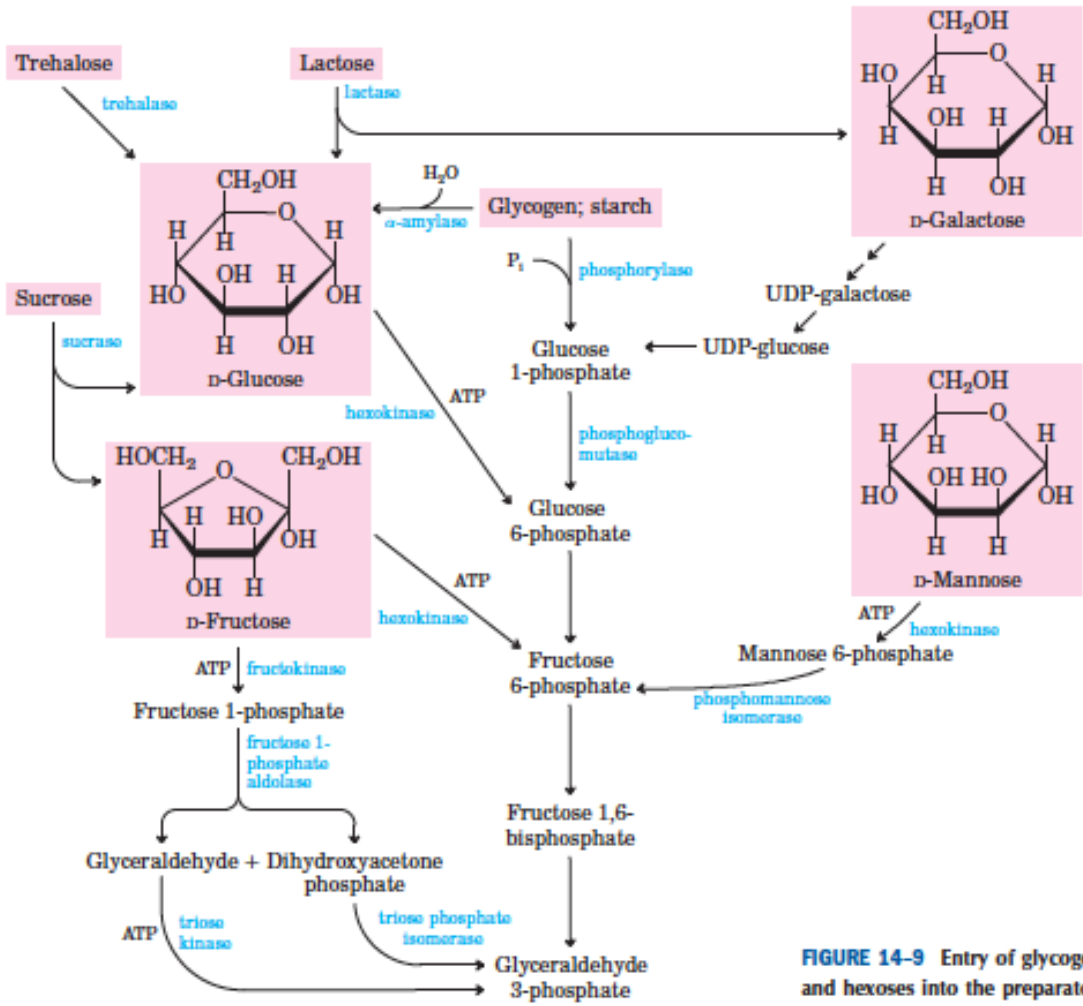


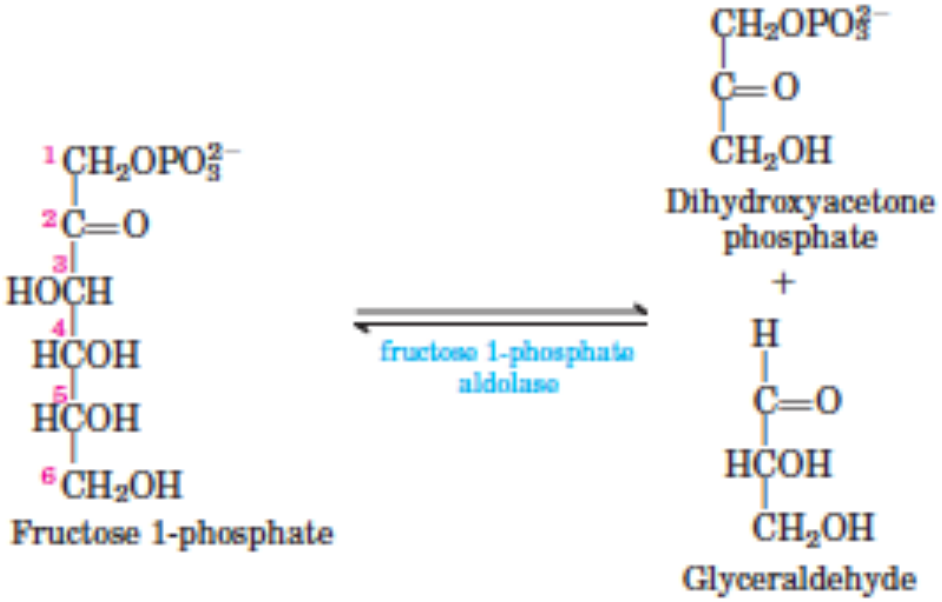
FIGURE 14–9 Entry of glycogen, starch, disaccharides, and hexoses into the preparatory stage of glycolysis.

Metabolismo de frutose no fígado é distinto de outros tecidos:

Frutoquinase, fígado-específica

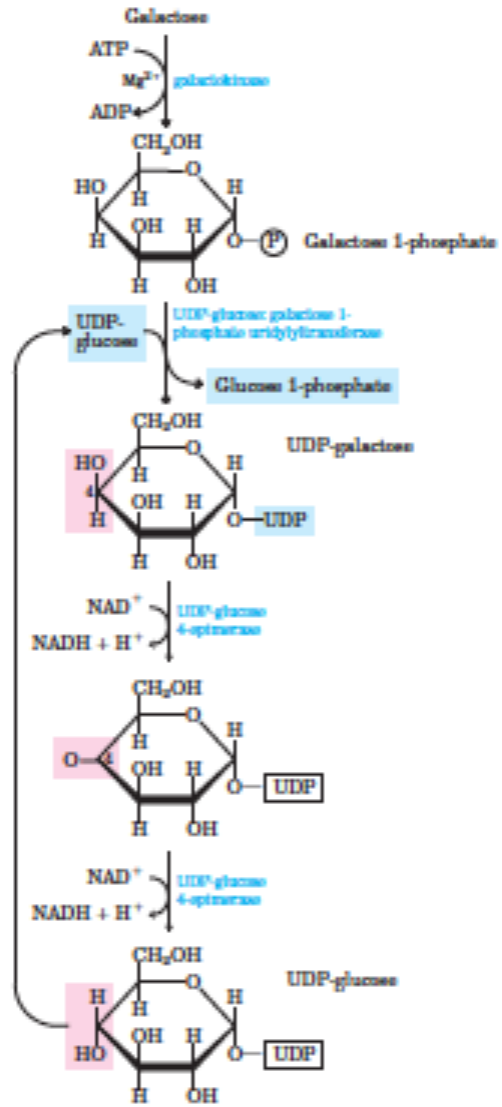
$$\text{Fructose} + \text{ATP} \xrightarrow{Mg^{2+}} \text{fructose 1-phosphate} + \text{ADP}$$

