Faculty of Pharmacy

# Biochemistry-2

Edited By:


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**Lecturer of Biochemistry**


# Lecture 2

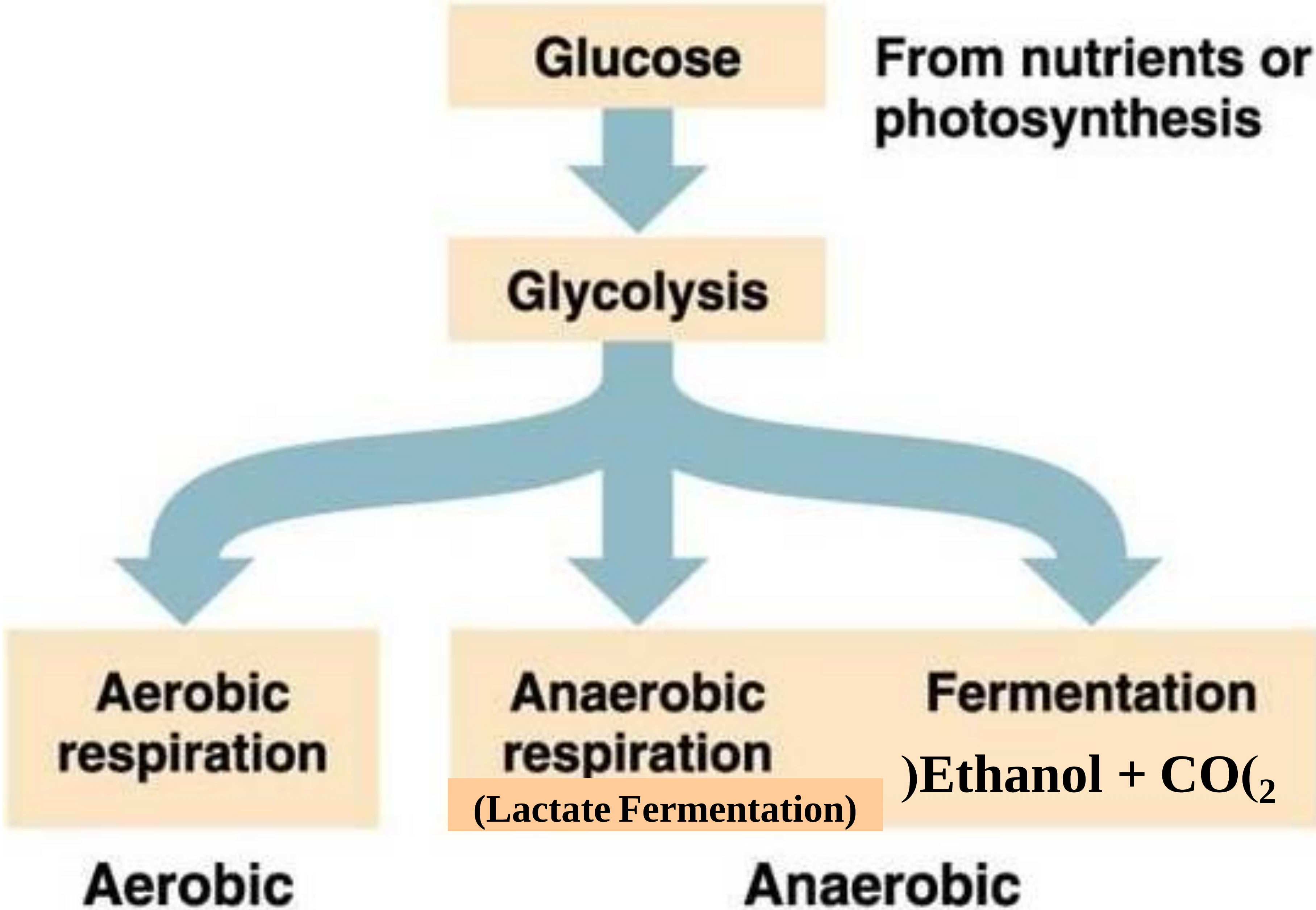
# GLYCOLYSIS (EMBDEN-MEYERHOF-PATHWAY)

- **Definition**: It is the oxidation of glucose into pyruvic acid (in presence of $O_2$), or lactic acid (in absence of $O_2$), the latter occurs only in muscular exercise due to $O_2$ lack, and in RBCs due to absence of mitochondria.
- Site It occurs in **the cytoplasm** of every cell.

# GLYCOLYSIS

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

# All cells undergo glycolysis



# GLYCOLYSIS

- **The first step in the metabolism of glucose is an enzyme-catalyzed phosphorylation forming glucose-6-phosphate. With the addition of phosphate group, the molecule acquires a negative charge and is then unable to diffuse out of the cell across the plasma membrane. This reaction has the effect of trapping glucose within the cell, where it is available for catabolism or storage.**

- **Phosphorylation of glucose is catalyzed by hexokinase or glucokinase.**

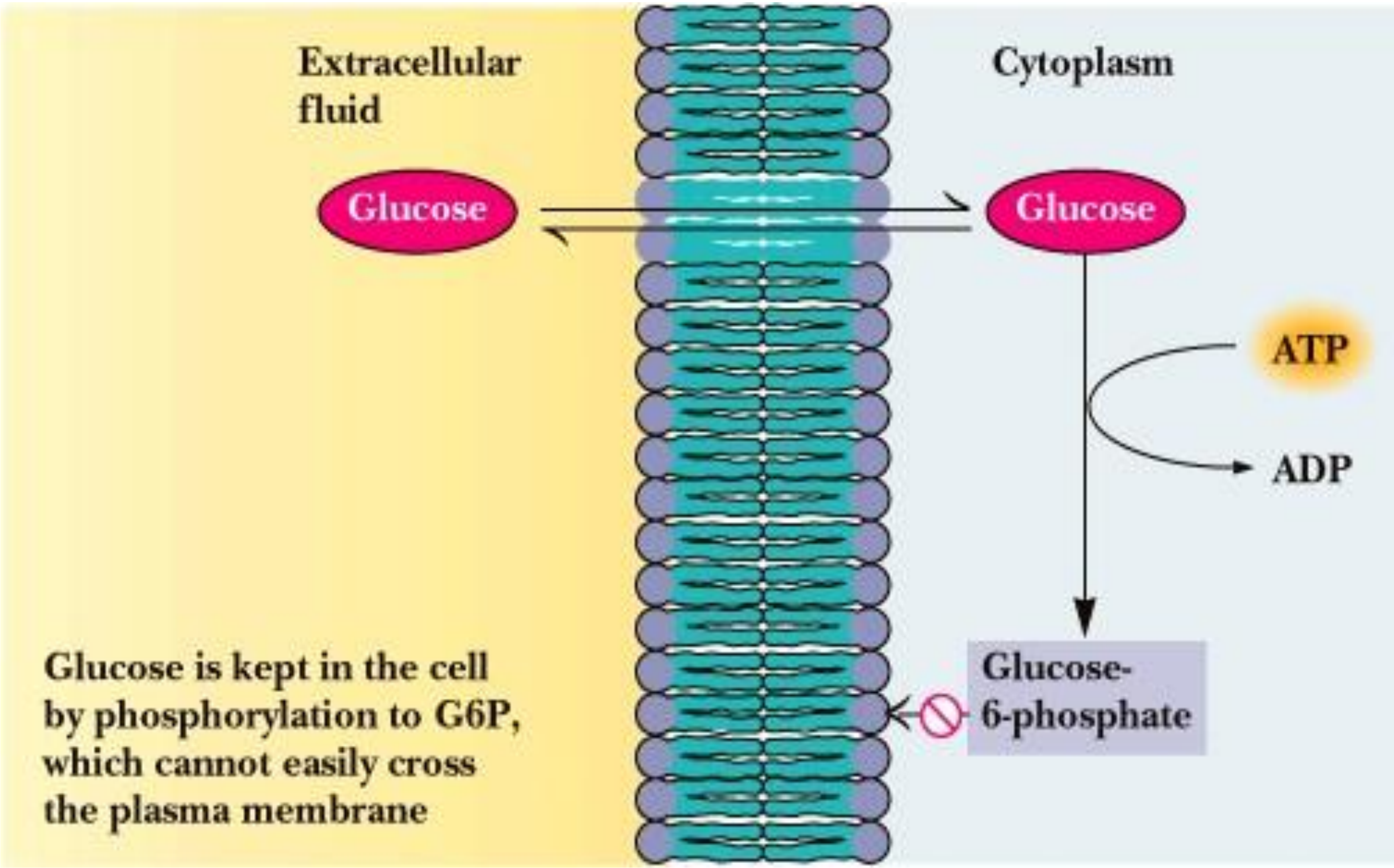
# Phosphorylation of Glucose

- **There are TWO enzymes**

Glucose + ATP $\xrightarrow{\text{Glucokinase}}$ Glucose–6–phosphate + ADP

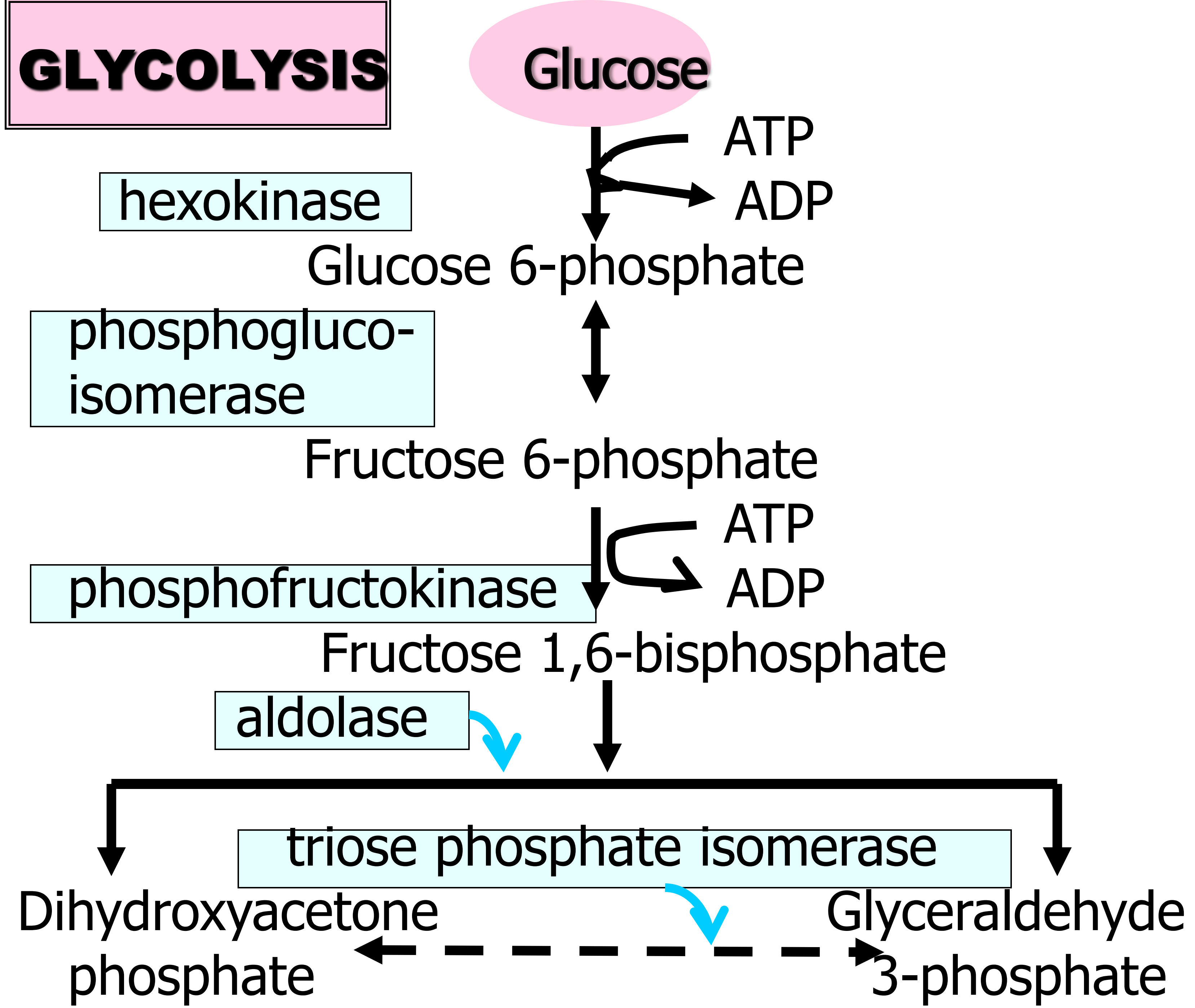
Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ Glucose–6–phosphate + ADP

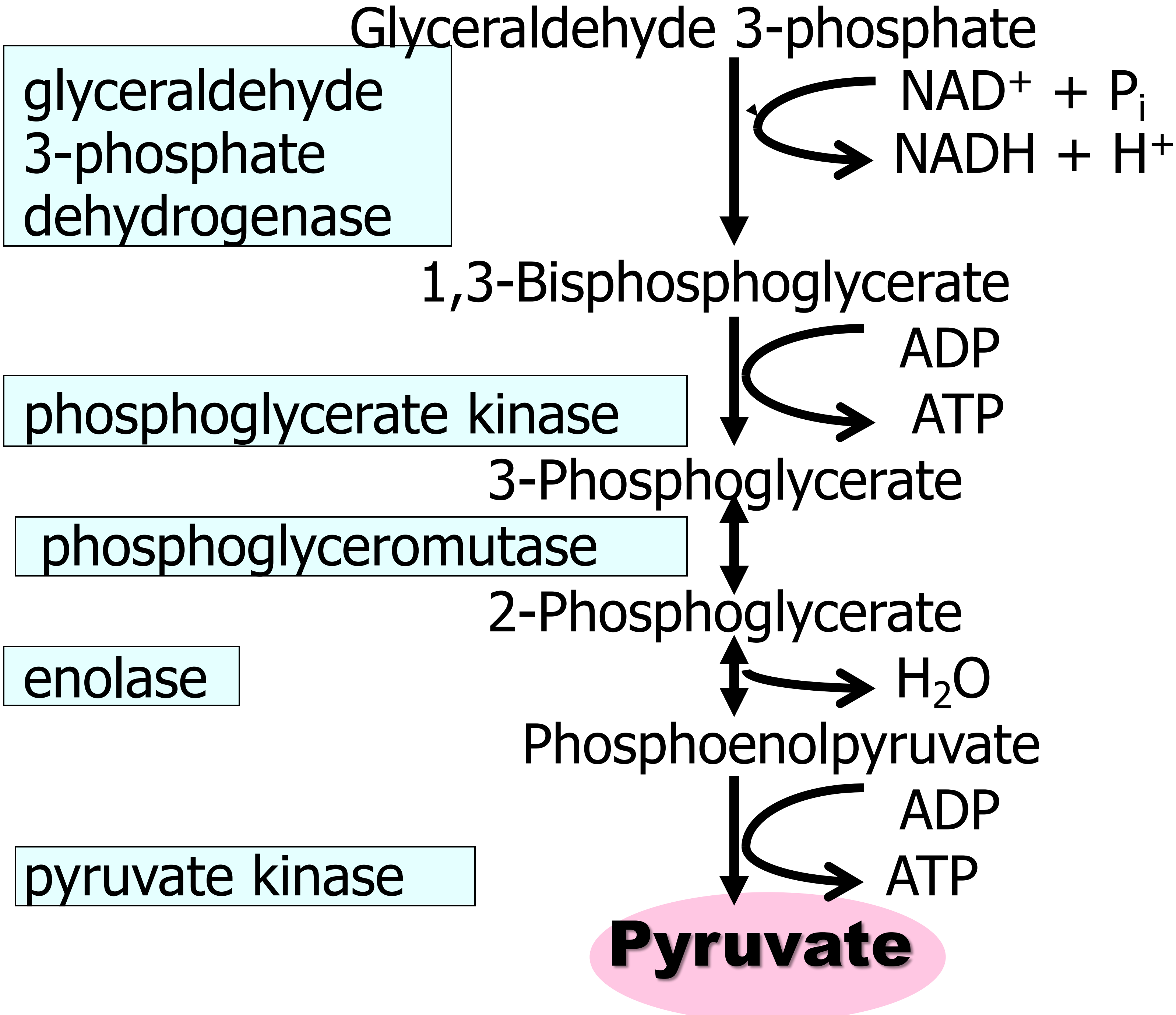
# Trapping of Glucose by phosphorylation