The fructose 1-phosphate is then cleaved to glyceraldehyde and dihydroxyacetone phosphate by **fructose 1-phosphate aldolase:**



$$\text{Glyceraldehyde} + \text{ATP} \xrightarrow{Mg^{2+}} \text{glyceraldehyde 3-phosphate} + \text{ADP}$$

Galactose requer uma via específica para entrar na via glicolítica



Destinos do NADH produzido na glicólise

Em aerobiose: $$2NADH + 2H^+ + O_2 \longrightarrow 2NAD^+ + 2H_2O$$

Na fosforilação oxidativa

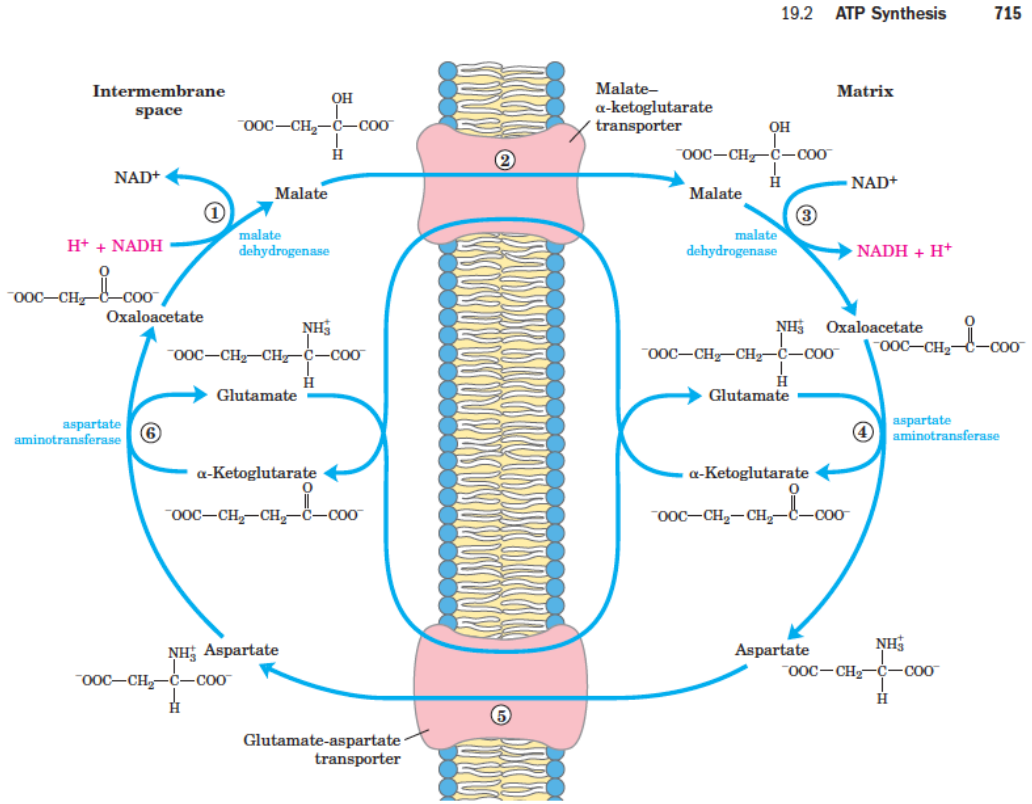
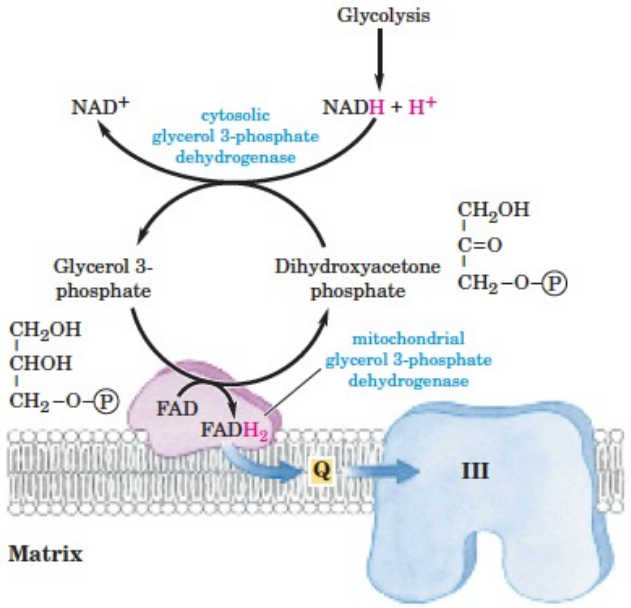


FIGURE 19–27 Malate-aspartate shuttle. This shuttle for transporting two reducing equivalents to NAD^+, and the resulting NADH is oxi-



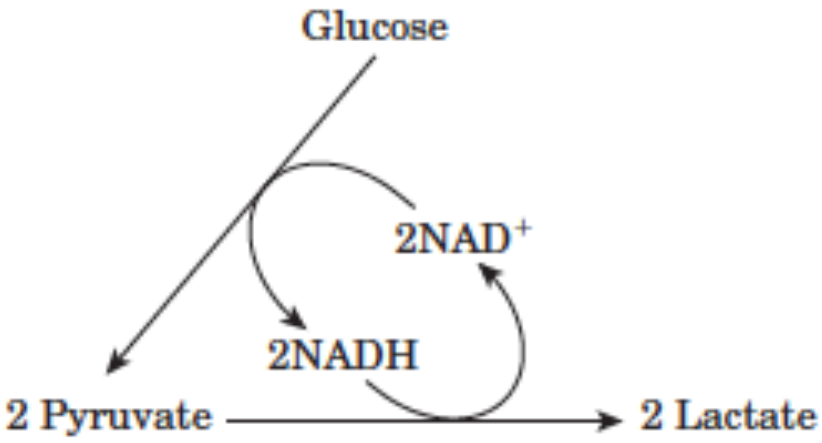
Destinos do NADH produzido na glicólise

Em aerobiose:

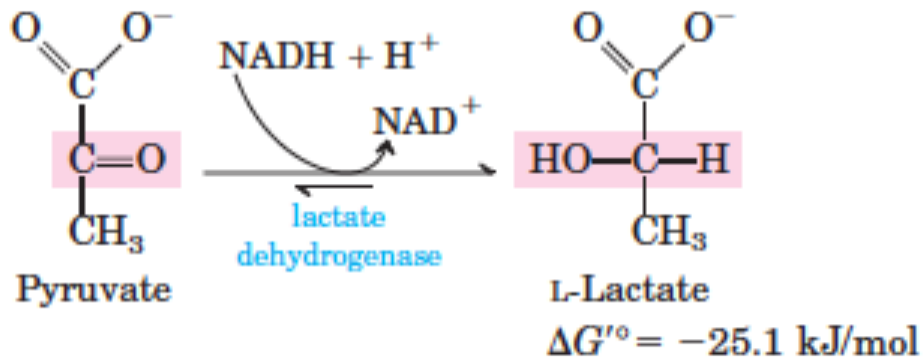
$$2NADH + 2H^+ + O_2 \longrightarrow 2NAD^+ + 2H_2O$$

Na fosforilação oxidativa

E em anaerobiose?