# Comparison between Hexokinase & Glucokinas

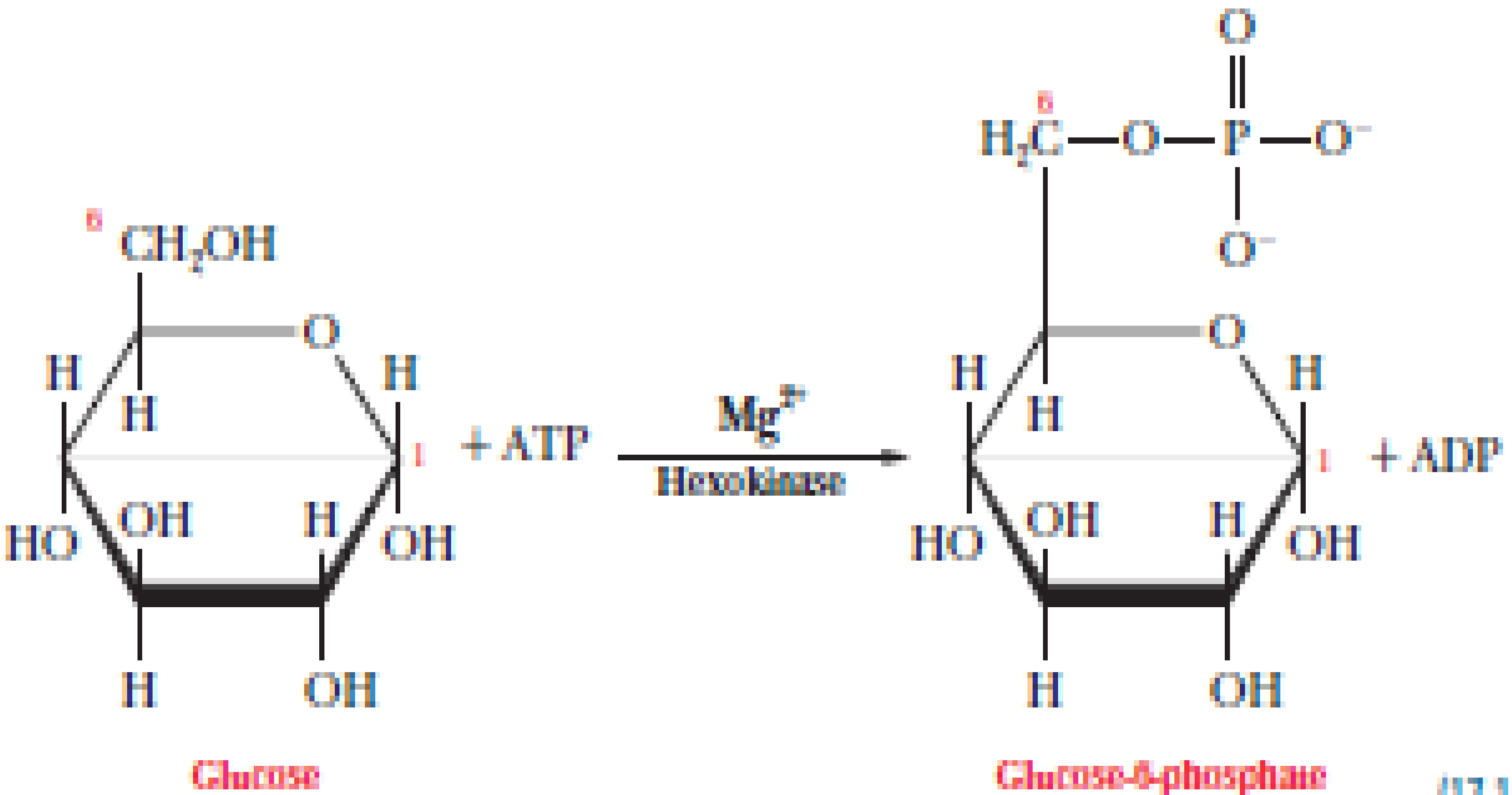
| No | Factor | Hexokinase | Glucokinase |
|---|---|---|---|
| 1 | Substrate | All Hexoses | Glucose only |
| 2 | Distribution | All tissues | Liver only |
| 3 | Product inhibition | Inhibited by Glucose-6-phosphate | Not inhibited by Glucose-6-phosphate |
| 4 | Km for glucose | Low Km (High affinity) | High Km (Low affinity) |
| 5 | Effect of Insulin | Not affected | Activated |
| 6 | Effect of Carbohydrate | Not affected | Activated |
| 7 | Effect of Starvation | Not affected | Inhibited |


GLYCOLYSIS
Glucose
ATP
ADP
hexokinase
Glucose 6-phosphate
phosphogluco-
isomerase
Fructose 6-phosphate
ATP
ADP
phosphofructokinase
Fructose 1,6-bisphosphate
aldolase
triose phosphate isomerase
Dihydroxyacetone
phosphate
Glyceraldehyde
3-phosphate


Glyceraldehyde 3-phosphate
glyceraldehyde 3-phosphate dehydrogenase
$NAD^+ + P_i$
$NADH + H^+$
1,3-Bisphosphoglycerate
phosphoglycerate kinase
ADP
ATP
3-Phosphoglycerate
phosphoglyceromutase
2-Phosphoglycerate
enolase
$H_2O$
Phosphoenolpyruvate
pyruvate kinase
ADP
ATP
Pyruvate

## *STEP 1. PHOSPHORYLATION*

Glucose + ATP → Glucose-6-phosphate + ADP



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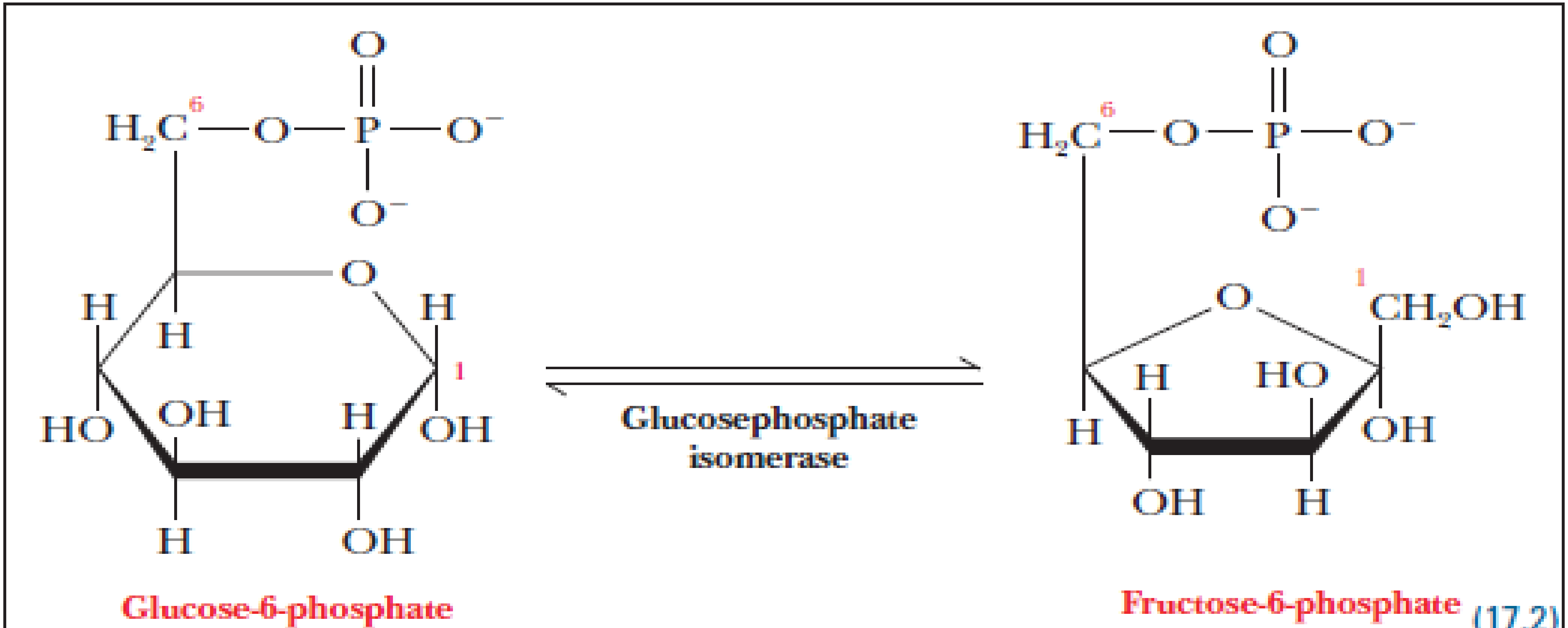
## STEP 2. ISOMERIZATION

of glucose-6-phosphate to give fructose-6-phosphate

Glucosephosphate isomerase is the enzyme that catalyzes this reaction

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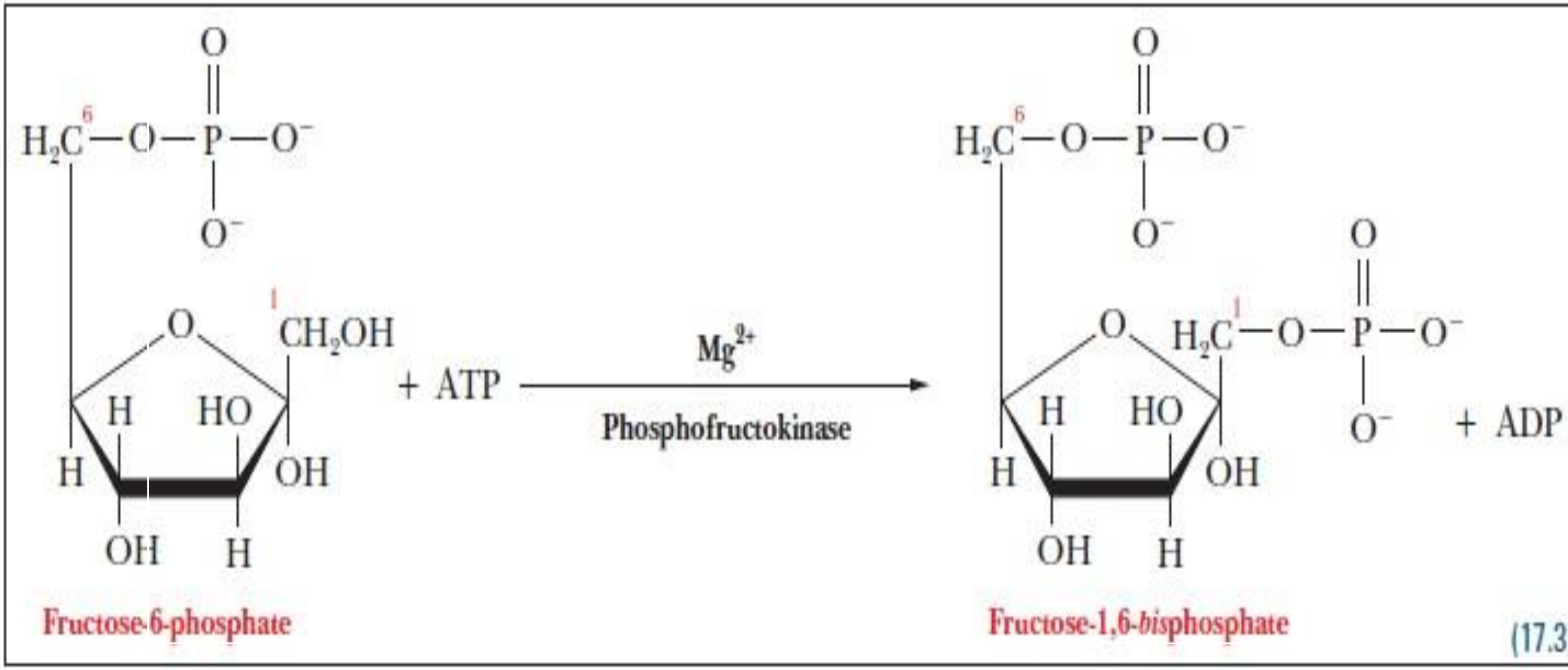
## STEP 3. *PHOSPHORYLATION*

of fructose-6-phosphate to give fructose-1,6-*bis*phosphate (ATP is the source of the phosphate group) + ADP

The phosphorylation of fructose-6-phosphate is **irreversible**, and **phosphofructokinase,** the enzyme that catalyzes it, is the key regulatory enzyme in glycolysis.

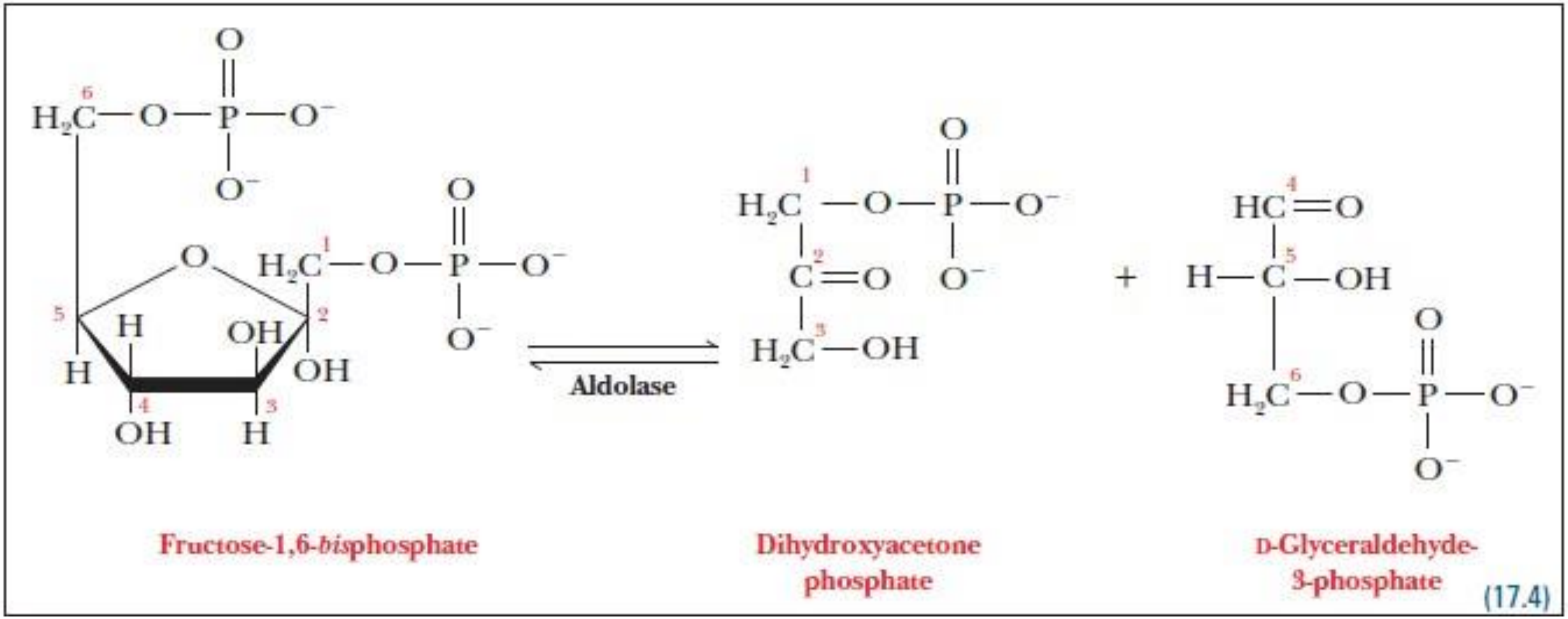


# STEP 4. *CLEAVAGE*

of fructose-1,6-*bis*phosphate to give two 3-carbon fragments,

the enzyme that catalyzes it is called **aldolase.**

**Conversion of Six-Carbon Glucose to Three-Carbon Glyceraldehyde-3-Phosphate**

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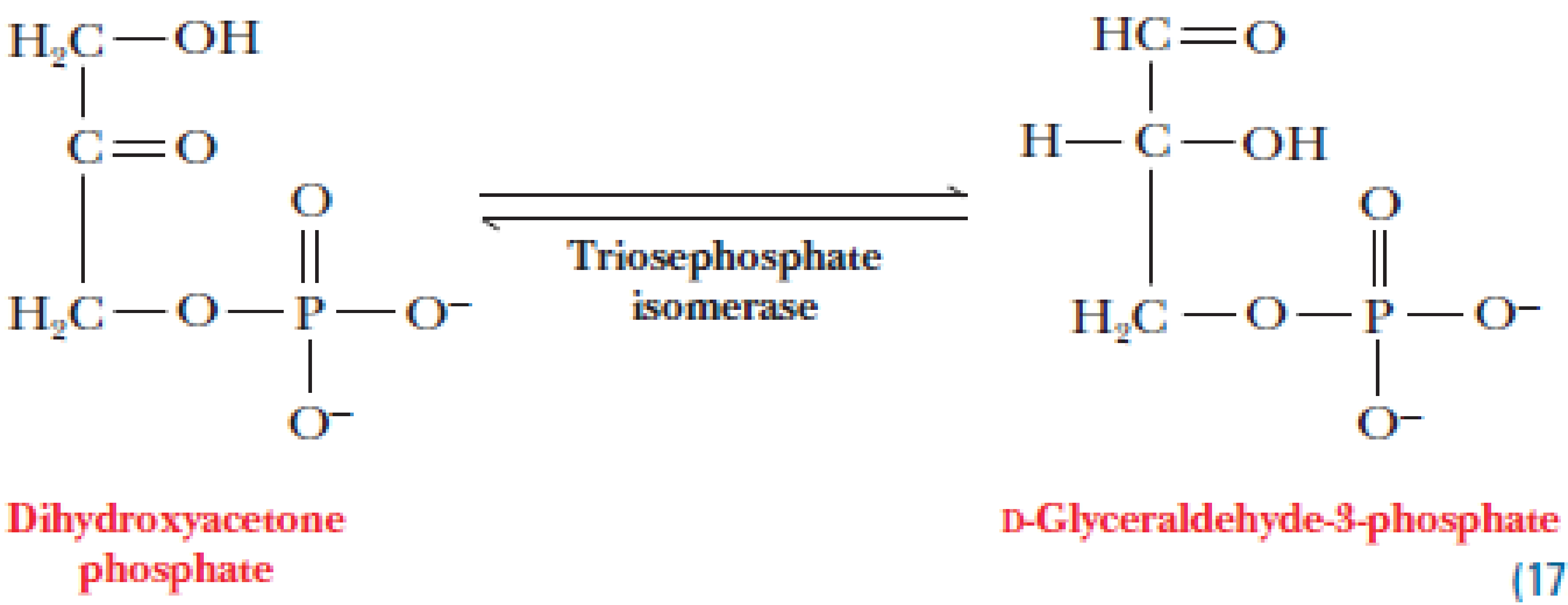
# STEP 5. *ISOMERIZATION*

of dihydroxyacetone phosphate to give glyceraldehyde- 3-phosphate.

The enzyme that catalyzes this reaction is **triosephosphate isomerase.** (Both dihydroxyacetone and glyceraldehyde are trioses)

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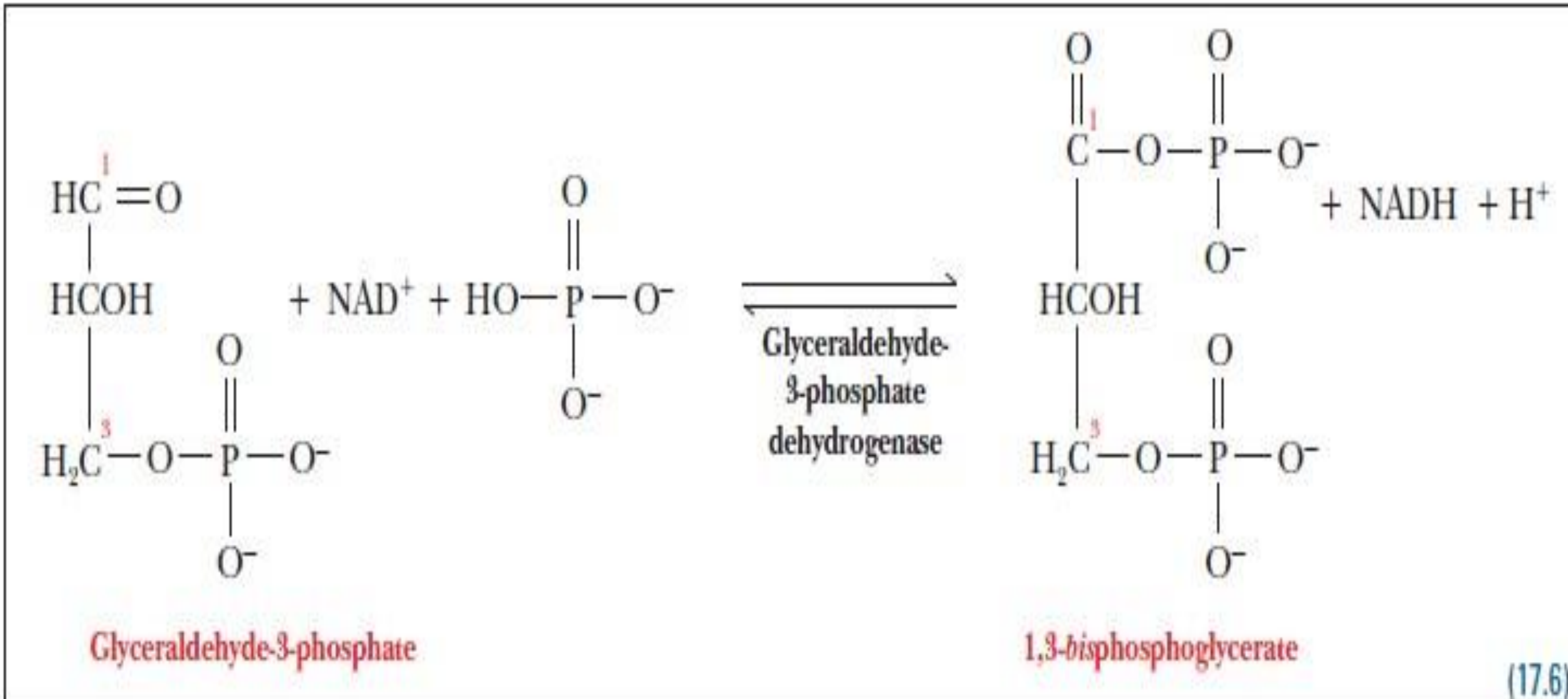
# STEP 6. *OXIDATION AND PHOSPHORYLATION*

of glyceraldehyde-3-phosphate to give 1,3-*bis*phosphoglycerate. The enzyme that catalyzes this reaction is **glyceraldehyde-3-phosphate dehydrogenase.**

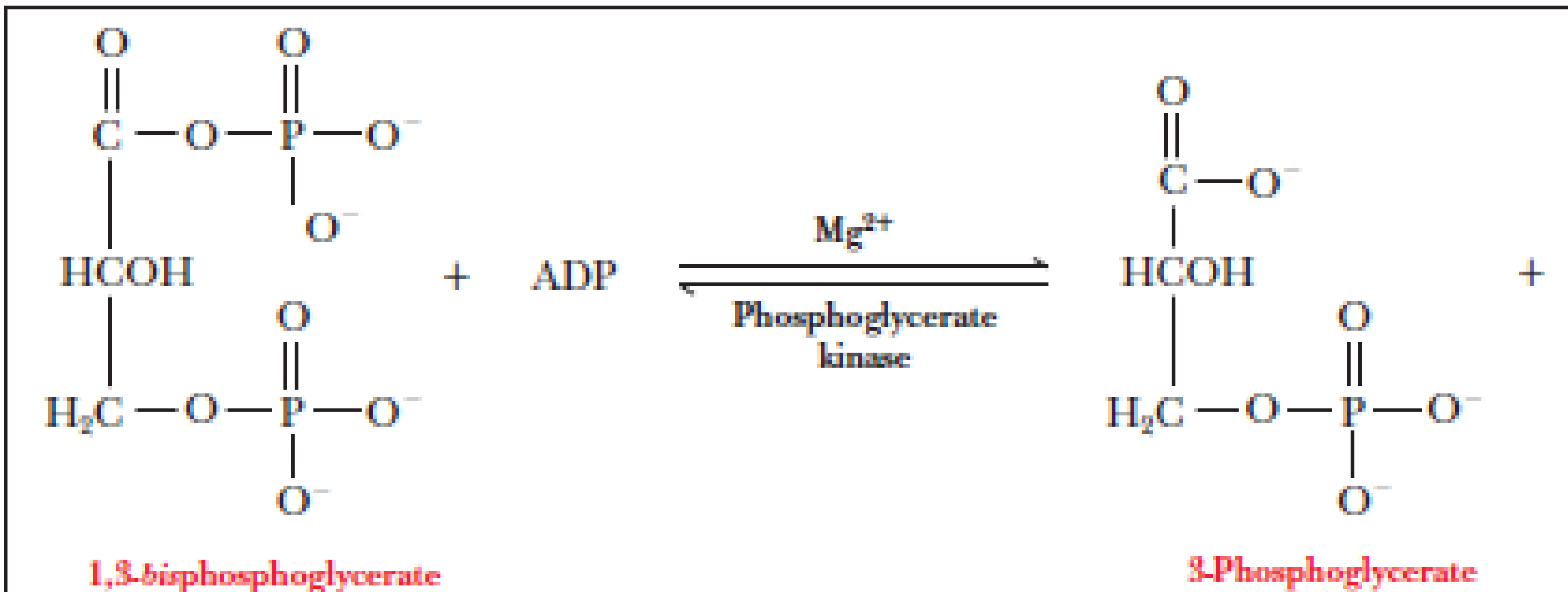
It is the first oxidation reduction reaction in glycolysis.



# *STEP 7. TRANSFER OF A PHOSPHATE GROUP*

from 1,3-*bis*phosphoglycerate to ADP (phosphorylation of ADP to ATP) to give 3phosphoglycerate **+ ATP.**

The enzyme that catalyzes this reaction is **phosphoglycerate kinase.** This transfer is typical of **substrate-level phosphorylation.**

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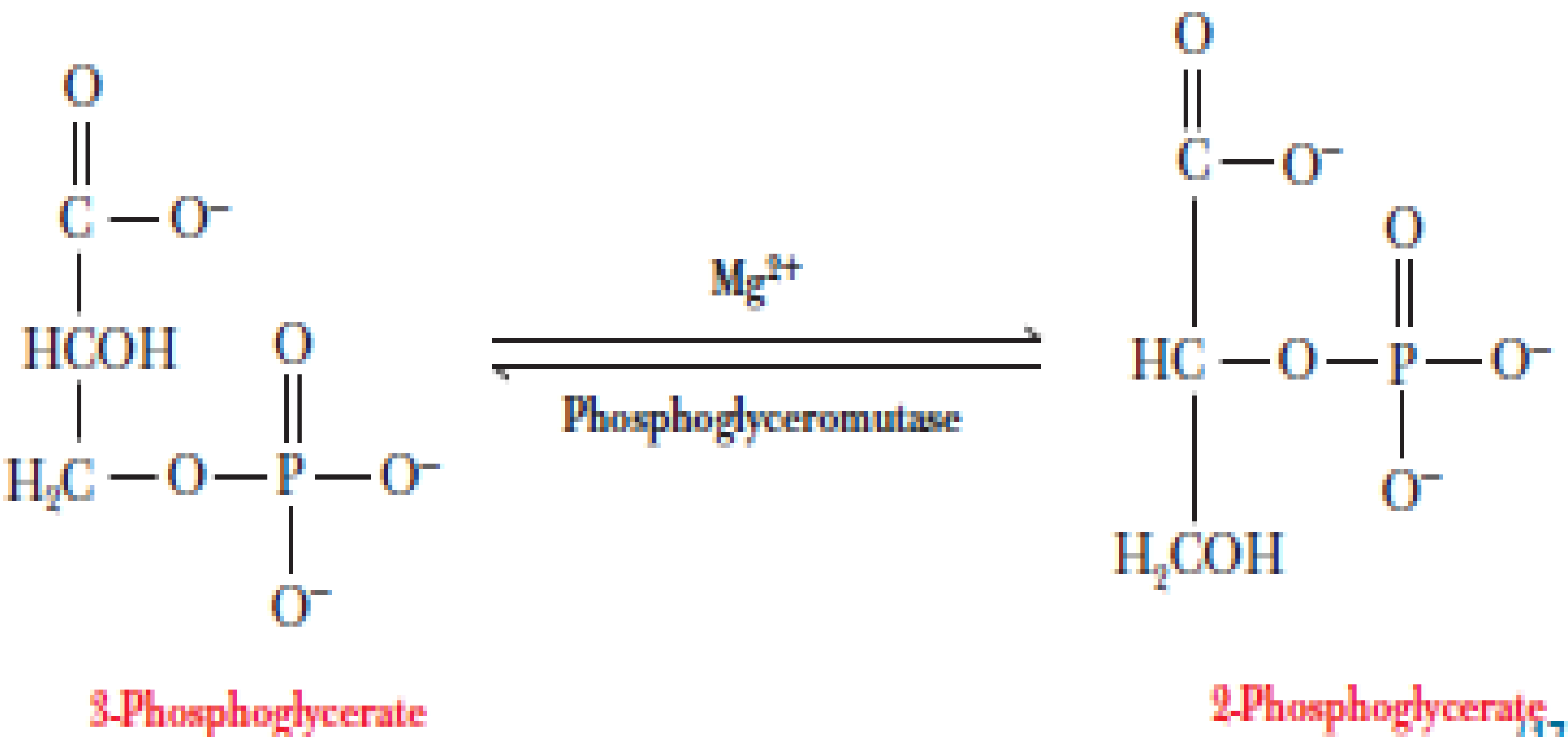
## *Step 8. Isomerization*

of 3-phosphoglycerate to give 2-phosphoglycerate. The enzyme that catalyzes this reaction is **phosphoglyceromutase.**

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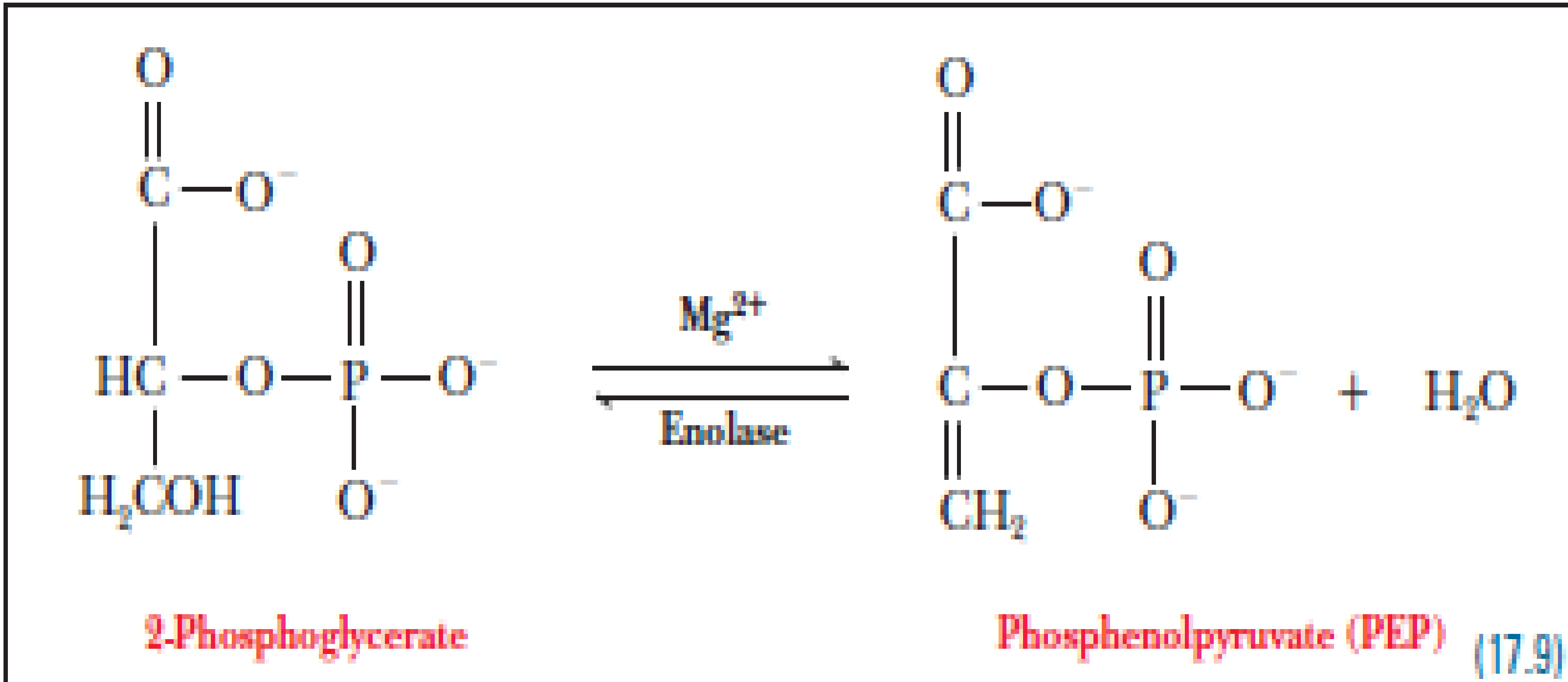
## Step 9. *Dehydration*

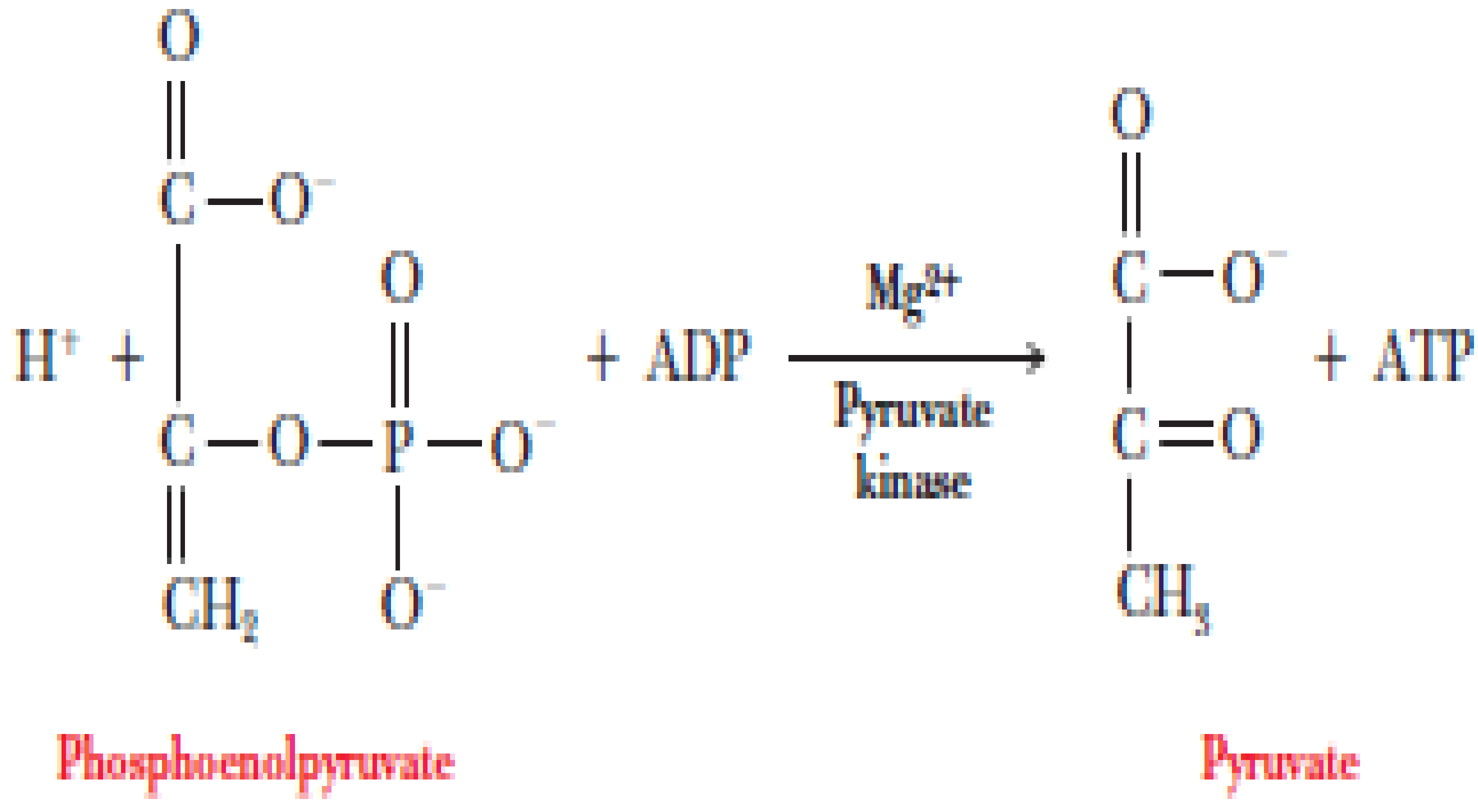
of 2-phosphoglycerate to give phosphoenolpyruvate.

The enzyme that catalyzes this reaction is **Enolase, it** requires Mg2+ as a cofactor.