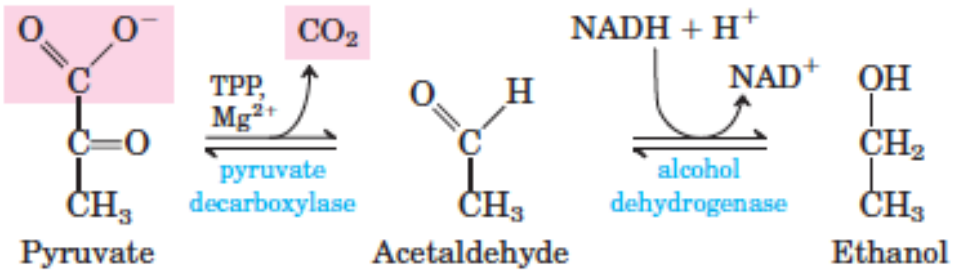


Redução de piruvato a lactato (fermentação láctica):



Equilíbrio favorece a formação de lactato

Fermentação alcoólica:

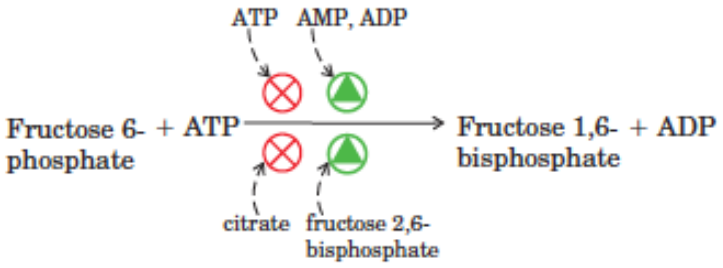


Presente em muitos organismos que metabolizam etanol, inclusive humanos. Responsável pela metabolização de etanol consumido, ou produzido por microrganismos comensais

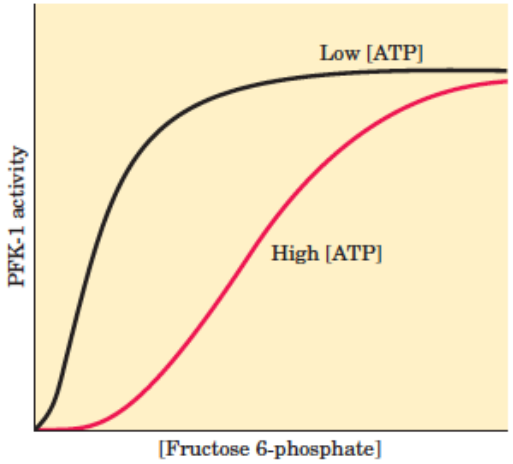
Regulação da via glicolítica

1. Os passos regulatórios são, em geral, os passos irreversíveis
2. Todos os passos regulatórios são catalisados por enzimas alostéricas

Fosfofrutoquinase



(c)



(b)

TABLE 15–1 Relative Changes in [ATP] and [AMP] When ATP Is Consumed

Adenine nucleotide	*Concentration before ATP depletion (mM)*	*Concentration after ATP depletion (mM)*	*Relative change*
ATP	5.0	4.5	10%
ADP	1.0	1.0	0
AMP	0.1	0.6	**600%**

$$2\ ADP \longrightarrow AMP + ATP$$

Piruvato quinase

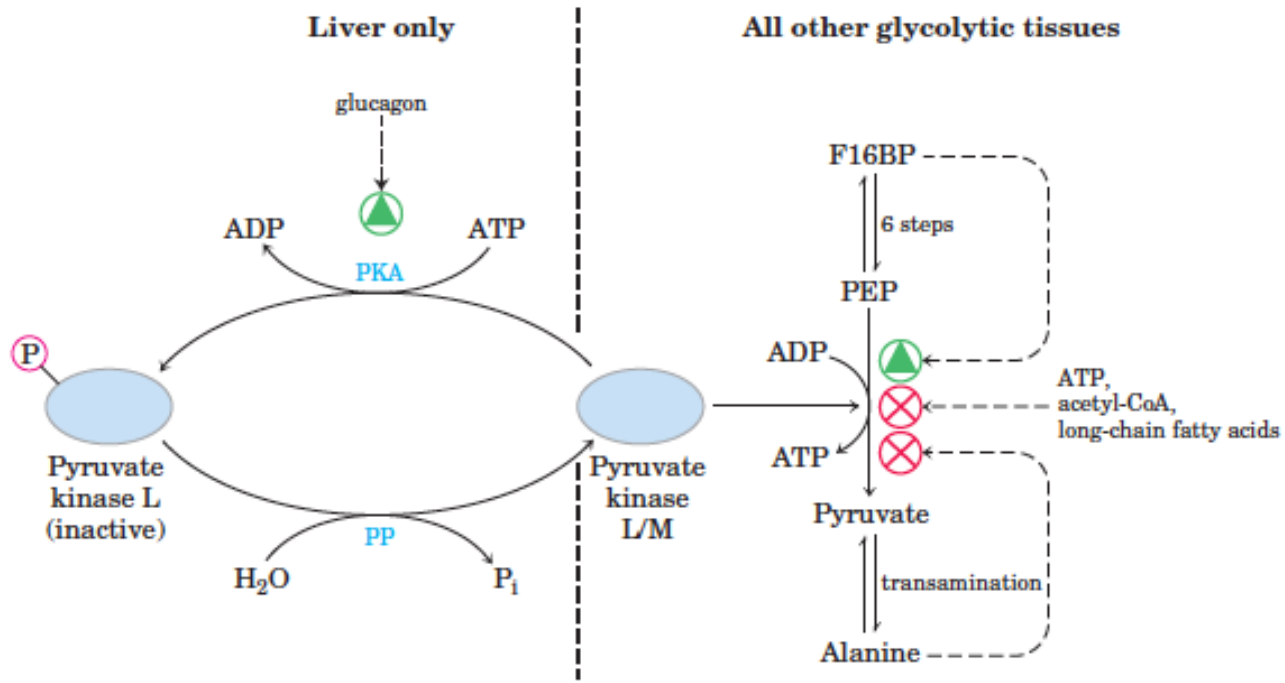
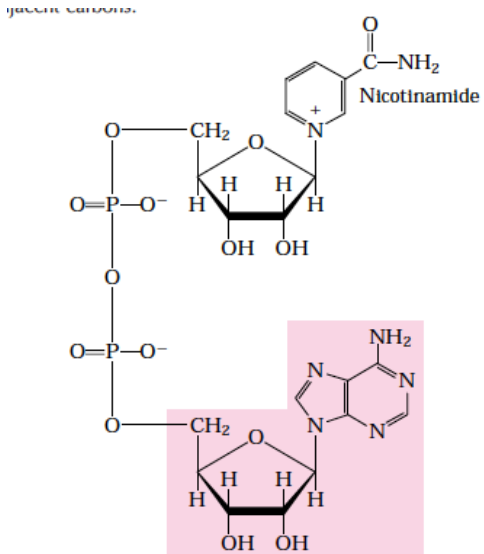
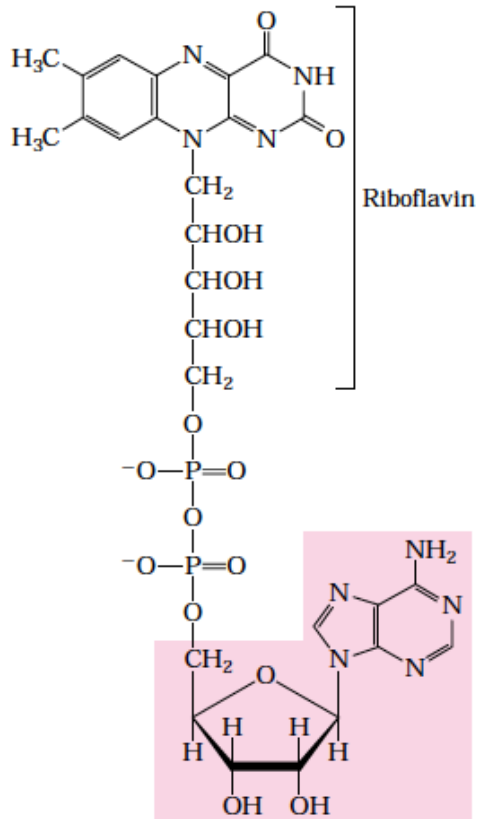