## *Step 10. Transfer*

of a phosphate group from phosphoenolpyruvate to ADP (phosphorylation of ADP to ATP) to give pyruvate **+ ATP**

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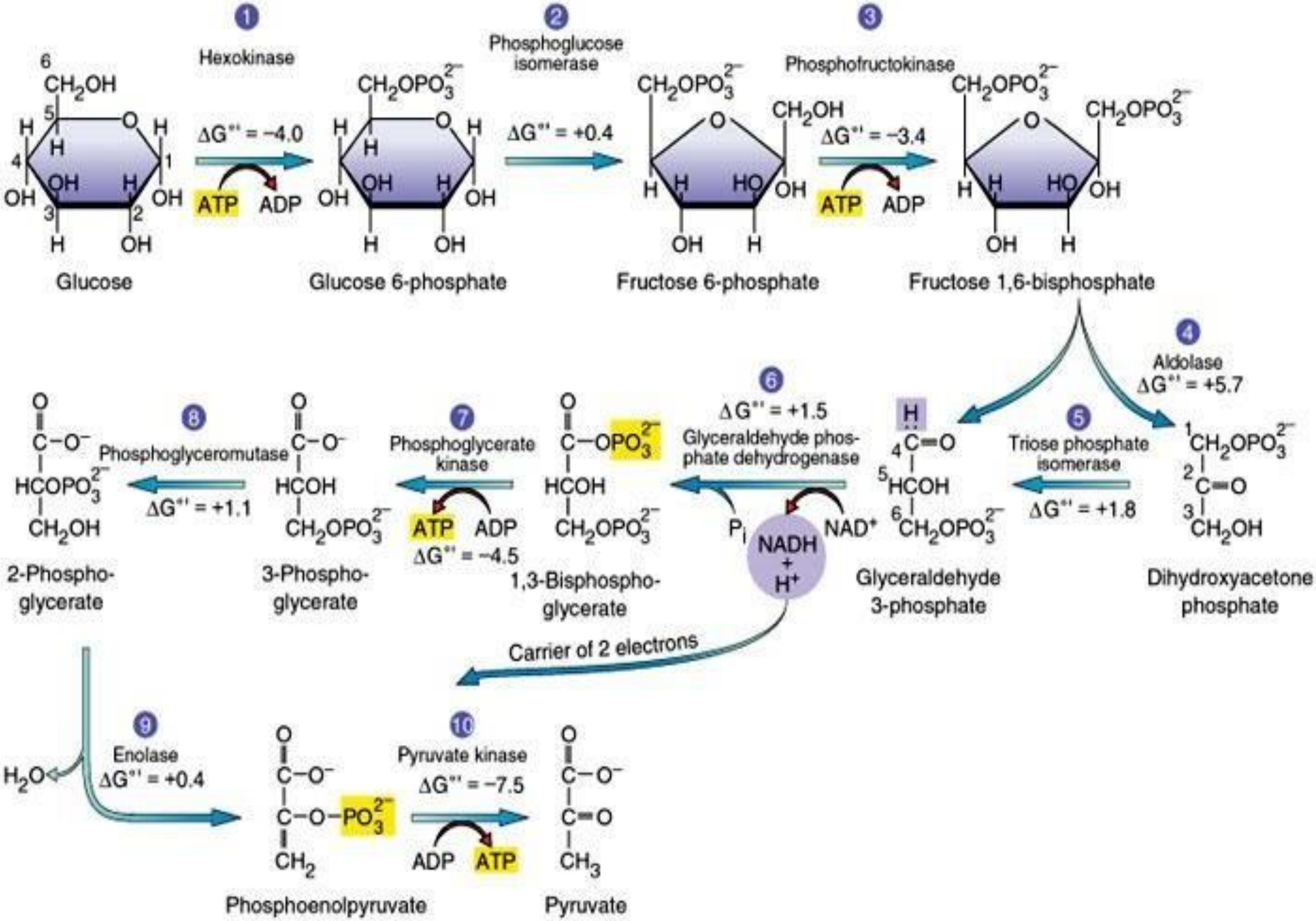


1
Hexokinase
ΔG°' = −4.0
ATP
ADP
2
Phosphoglucose isomerase
ΔG°' = +0.4
3
Phosphofructokinase
ΔG°' = −3.4
ATP
ADP
Glucose
Glucose 6-phosphate
Fructose 6-phosphate
Fructose 1,6-bisphosphate
4
Aldolase
ΔG°' = +5.7
5
Triose phosphate isomerase
ΔG°' = +1.8
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate
6
ΔG°' = +1.5
Glyceraldehyde phosphate dehydrogenase
Pi
NAD+
NADH + H+
1,3-Bisphosphoglycerate
7
Phosphoglycerate kinase
ATP
ADP
ΔG°' = −4.5
3-Phosphoglycerate
8
Phosphoglyceromutase
ΔG°' = +1.1
2-Phosphoglycerate
Carrier of 2 electrons
9
Enolase
ΔG°' = +0.4
H2O
Phosphoenolpyruvate
10
Pyruvate kinase
ΔG°' = −7.5
ADP
ATP
Pyruvate

Net energy produced is 2 ATP

In addition, the two pyruvate can go on to the citric acid cycle to produce more energy.

# Overall glycolysis

$$\text{glucose} + 2\ \text{ATP} + 2\ \text{ADP} + 2\ PO_4^{=} + 2\ NAD^{+}$$

$$\downarrow \text{10 enzymes}$$

$$2\ \text{pyruvate} + 2\ \text{NADH} + 2\ H_2O + 4\ \text{ATP}$$

# Site of ATP production

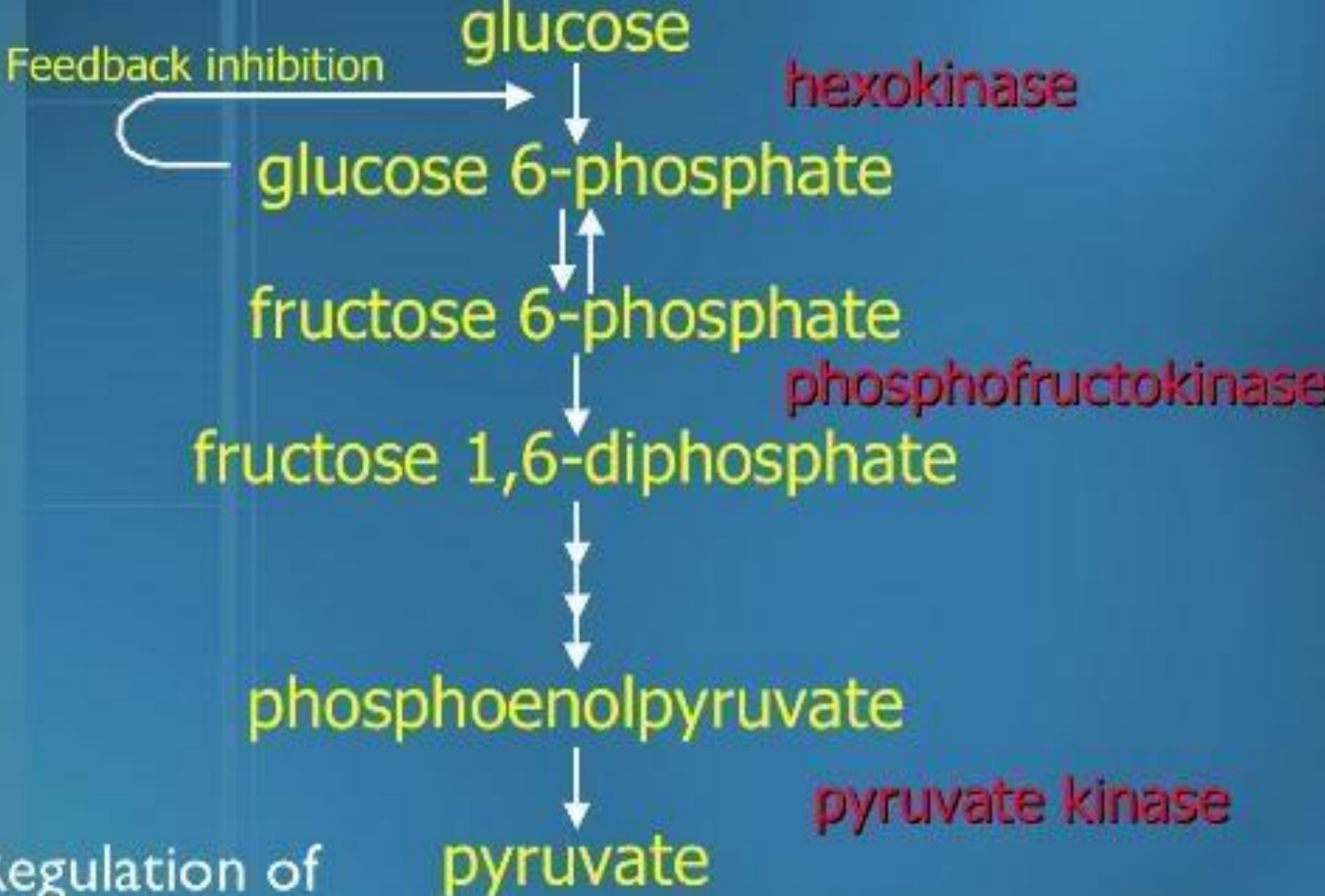
1- Respiratory chain (2NADH)

2- Substrate level phosphorelation (2 ATP)

# REGULATION OF GLYCOLYSIS

Three irreversible kinase reactions primarily drive glycolysis forward.

- hexokinase or glucokinase
- phosphofructokinase
- pyruvate kinase




Regulation of glycolysis

# 1- Hormonal regulation

a- **Insulin**: stimulates synthesis of all key enzymes of glycolysis. It is secreted after meal (in response to high glucose level)

B- Glucagon: inhibits the activity of all key enzymes of glycolysis . It is secreted in response to low glucose level

# 2- energy regulation

A- high level of ATP

inhibits PFK-1 and pyruvate kinase

B- high level of ADP and AMP

stimulatePFK-1

# 3- substrate regulation

A- glucose -6-phosphate inhibits hexokinase (and not glucokinase)

B- fructose 2,6 bisphosphate stimulates phosphofructokinase-1

C- citrate inhibits phosphofructokinase-1

D- fructose 1, 6 bisphosphate stimulates pyruvate kinase

# Regulation of Glycolysis

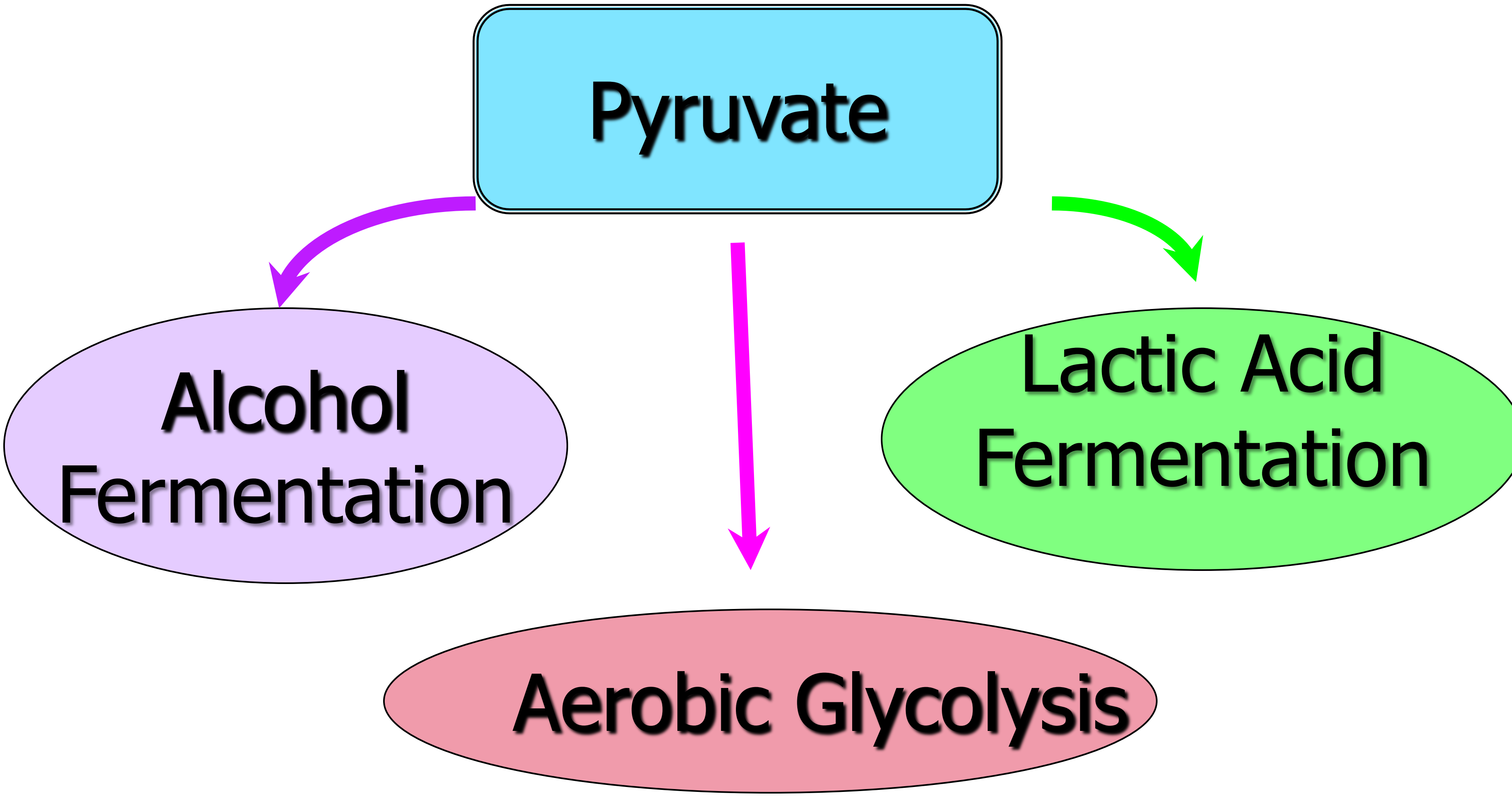
**1- After carbohydrate meal:**

**Blood glucose level ↑ → Stimulates insulin secretion → Increases synthesis of glucokinase, phosphofructokinase & pyruvate kinase**

**→ Enhances glycolysis**

**2- During fasting:**

**Blood glucose level ↓ → Inhibits insulin secretion & stimulates glucocorticoid secretion → Increases the synthesis of the four enzymes that reverse glycolysis (Stimulate gluconeogenesis)**



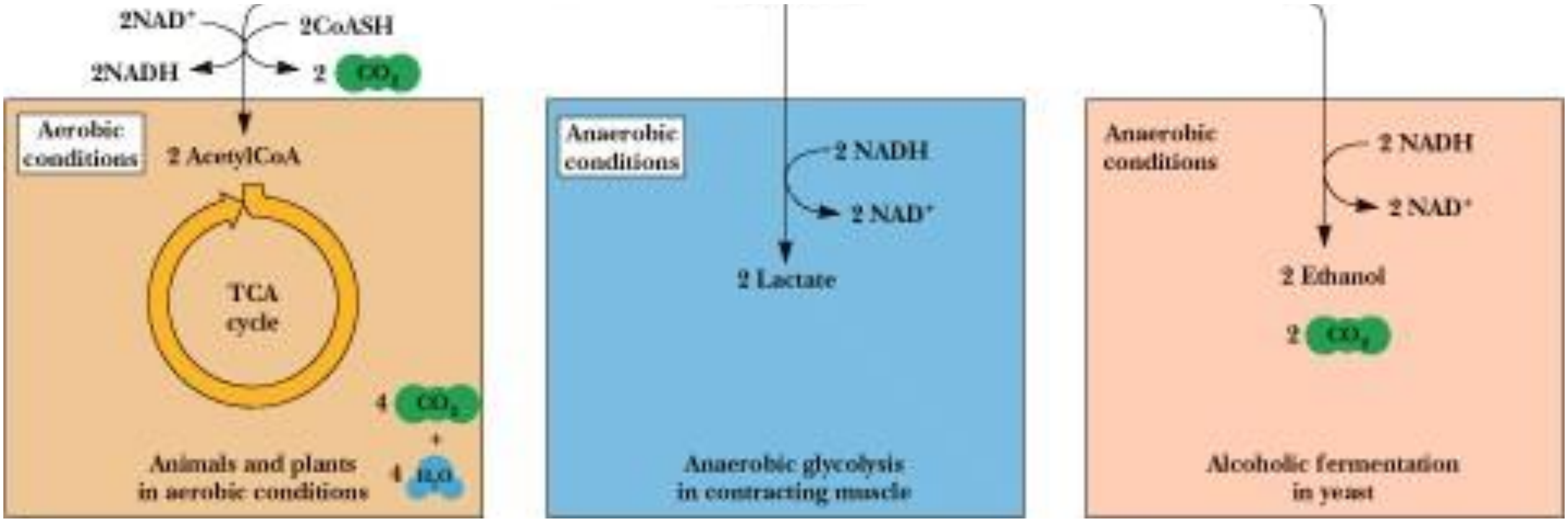

# Fate of Pyruvate

# Fate of Pyruvate

Pyruvate DH

Lactate DH

Pyruvate Decarboxylase &Alcohol DH




In Mitochondria

In Cytoplasm

# Fermentation

An anaerobic process beyond glycolysis.

- In our body it is used to make $NAD^+$ when there is not enough oxygen.
- $NAD^+$ must be regenerated from NADH or glycolysis will stop.
- There are several types of fermentation, We'll look briefly at two: lactate and ethanol

# Lactate fermentation

Lactate

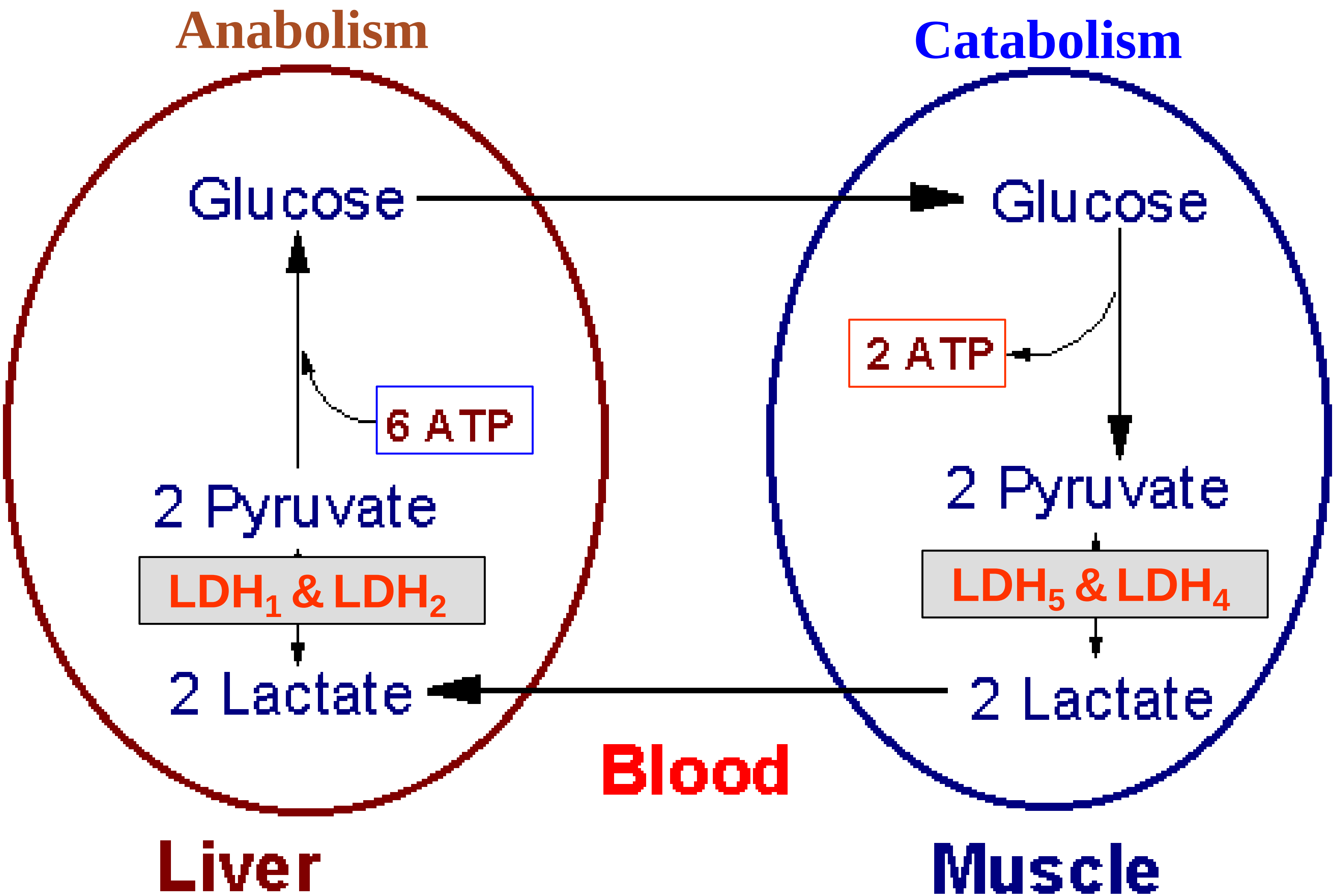
Produced by muscles when the body can't supply enough $O_2$.


Anaerobic conversion of pyruvate to lactate permits regeneration of $NAD^+$.

Body can then make more ATP - at a cost. Creates an oxygen debt.

**We** Must use extra $O_2$ to oxidize lactate later

# The Cori Cycle



# Different forms of Lactate Dehydrogenase

- **Lactate DH in Heart & Muscles:**

**Heart ($H_4$)**

$LDH_1$

| | |
|---|---|
| H | H |
| H | H |

**Muscle ($M_4$)**

$LDH_5$

| | |
|---|---|
| M | M |
| M | M |

- **Lactate DH in different tissues:**

**$H_3M$**

$LDH_2$

| | |
|---|---|
| H | H |
| H | M |

**$H_2M_2$**

$LDH_3$

| | |
|---|---|
| H | H |
| M | M |

**$HM_3$**

$LDH_4$

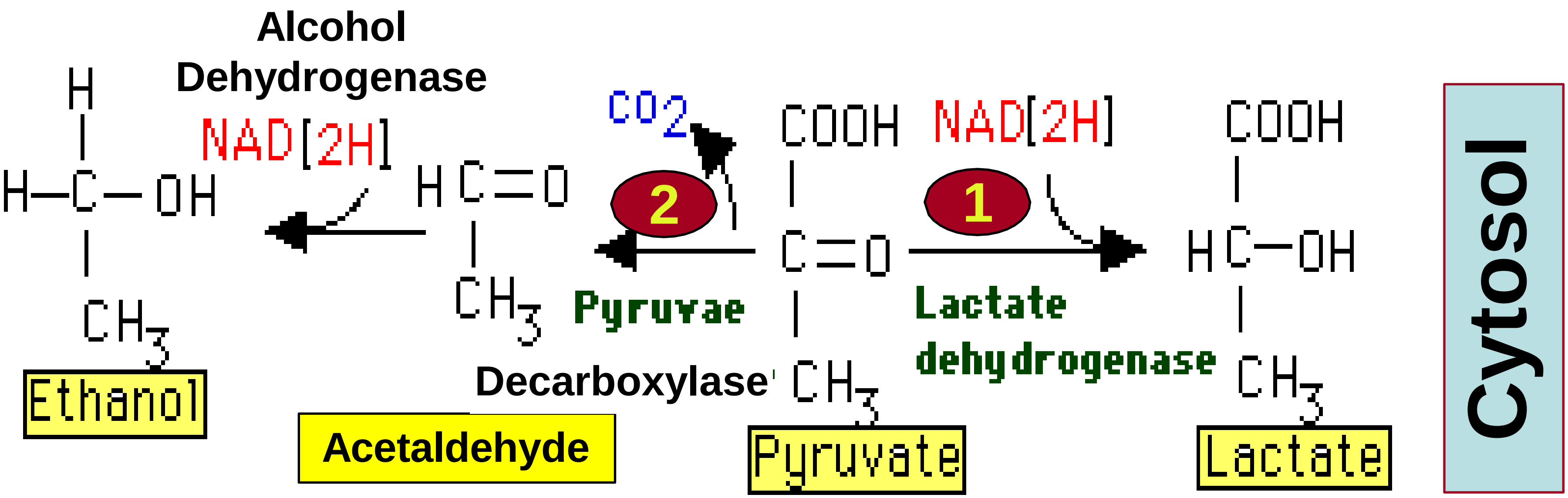
| | |
|---|---|
| H | M |
| M | M |

- The liver releases some of the glucose.
- The liver uses most of this lactate to make glycogen.
- Glycogen can be broken down into glucose when needed.

Used by some anaerobic bacteria to obtain additional energy from glucose.

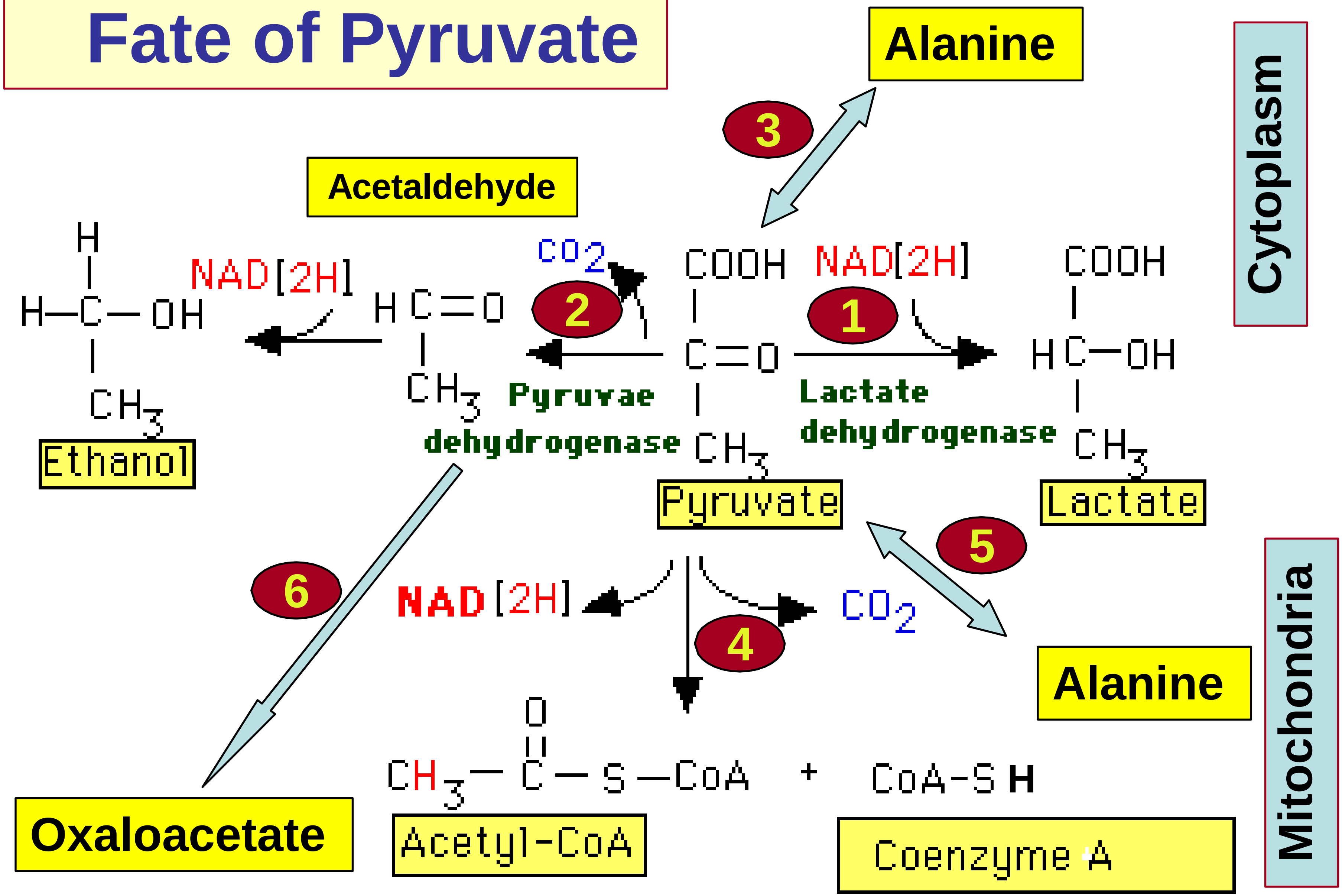
We do the opposite to remove ethanol.

# Alcohol fermentation




# Lactate & Ethanol Fermentation

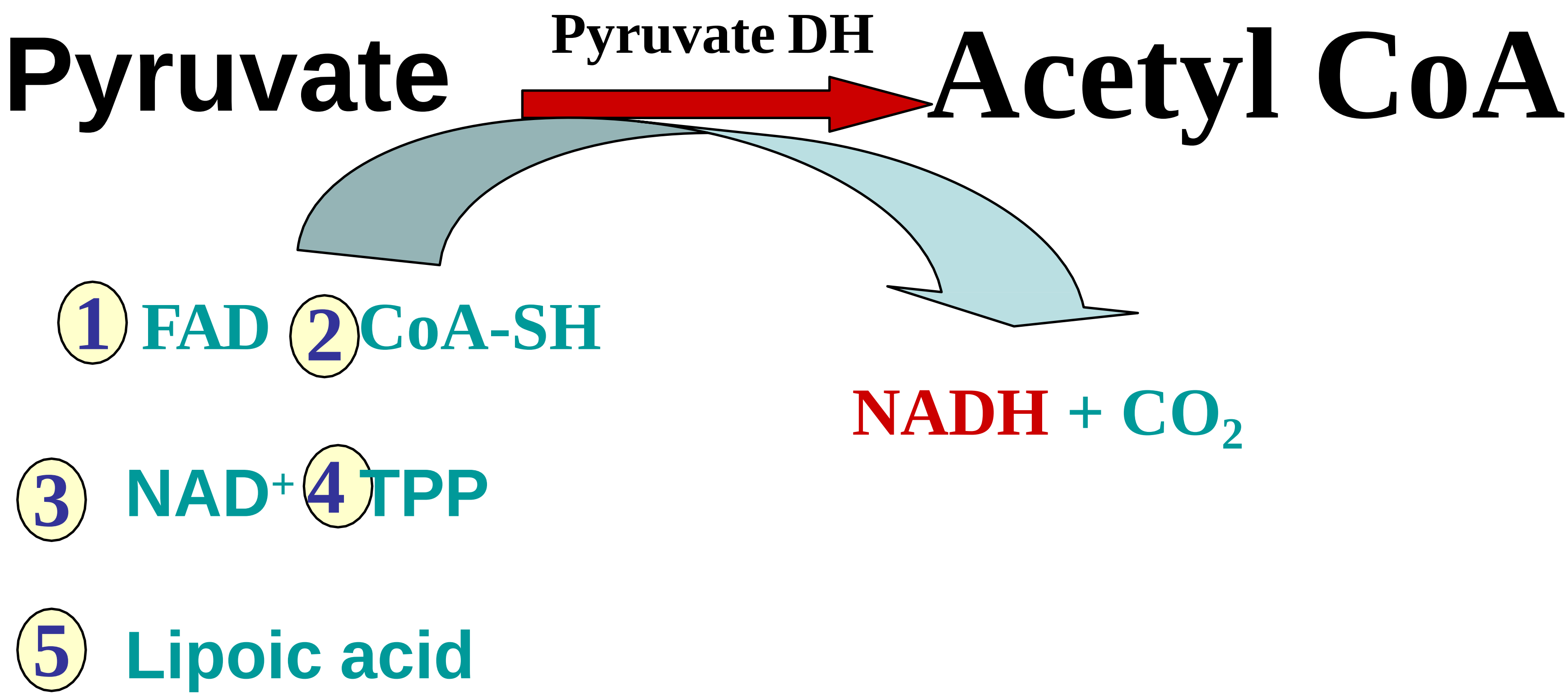
# Fate of Pyruvate



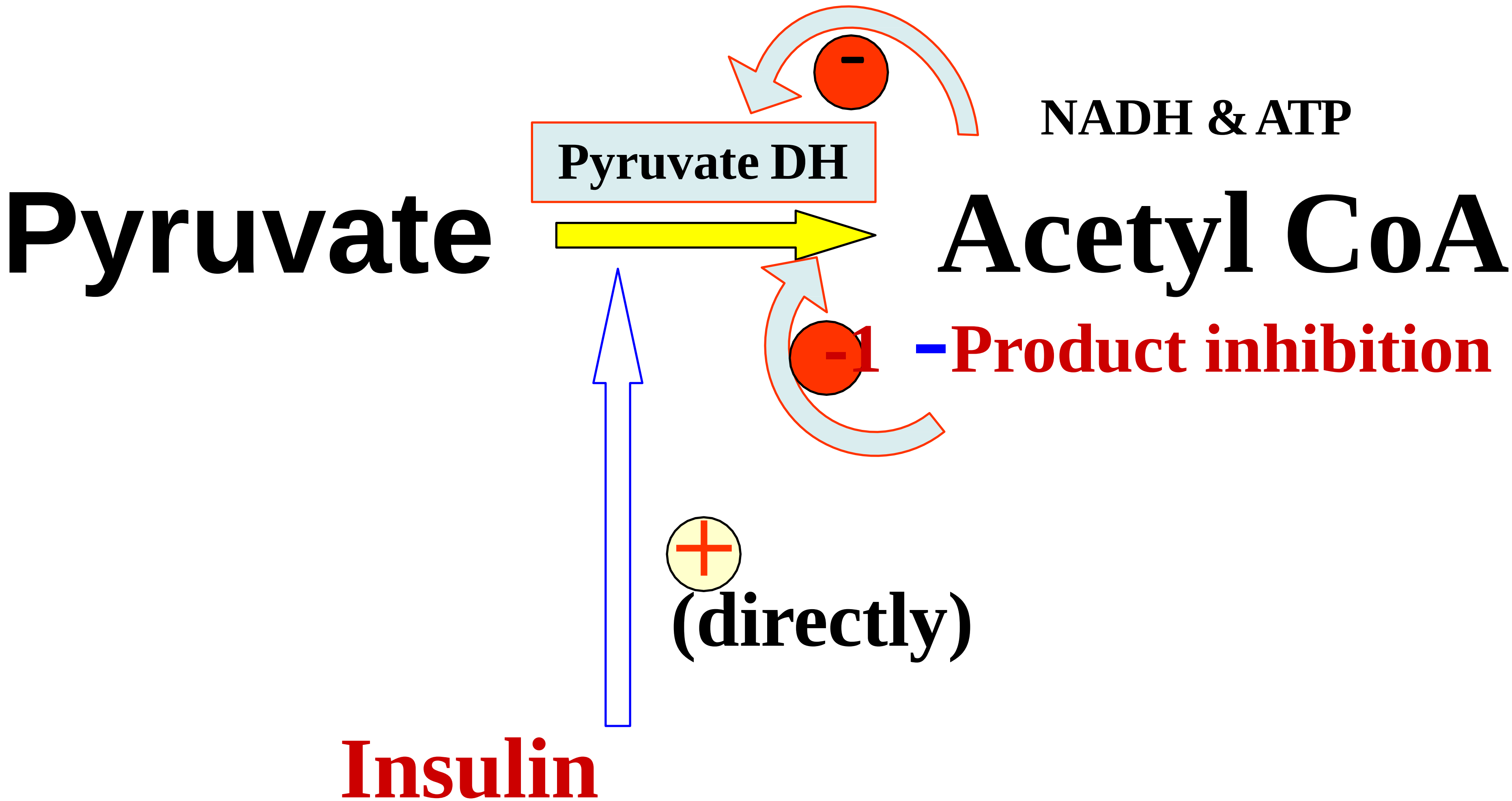
# Pyruvate Dehydrogenase:
# A multienzyme complex has 3 Functions:

1. **Decarboxylation:** Removal of $CO_2$ (Decarboxylase , thiamine pyrophosphate (TPP) as coenzyme)
2. **Oxidation** of the remaining two-carbon compound and **reduction** of NAD+ (Dehydrogenase, CoASH, FAD & $NAD^+$).
3. **Trans-acetylating function:** Attachment of CoA with a high energy thio-ester bond to form Acetyl CoA (Transacetylase, Lipoic acid).