FIGURE 15–19 Regulation of pyruvate kinase. The enzyme is allosterically inhibited by ATP, acetyl-CoA, and long-chain fatty acids (all signs of an abundant energy supply), and the accumulation of fructose 1,6-bisphosphate triggers its activation. Accumulation of alanine, which can be synthesized from pyruvate in one step, allosterically inhibits pyruvate kinase, slowing the production of pyruvate by glycolysis. The liver isozyme (L form) is also regulated hormonally; glucagon activates cAMP-dependent protein kinase (PKA; see Fig. 15–25), which phosphorylates the pyruvate kinase L isozyme, inactivating it. When the glucagon level drops, a protein phosphatase (PP) dephosphorylates pyruvate kinase, activating it. This mechanism prevents the liver from consuming glucose by glycolysis when the blood glucose concentration is low; instead, liver exports glucose. The muscle isozyme (M form) is not affected by this phosphorylation mechanism.

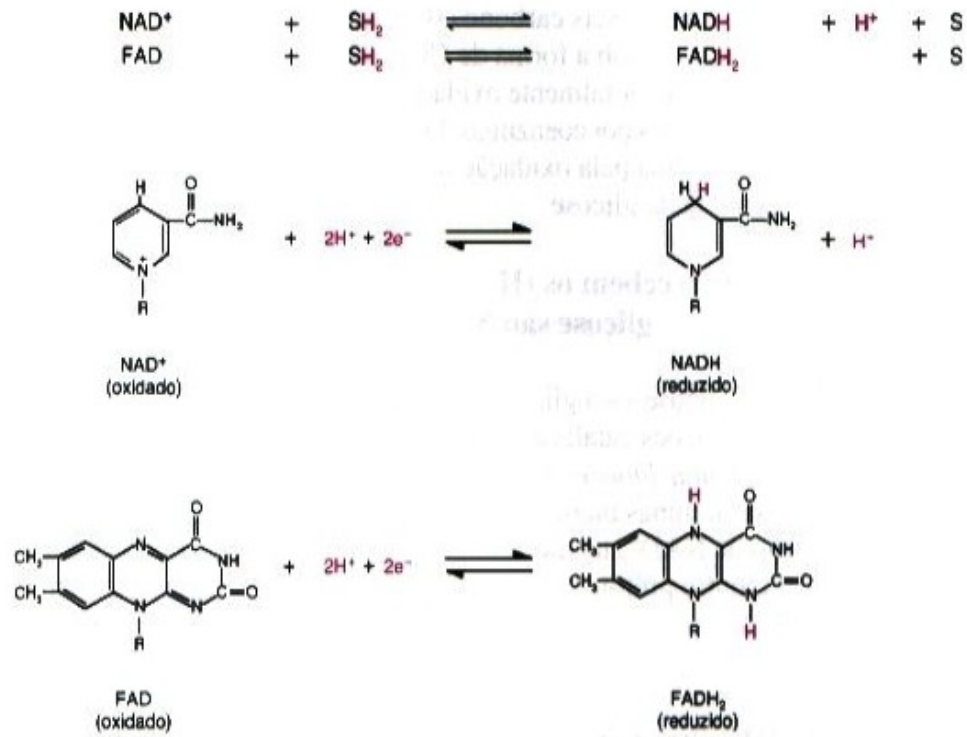
Durante a degradação oxidativa da glicose, os elétrons são transferidos para coenzimas



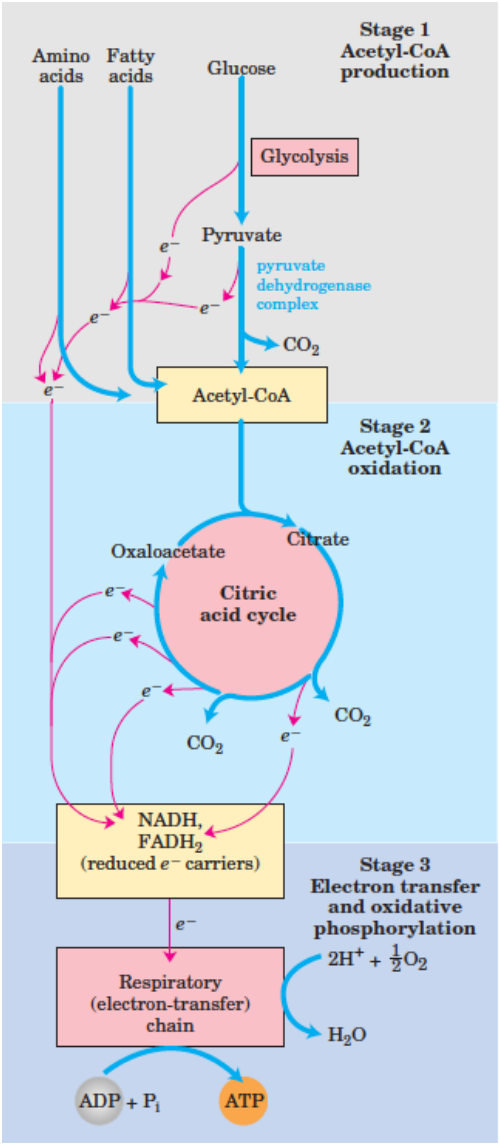
Nicotinamide adenine dinucleotide (NAD^+)



Flavin adenine dinucleotide (FAD)



O ciclo de Krebs integra o metabolismo degradativo dos principais componentes da dieta



(Photograph courtesy of Gil Hardy, Oxford Brookes University, UK.)