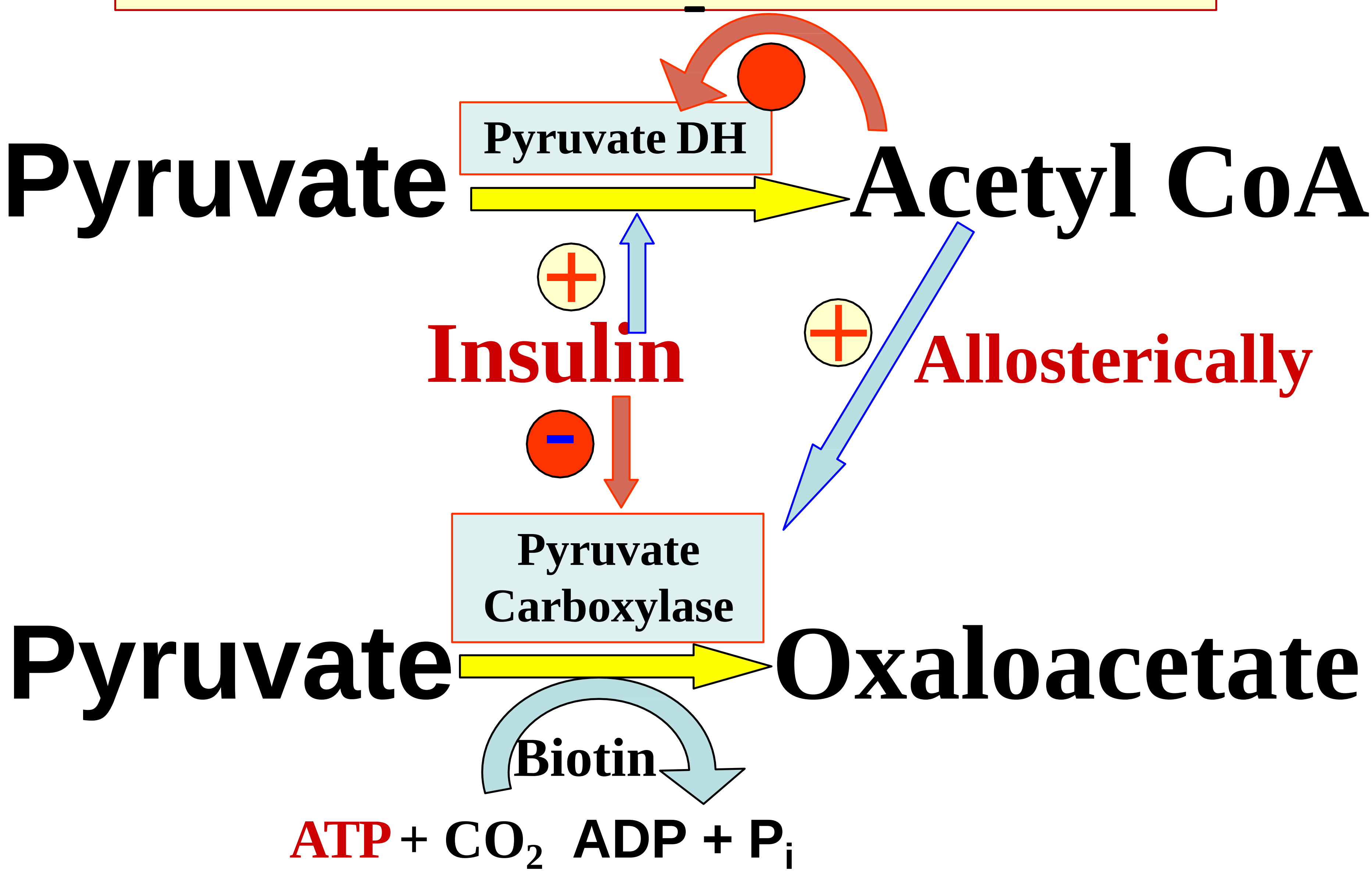
$$\text{Pyruvate} + NAD^+ + \text{CoASH} \longrightarrow \text{Acetyl CoA} + \text{NADH} + CO_2$$

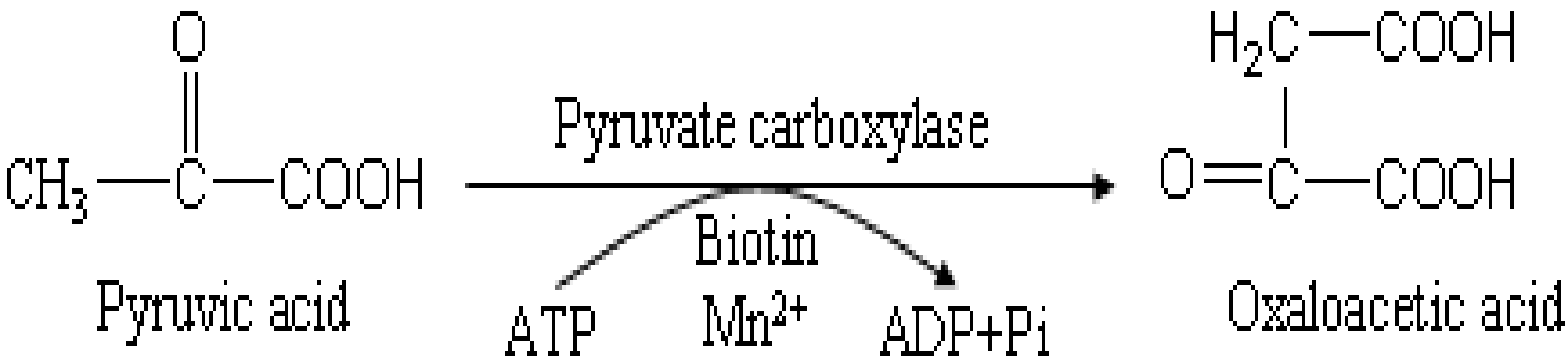

Pyruvate
Pyruvate DH
Acetyl CoA
1 FAD
2 CoA-SH
NADH + $CO_2$
3 $NAD^+$
4 TPP
5 Lipoic acid

# Regulation of Pyruvate DH

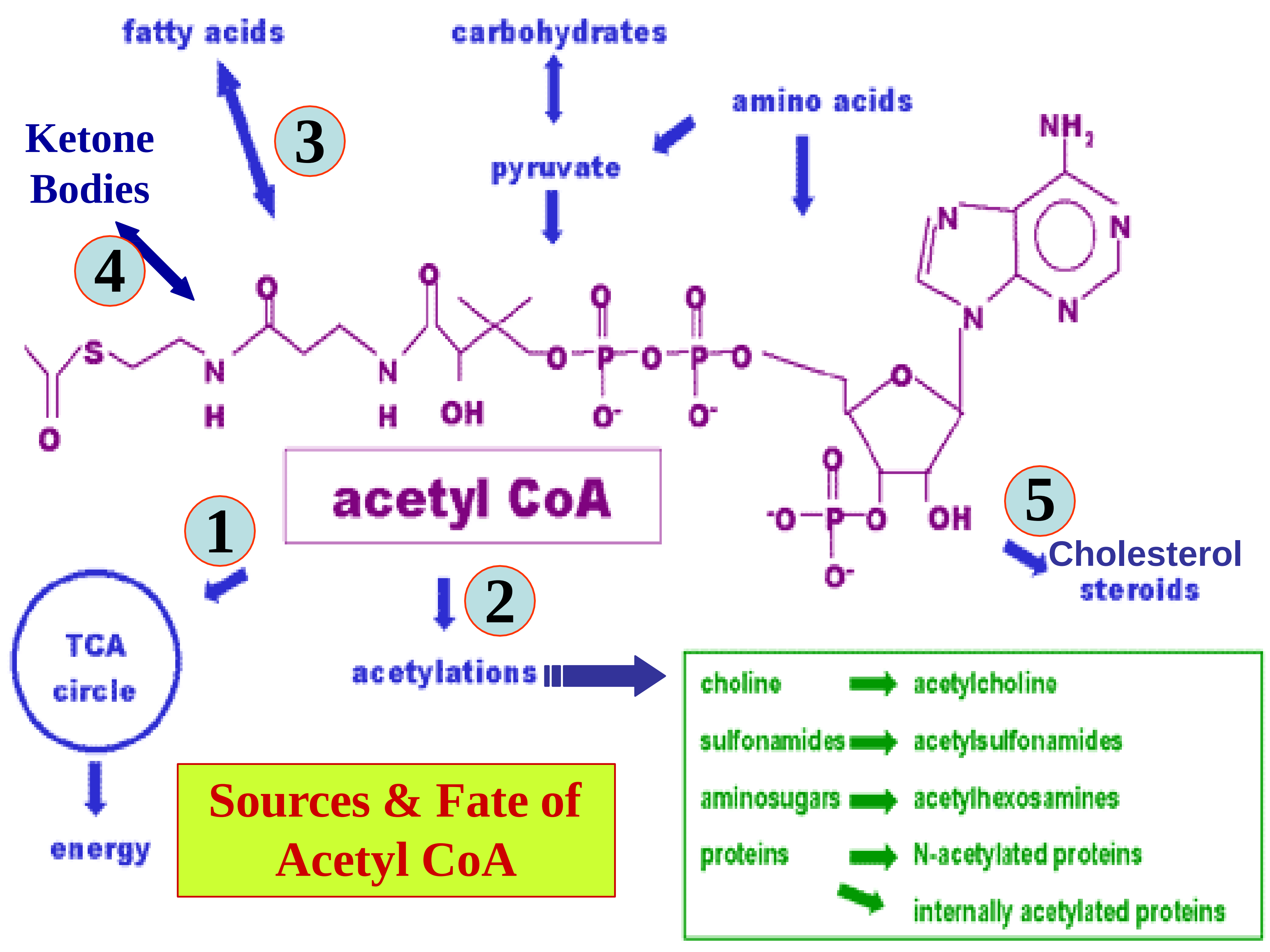


# Carboxylation of Pyruvate





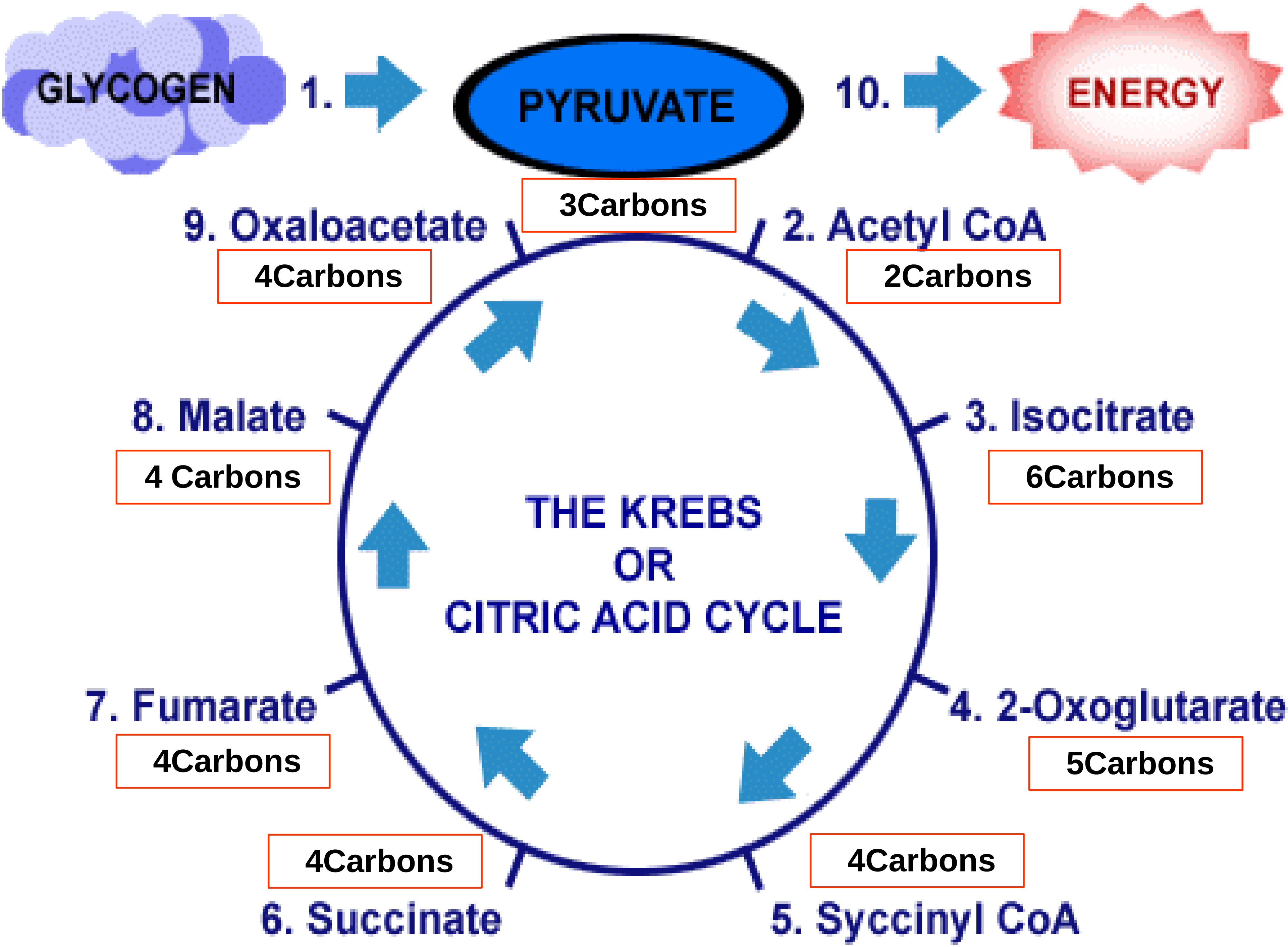
O
CH3 C COOH
Pyruvic acid
Pyruvate carboxylase
Biotin
ATP
Mn2+
ADP+Pi
H2C COOH
O C COOH
Oxaloacetic acid

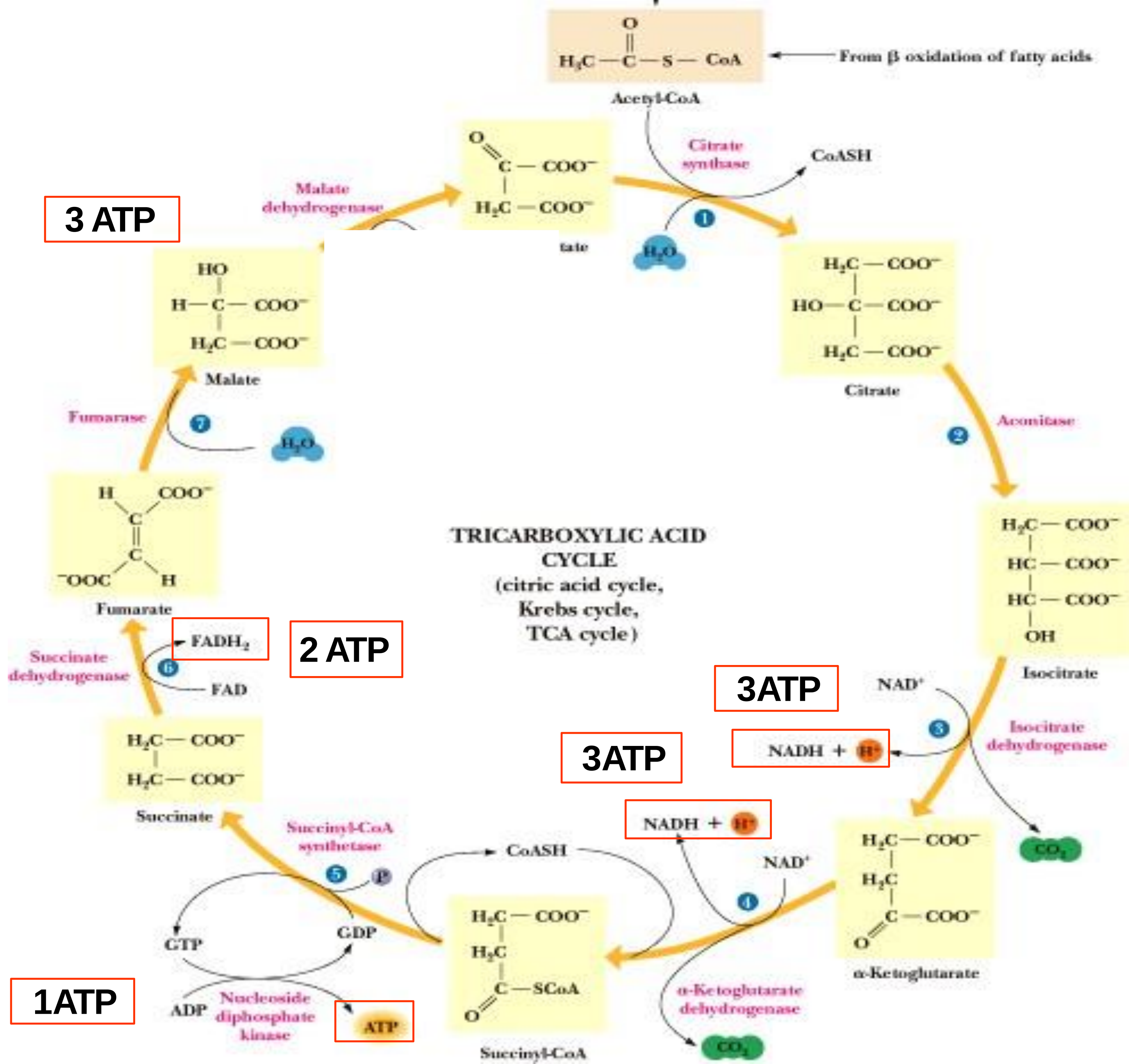

fatty acids
carbohydrates
amino acids
pyruvate
Ketone Bodies
3
4
NH2
N
N
N
N
S
O
N
H
O
N
H
OH
O
O
P
O
P
O
O
O
O-
O-
O
O
-O
P
O
O-
OH
acetyl CoA
1
2
5
Cholesterol
steroids
TCA circle
energy
acetylations
choline
acetylcholine
sulfonamides
acetylsulfonamides
aminosugars
acetylhexosamines
proteins
N-acetylated proteins
internally acetylated proteins
Sources & Fate of Acetyl CoA

# The Citric Acid Cycle

The citric acid cycle is the final common pathway for the oxidation of fuel molecules: amino acids, fatty acids, & carbohydrates.

- Most fuel molecules enter the cycle as acetyl coenzyme A
- It is the gateway to aerobic metabolism for any molecule that can be transformed into an acetyl group or dicarboxylic acid,
- Also known as, Krebs Cycle, & Tricarboxylic Acid Cycle (TCA)

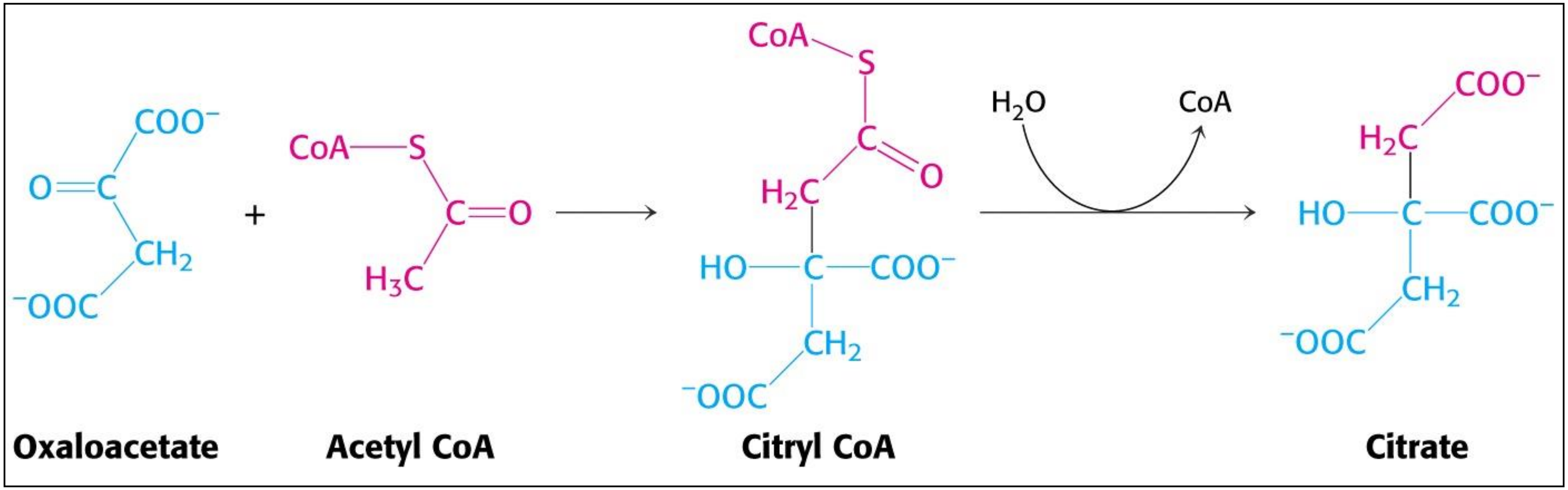

GLYCOGEN
1.
PYRUVATE
3Carbons
10.
ENERGY
9. Oxaloacetate
4Carbons
2. Acetyl CoA
2Carbons
8. Malate
4 Carbons
3. Isocitrate
6Carbons
THE KREBS
OR
CITRIC ACID CYCLE
7. Fumarate
4Carbons
4. 2-Oxoglutarate
5Carbons
4Carbons
6. Succinate
4Carbons
5. Syccinyl CoA



From β oxidation of fatty acids
Acetyl-CoA
Citrate synthase
CoASH
Malate dehydrogenase
3 ATP
Malate
Citrate
Fumarase
Aconitase
Fumarate
TRICARBOXYLIC ACID CYCLE (citric acid cycle, Krebs cycle, TCA cycle)
Isocitrate
FADH2
FAD
2 ATP
Succinate dehydrogenase
NAD+
3ATP
NADH + H+
Isocitrate dehydrogenase
3ATP
Succinate
NADH + H+
NAD+
CO2
Succinyl-CoA synthetase
CoASH
α-Ketoglutarate
GTP
GDP
ADP
Nucleoside diphosphate kinase
ATP
1ATP
α-Ketoglutarate dehydrogenase
CO2
Succinyl-CoA

# Citrate Cycle: step 1 (citrate formation)

Enzyme: Citrate synthase

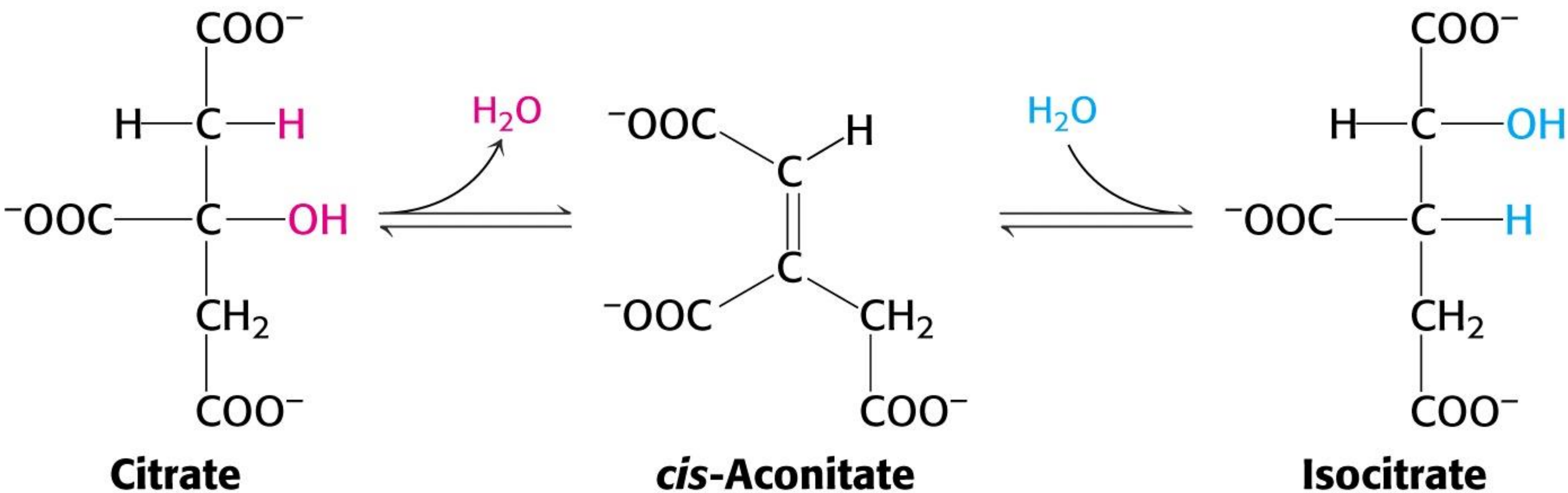



Condensation reaction

Hydrolysis reaction

# Citrate isomerized to Isocitate: step 2

Enzyme: aconitase

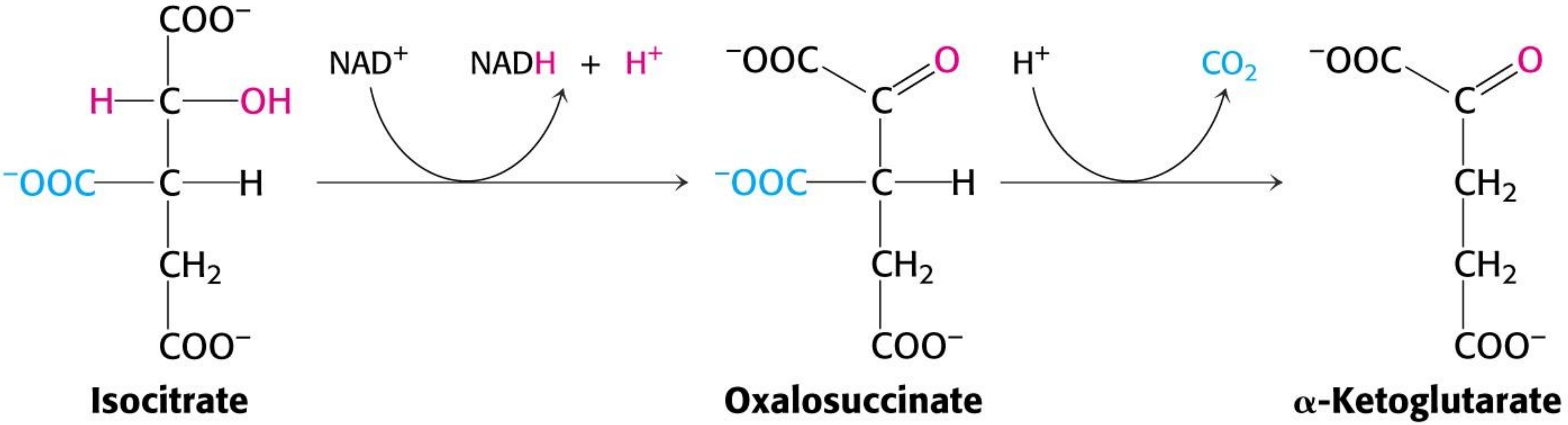



Dehydration

Hydration

# Isocitrate to α-ketoglutarate: step 3

Enzyme: isocitrate dehydrogenase

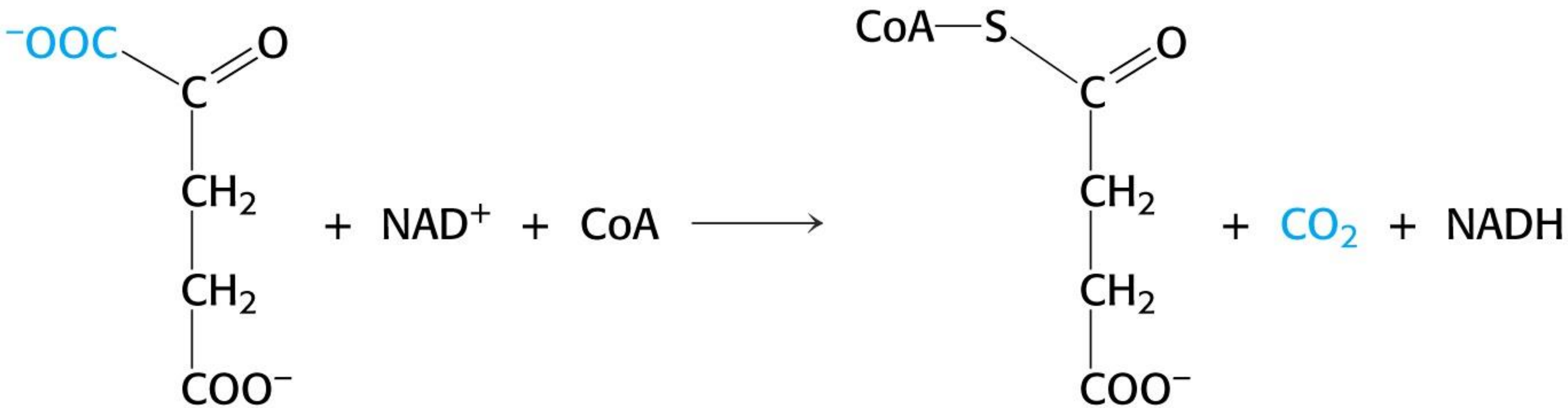



1st NADH produced

1st $CO_2$ removed

# Succinyl CoA formation: step4

Enzyme: $\alpha$-ketoglutarate dehydrogenase



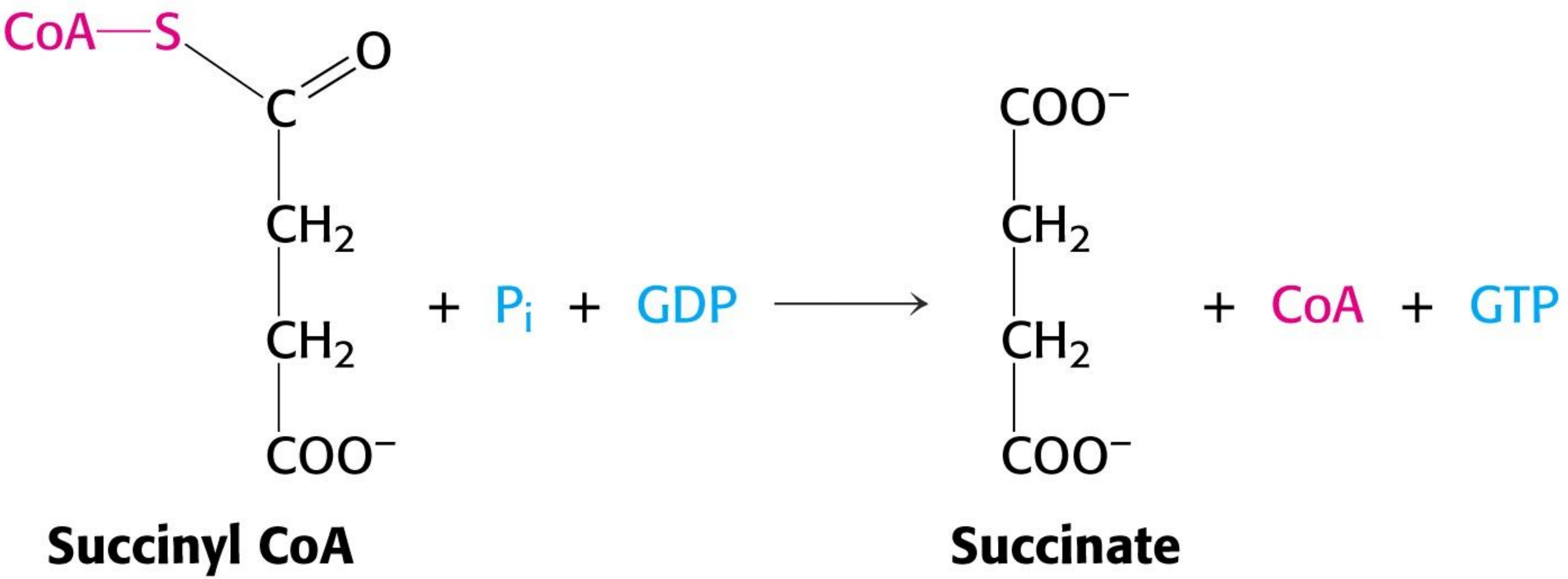

**$\alpha$-Ketoglutarate** **Succinyl CoA**

2nd NADH produced

2nd $CO_2$ removed

# Succinate formation: step5

Enzyme: succinyl CoA synthetase

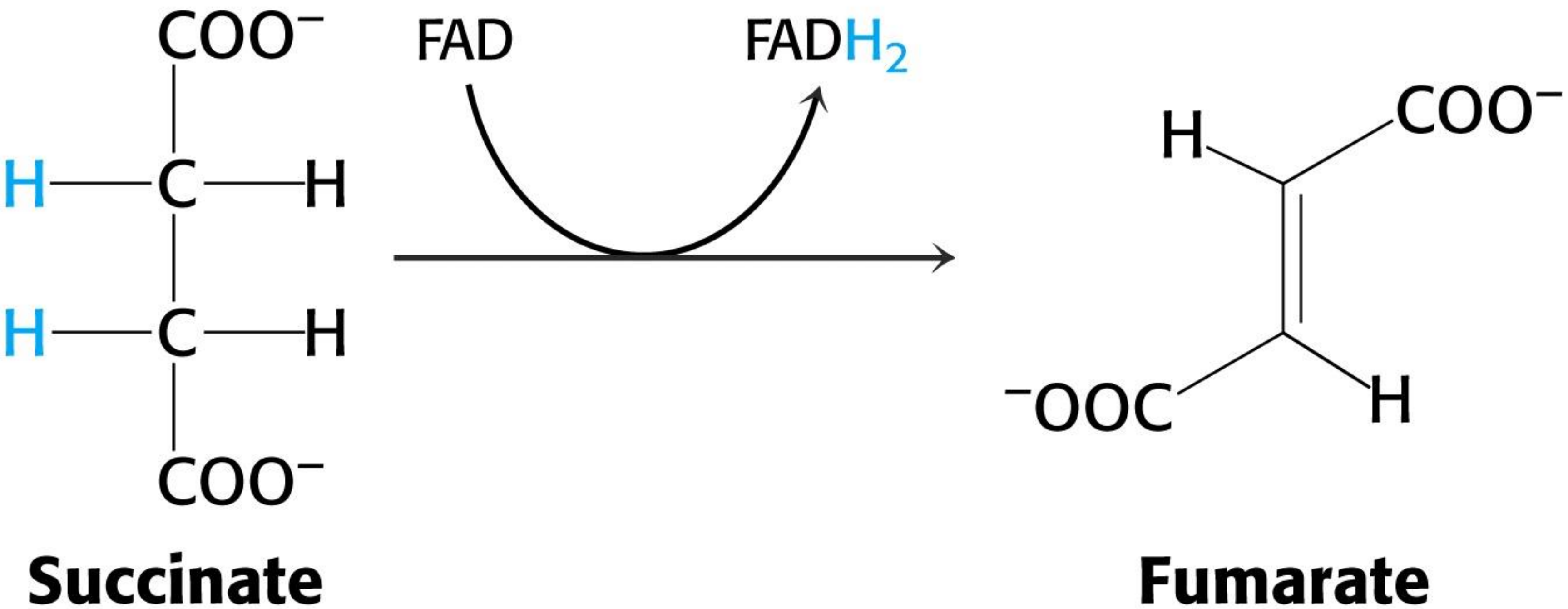



GTP produced

GTP + ADP ⇒ GDP + ATP (NPTase)