Fonte: https://www.nature.com/articles/35043073

A oxidação completa de glicose no ciclo de Krebs permite a obtenção de quantidade maior de ATP

Glicólise

$$\text{Glucose} + 2NAD^+ + 2ADP + 2P_i \longrightarrow 2\ \text{pyruvate} + 2NADH + 2H^+ + 2ATP + 2H_2O$$

Glicólise + Ciclo de Krebs + Fosforilação oxidativa

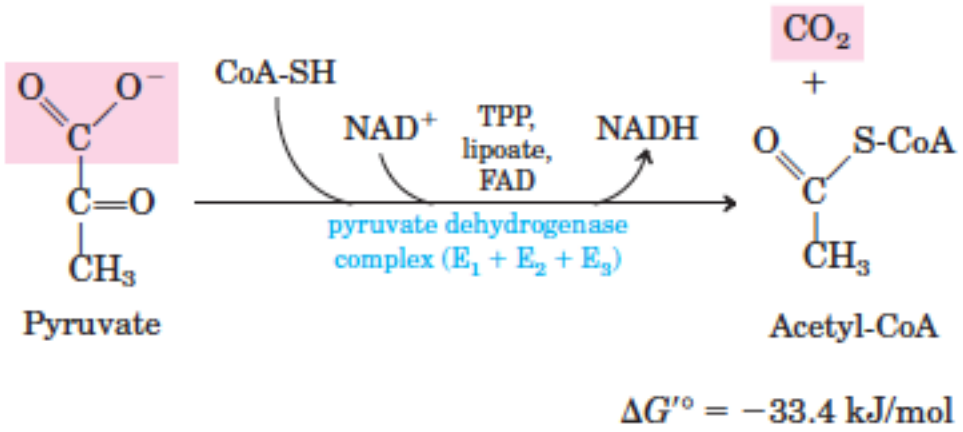
$$C_6H_{12}O_6 + 6\ O_2 + n\ ADP + n\ Pi \rightarrow 6\ CO_2 + 6\ H_2O + n\ ATP$$

n ~ 30 ou 32

(NADH como intermediário)

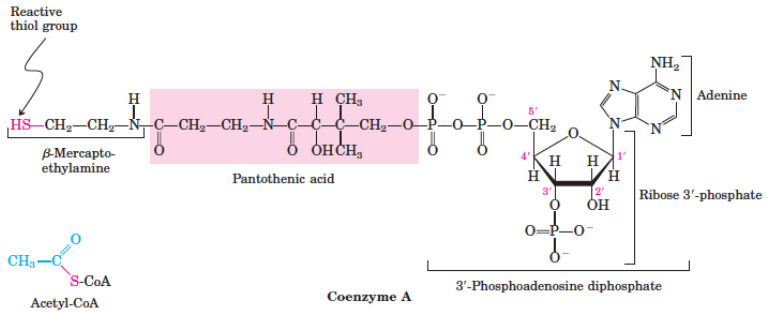
O piruvato entra no Ciclo de Krebs na forma de Acetil-CoA

Oriundo da degradação de glicose, outros carboidratos e alguns aminoácidos

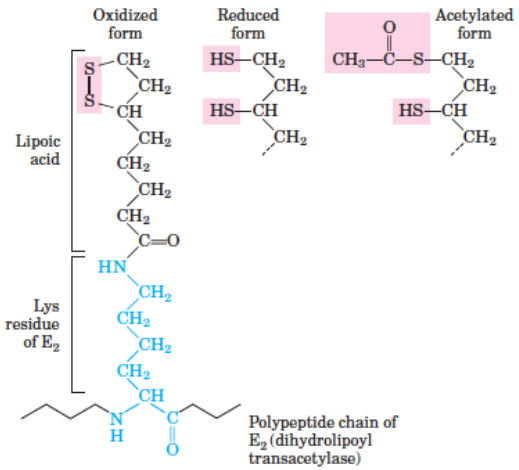


- A enzima PDH está localizada na matriz mitocondrial, onde ocorre a reação
- O piruvato é transportado para dentro da mitocôndria pelo transportador piruvato translocase
- A atividade de PDH é inibida quando as razões ATP/ADP e NADH/NAD^+ estão aumentadas

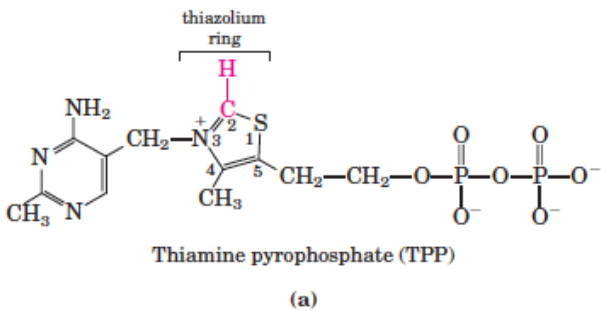
A atividade de PDH depende de 3 coenzimas, derivadas de vitaminas obtidas na dieta



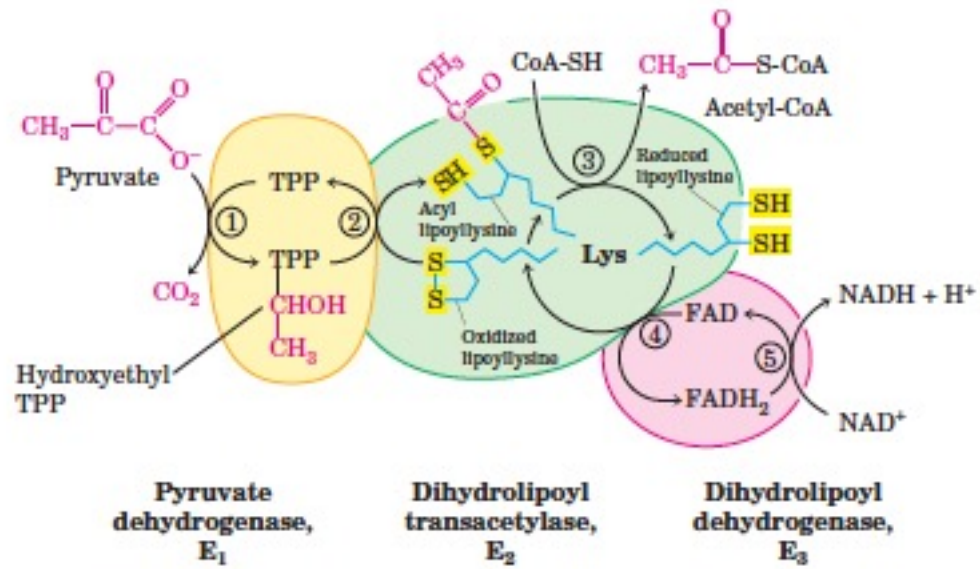
Ácido pantotênico ou vitamina B5



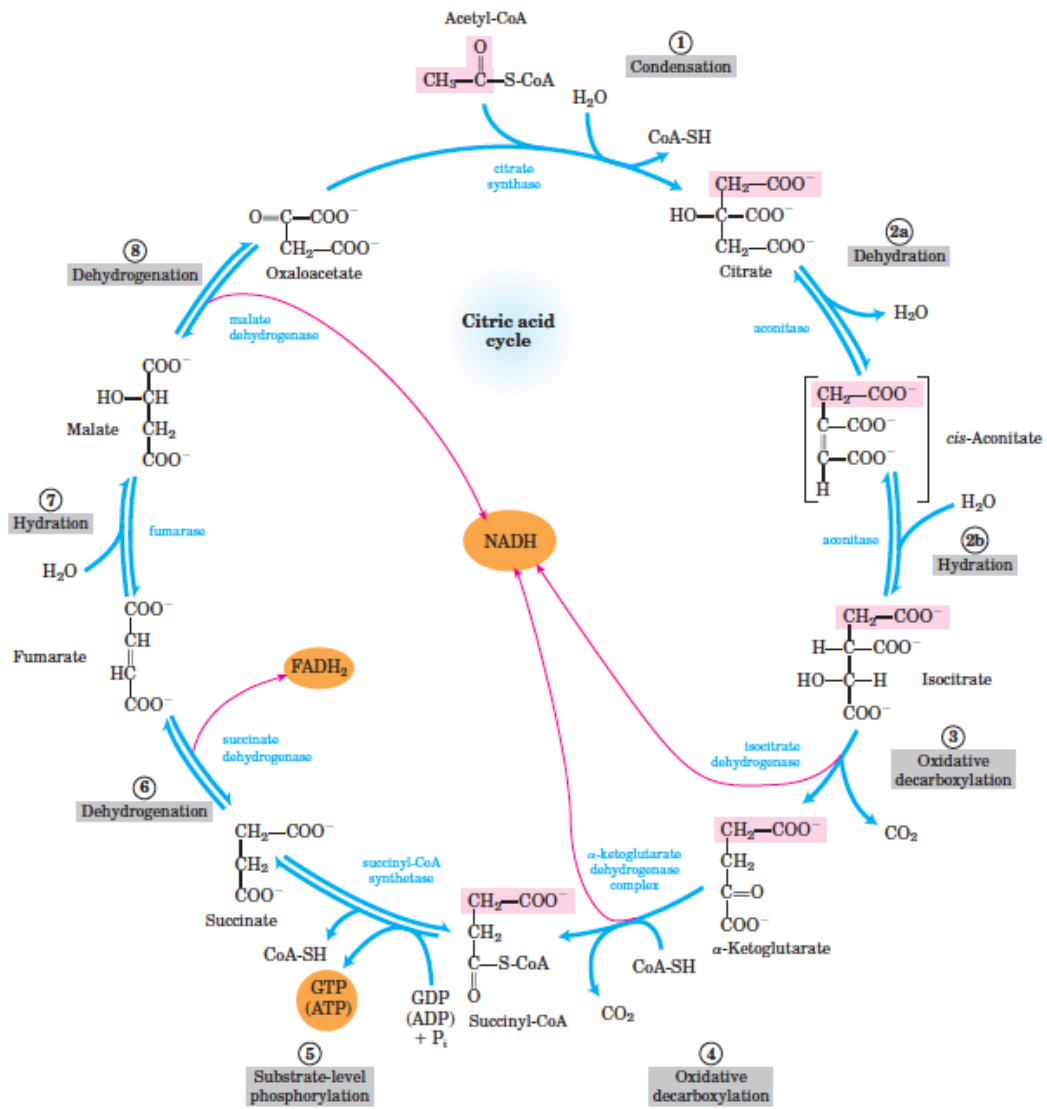
Ácido lipóico



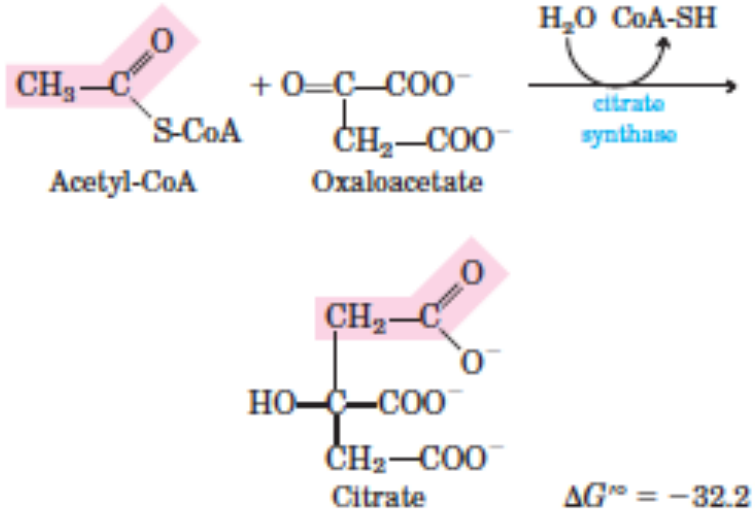
Tiamina ou vitamina B1



Reações do Ciclo de Krebs

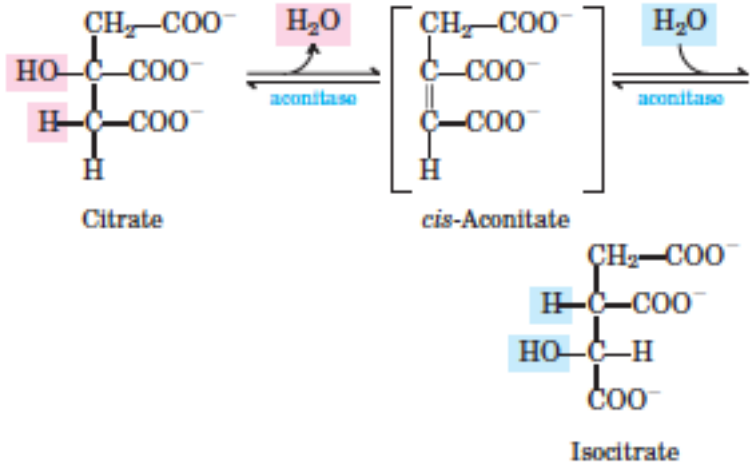


1 Formation of Citrate The first reaction of the cycle is the condensation of acetyl-CoA with **oxaloacetate** to form **citrate,** catalyzed by **citrate synthase:**



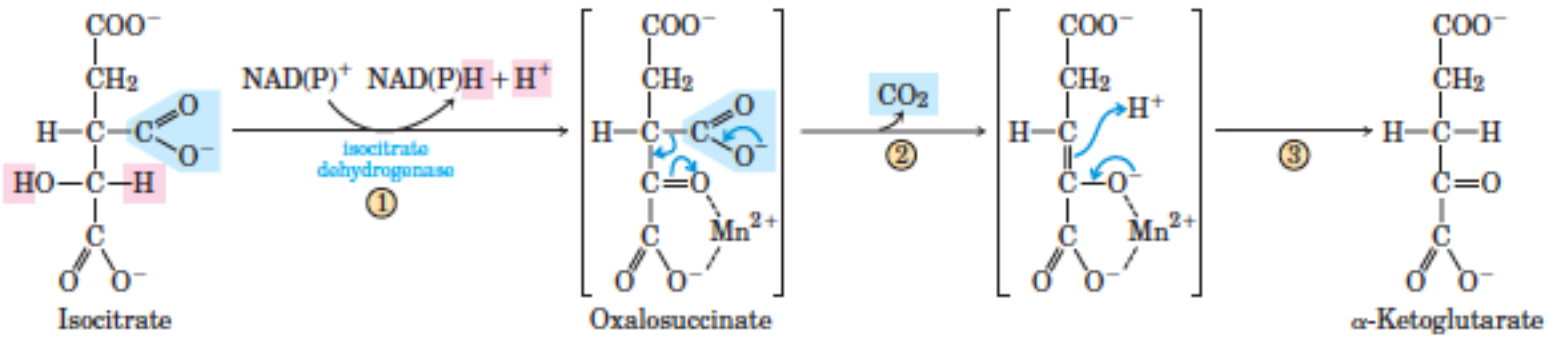
$\Delta G'^{\circ} = -32.2$ kJ/mol

2 Formation of Isocitrate via cis-Aconitate The enzyme **aconitase** (more formally, **aconitate hydratase**)

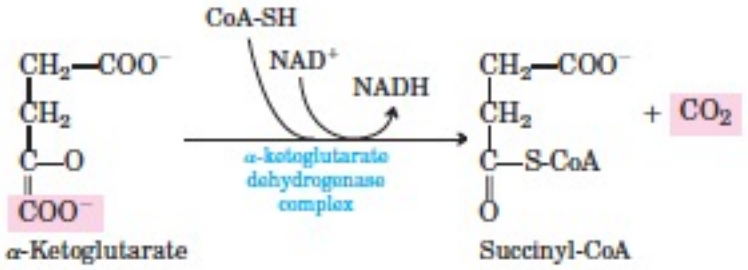


$\Delta G'^{\circ} = 13.3$ kJ/mol

③ ***Oxidation of Isocitrate to α-Ketoglutarate and CO_2*** In the next step, **isocitrate dehydrogenase** catalyzes oxidative decarboxylation of isocitrate to form **α-ketoglutarate** (Fig. 16–11). Mn^{2+} in the active site interacts

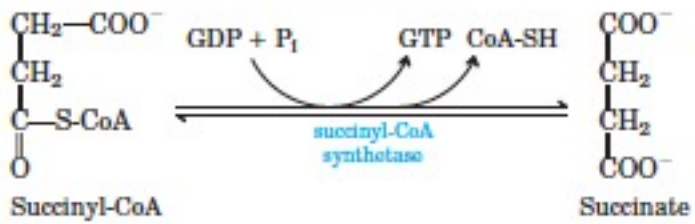


④ ***Oxidation of α-Ketoglutarate to Succinyl-CoA and CO_2*** The next step is another oxidative decarboxylation, in which α-ketoglutarate is converted to **succinyl-CoA** and CO_2 by the action of the **α-ketoglutarate dehydrogenase complex;** NAD^+ serves as electron accep-



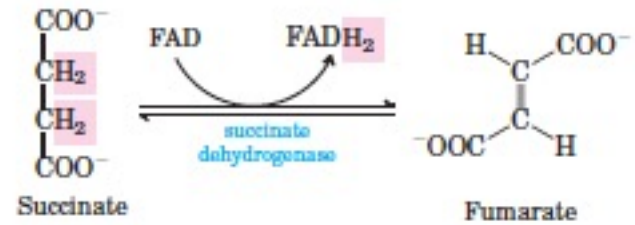
$\Delta G'^{\circ} = -33.5$ kJ/mol

⑤ *Conversion of Succinyl-CoA to Succinate* Succinyl-CoA, like acetyl-CoA, has a thioester bond with a strongly negative standard free energy of hydrolysis ($\Delta G'^{\circ} \approx -36$ kJ/mol). In the next step of the citric acid cycle, energy released in the breakage of this bond is used to drive the synthesis of a phosphoanhydride bond in either GTP or ATP, with a net $\Delta G'^{\circ}$ of only -2.9 kJ/mol. **Succinate** is formed in the process:



$\Delta G'^{\circ} = -2.9$ kJ/mol

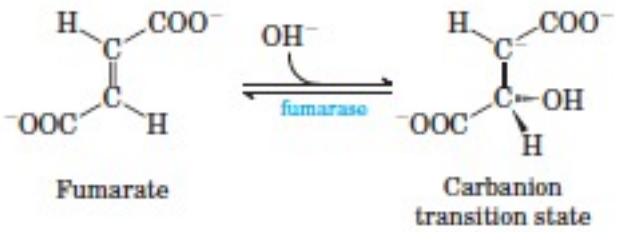
⑥ *Oxidation of Succinate to Fumarate* The succinate formed from succinyl-CoA is oxidized to **fumarate** by the flavoprotein **succinate dehydrogenase:**



$\Delta G'^{\circ} = 0$ kJ/mol

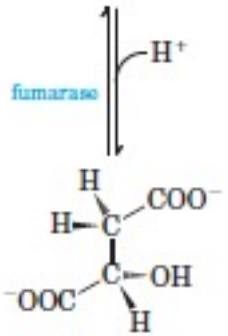
Esta reação acopla diretamente o Ciclo de Krebs à cadeia transportadora de elétrons, pois a enzima SDH está ancorada na membrana mitocondrial e transfere os elétrons do FADH2 para a coenzima Q. SDH também é conhecida como Complexo II da ETC

7 *Hydration of Fumarate to Malate* The reversible hydration of fumarate to **L-malate** is catalyzed by **fumarase**



Fumarate

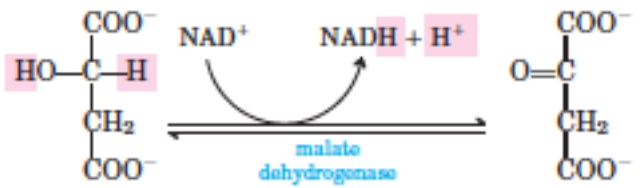
Carbanion transition state



Malate

$\Delta G'^{\circ} = -3.8$ kJ/mol

8 *Oxidation of Malate to Oxaloacetate* In the last reaction of the citric acid cycle, NAD-linked **L-malate dehydrogenase** catalyzes the oxidation of L-malate to oxaloacetate:



L-Malate

Oxaloacetate

$\Delta G'^{\circ} = 29.7$ kJ/mol

Conservação de energia na forma de ATP e coenzimas reduzidas

Note que:

- Para cada acetil-CoA que entra no Ciclo de Krebs, são produzidos 3 NADH, 1 $FADH_2$ e 1 ATP
- Assim, 1 molécula de glicose produz, no Ciclo de Krebs, 6NADH, 2 $FADH_2$ e 2 ATP
- Lembre também que a geração de acetil-CoA à partir de piruvato já produziu um NADH/piruvato
- Assim, para cada glicose são mais 2 NADH

Glicose $\xrightarrow[\text{2 ATP, 2 NADH}]{}$ 2 piruvato $\xrightarrow[\text{2 NADH}]{}$ 2 acetil-CoA $\xrightarrow[\text{2 ATP, 6 NADH, 2 } FADH_2]{}$ 6 CO_2

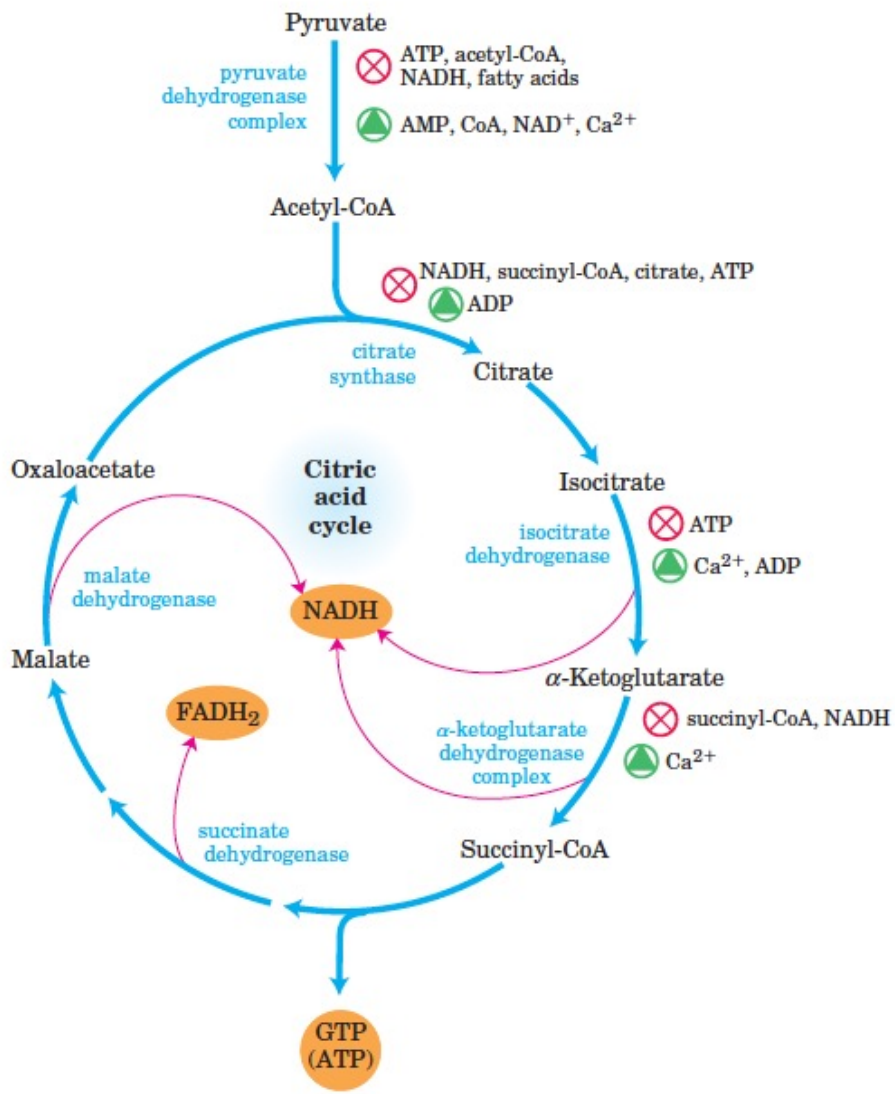
Total: para cada glicose são produzidos 4 ATP, 10 NADH e 2 $FADH_2$

TABLE 16–1 Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

Reaction	*Number of ATP or reduced coenzyme directly formed*	*Number of ATP ultimately formed**
Glucose ⟶ glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate	2 NADH	3 or 5†
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate ⟶ 2 pyruvate	2 ATP	2
2 Pyruvate ⟶ 2 acetyl-CoA	2 NADH	5
2 Isocitrate ⟶ 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate ⟶ 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA ⟶ 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate ⟶ 2 fumarate	2 $FADH_2$	3
2 Malate ⟶ 2 oxaloacetate	2 NADH	5
Total		30–32

- NADH e $FADH_2$ serão usados na fosforilação oxidativa para gerar ATP
- † o número de ATPs gerados vai depender da lançadeira usada para transportar os equivalentes de redução do citosol para a mitocôndria

A velocidade do Ciclo de Krebs é regulada em 4 passos enzimáticos distintos



O Ciclo de Krebs também fornece intermediários para a síntese de várias moléculas importantes para o metabolismo (função anaplerótica)

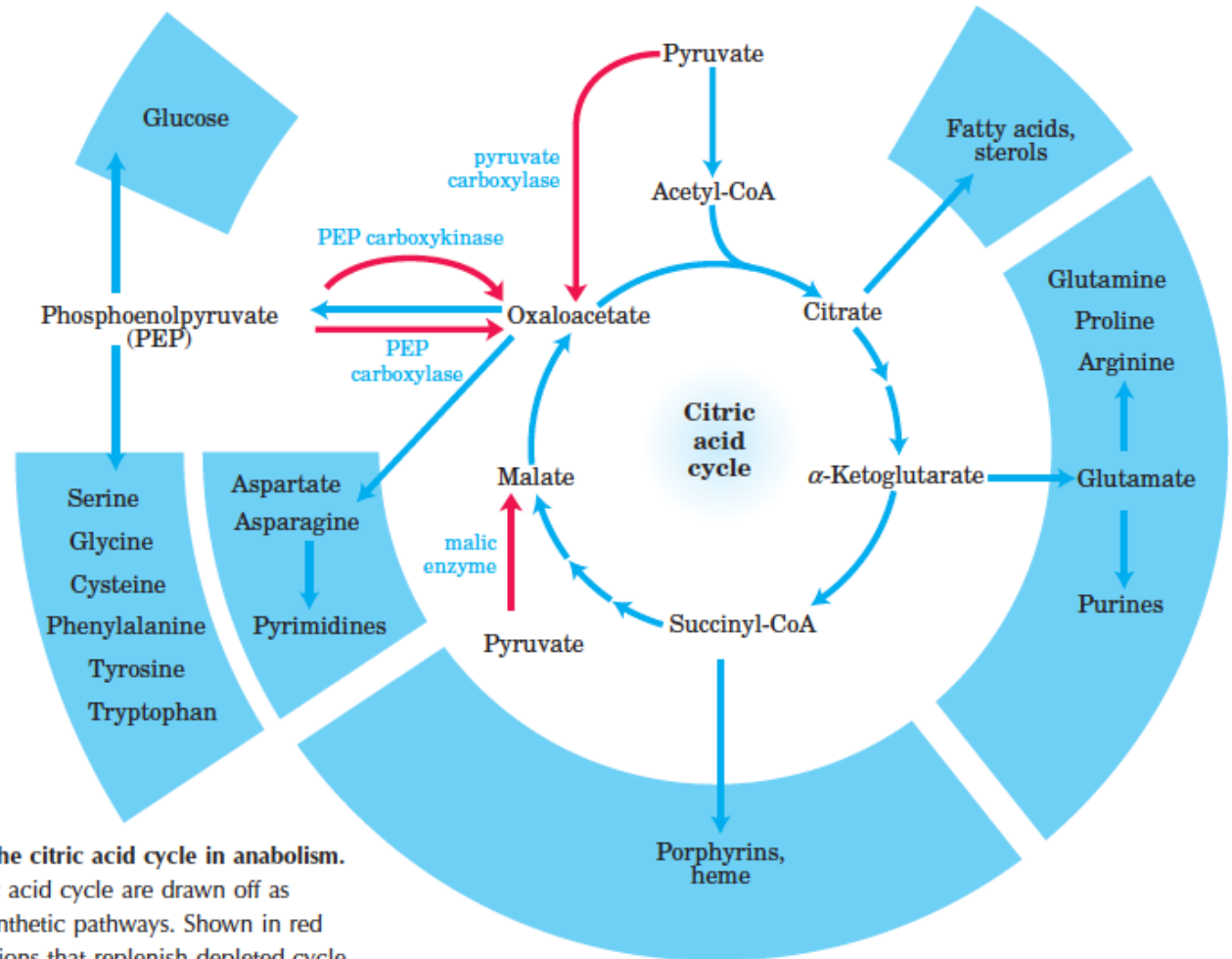


FIGURE 16–15 Role of the citric acid cycle in anabolism. Intermediates of the citric acid cycle are drawn off as precursors in many biosynthetic pathways. Shown in red are four anaplerotic reactions that replenish depleted cycle intermediates (see Table 16–2).