# Succinate to Fumarate: step 6

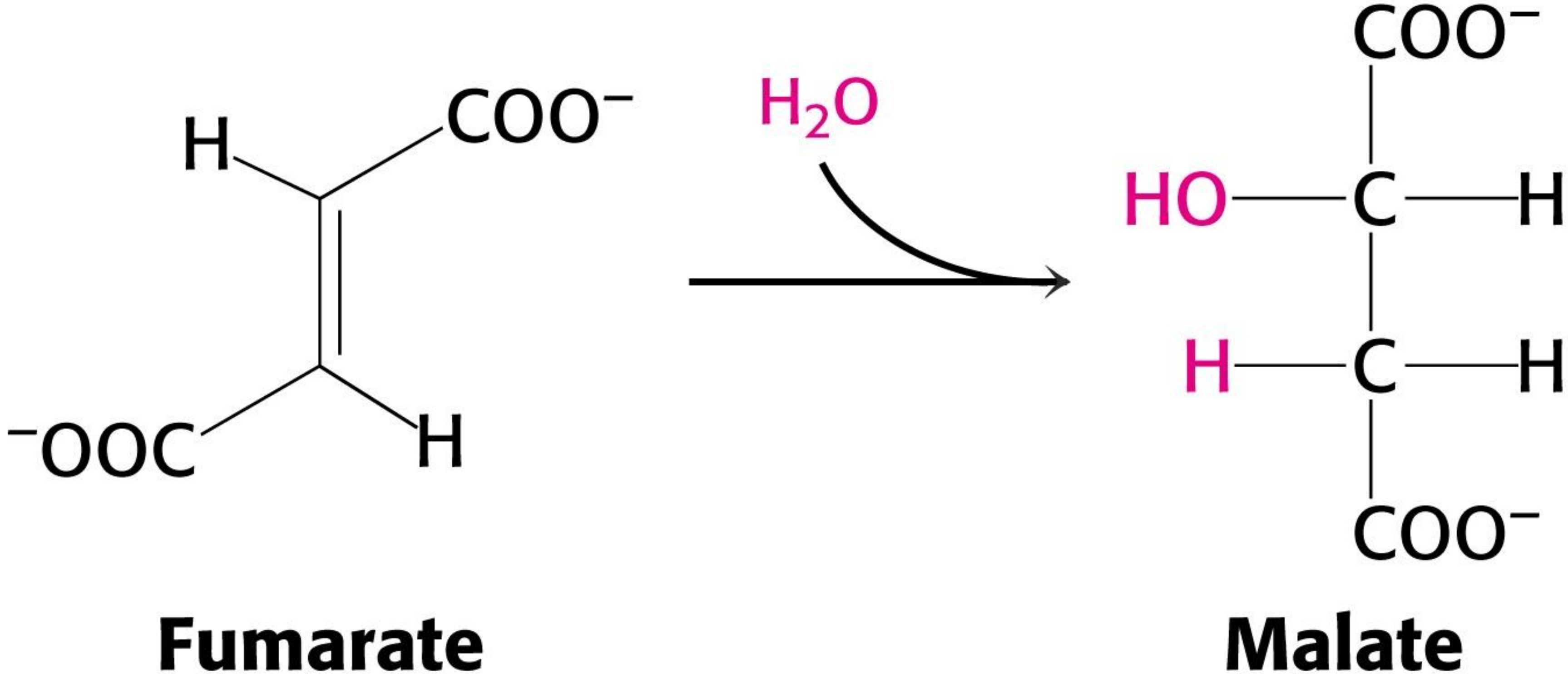
Enzyme: succinate dehydrogenase




$FADH_2$ produced

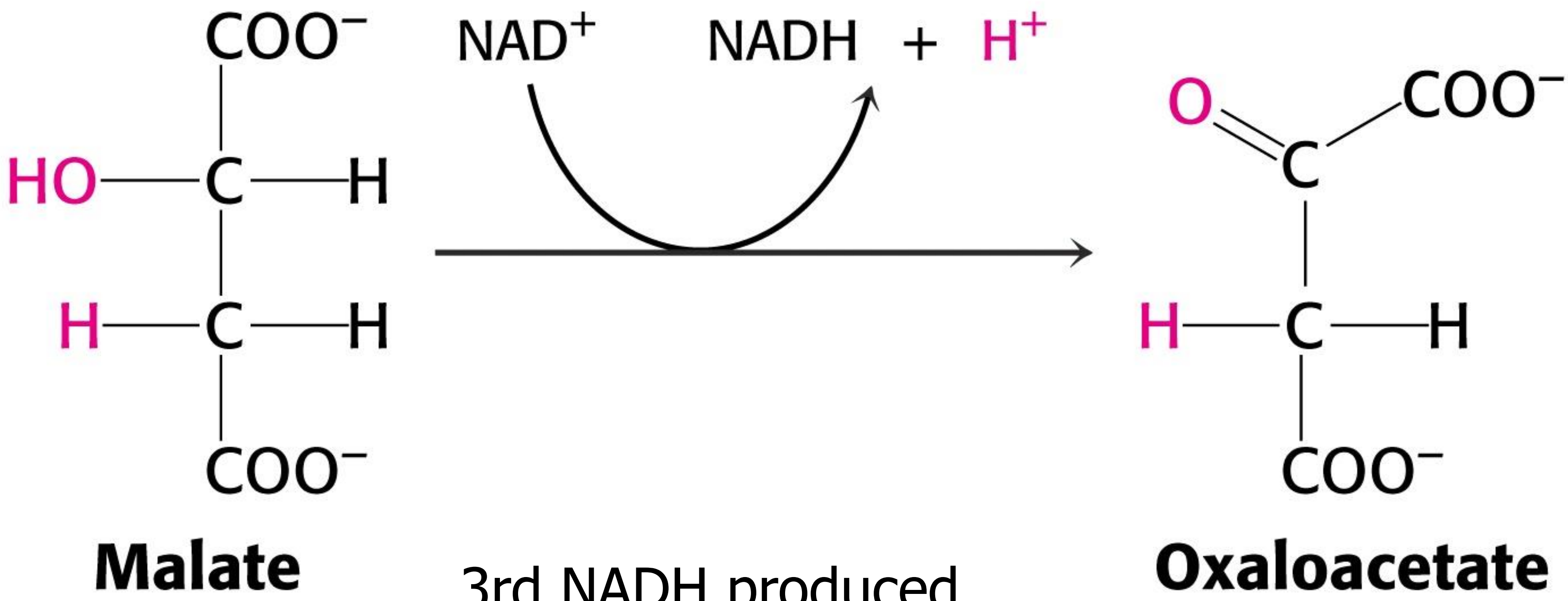
# Fumarate to Malate: step 7

Enzyme: fumarase



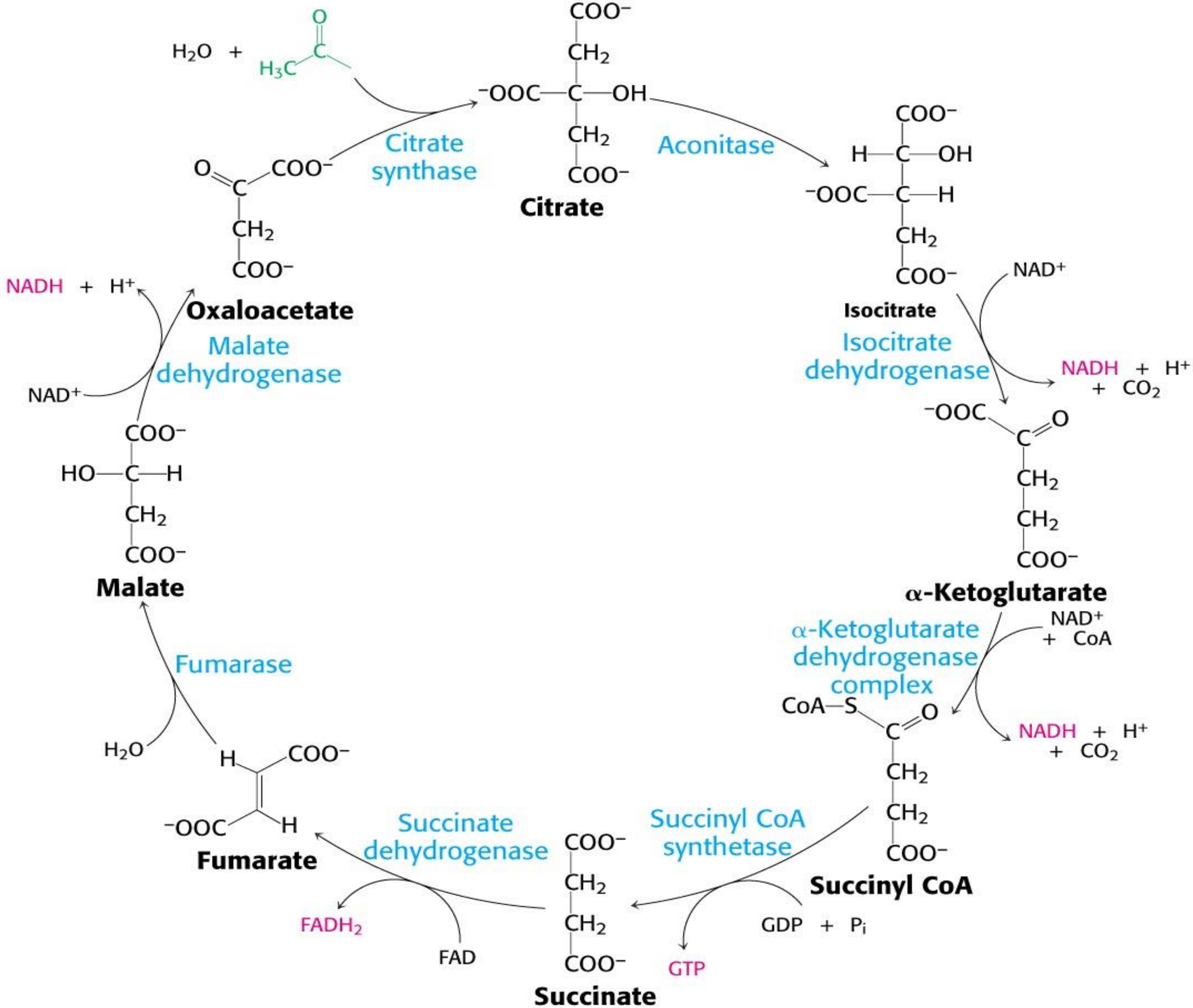
# Malate to Oxalate: step 8

Enzyme: malate dehydrogenase




3rd NADH produced

# The citric acid cycle



# Comments & Biological Significance of TCA Cycle

- **It is the final pathway for oxidation (3rd stage) of all foodstuffs to $CO_2$ + $H_2O$ + Energy.**
- **It is important for the interconversion of carbohydrates, fats & proteins.**
- **All reactions are reversible except: Citrate synthase, Isocitrate DH & $\alpha$-Ketoglutarate DH.**
- **The rate limiting enzyme is Citrate synthase.**

# Comments & Biological Significance of TCA Cycle

- **TCA is the major source of succinyl Co A which used for:**
  - **Heme synthesis.**
  - **Ketolysis.**
  - **Detoxication reactions.**

# Regulation of Kreb's Cycle

1. **Insulin activates pyruvate DH & inhibits pyruvate carboxylase, thus directing pyruvate towards complete oxidation through kreb's cycle.**
2. **Acetyl Co A inhibits pyruvate DH & activates pyruvate carboxylase, thus directing pyruvate & glucose towards formation of oxaloacetate to combine with excess Acetyl Co A for the optimal activity of kreb's cycle.**

- **During starvation (glucose supply is low & fat oxidation provides excess Acetyl Co A), so oxaloacetate is required.**

# Regulation of Kreb's Cycle

3. So, Kreb's cycle is <u>inhibited</u> by:

   a) Starvation (<u>No carbohydrates</u>).

   b) Diabetes mellitus (<u>No insulin</u>).

   c) Anaerobic conditions (<u>No oxygen</u>).

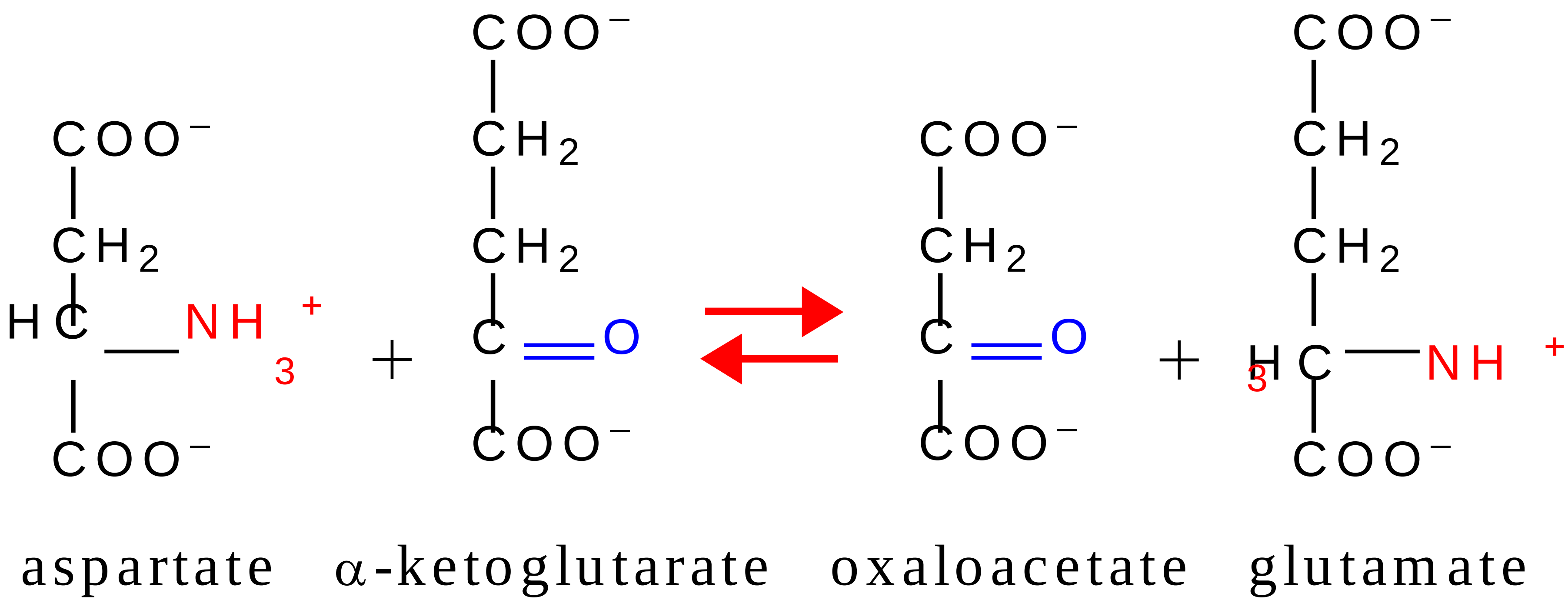
4. It is inhibited *in vitro* by fluroacetate & iodoacetate which form flurocitrate & iodocitrate that inhibits aconitase.

5. Malonic acid is a competitive inhibitor of succinate dehydrogenase.

6. Arsenite inhibits Kreb's cycle.

# Sources of Oxaloacetate

1. **Pyruvate, in mitochondria (Pyruvate carboxylase).**
2. **Malate, in mitochondria, by malate DH.**
3. **Citric acid, in cytoplasm (ATP-Citrate lyase)**
4. **Aspartic acid, by transamination in both cytoplasm & mitochondria.**




Aminotransferase (Transaminase)

# Fate of $CO_2$

1. Excretion through lungs (main fate).
2. Combined with ammonia to form urea.
3. Combined with ammonia to form pyrimidine.
4. Enters in the formation of $C_6$ of purines.
5. Fixation into organic acids (Carboxylation):
    1. Pyruvic acid + $CO_2$ → Oxaloacetic acid.
    2. Acetyl Co A + $CO_2$ → Malonyl Co A.
    3. Propionyl Co A + $CO_2$ → Methylmalonyl Co A.

# Summary of Products in the Citric Acid Cycle

In the citric acid cycle:

- Oxaloacetate bonds with an acetyl group to form citrate.
- Two decarboxylations remove two carbons as $2CO_2$.
- Four oxidations provide hydrogen for 3NADH and one $FADH_2$.
- A direct phosphorylation forms GTP.

# ATP from Glycolysis

| Reaction Pathway | ATP for One Glucose |
|---|---|
| **ATP from Glycolysis** | |
| Activation of glucose | -2 ATP |
| Oxidation of 2 NADH | 6 ATP |
| Direct ADP phosphorylation (two triose) | 4 ATP |

**Summary:**

$$\underset{\text{glucose}}{C_6H_{12}O_6} \longrightarrow 2\ \text{pyruvate} + 2H_2O + 8\ \text{ATP}$$

# ATP from Two Pyruvate

**Under aerobic conditions:**

- 2 pyruvate are oxidized to 2 acetyl CoA and 2 NADH.
- 2 NADH enter electron transport to provide 6 ATP.

**Summary:**

$$2 \text{ Pyruvate} \longrightarrow 2 \text{ Acetyl CoA} + 6 \text{ ATP}$$

# ATP from Citric Acid Cycle

| Reaction Pathway | ATP for One Glucose |
|---|---|
| **ATP from Citric Acid Cycle** | |
| Oxidation of 2 isocitrate (2NADH) | 6 ATP |
| Oxidation of 2 α-ketoglutarate (2NADH) | 6 ATP |
| 2 Direct substrate phosphorylations (2GTP) | 2 ATP |
| Oxidation of 2 succinate ($2FADH_2$) | 4 ATP |
| Oxidation of 2 malate (2NADH) | 6 ATP |

**Summary:** 2Acetyl CoA $\longrightarrow$ $4CO_2 + 2H_2O$ + 24 ATP

# ATP from Glucose

One glucose molecule undergoing complete oxidation provides:

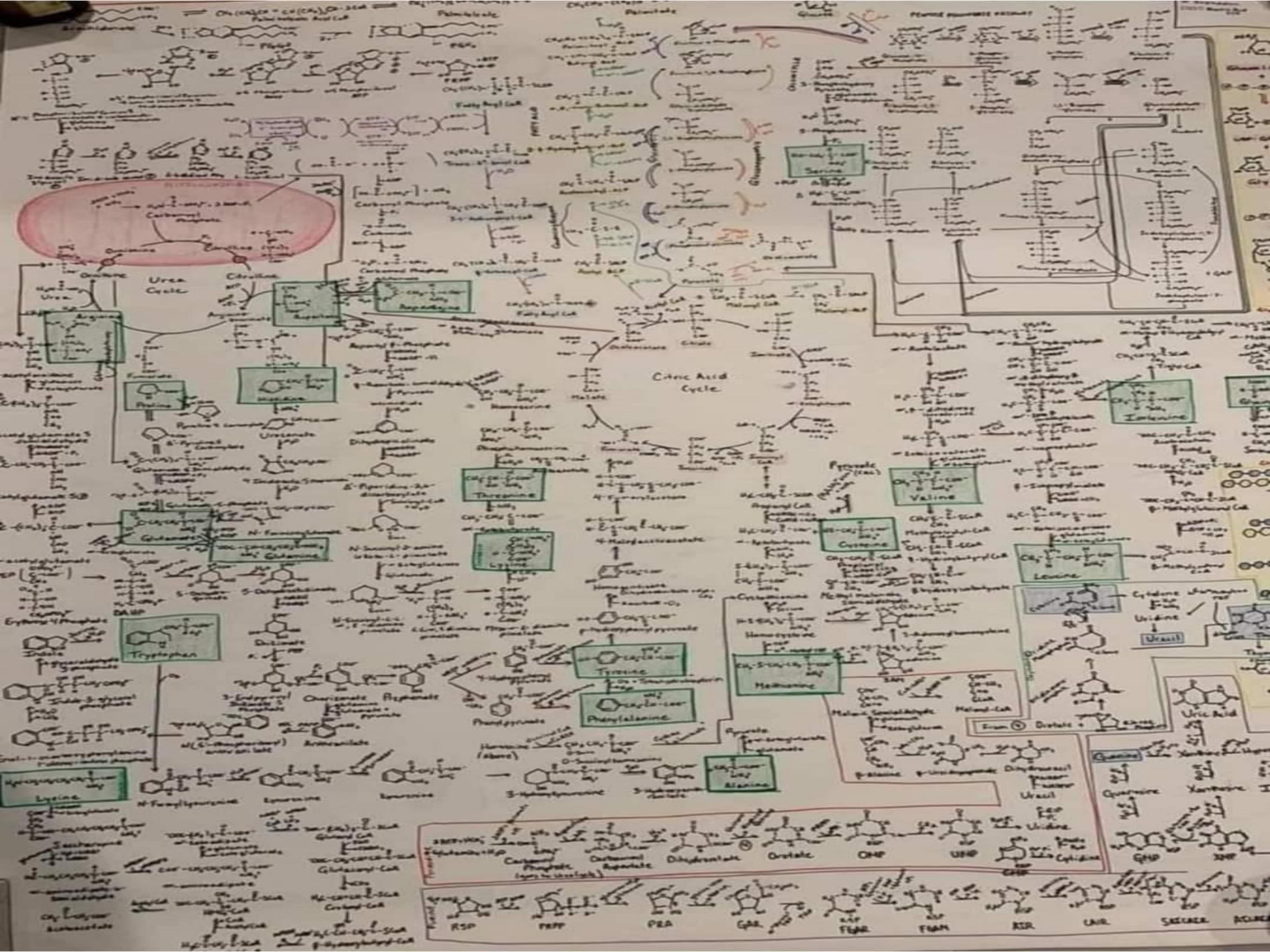
| | |
|---|---|
| From glycolysis | 8 ATP |
| From 2 Pyruvate | 6 ATP |
| From 2 Acetyl CoA | 24 ATP |

**Overall ATP Production for One Glucose:**

$$C_2H_{12}O_6 + 6O_2 + 36ADP + 36P_i \longrightarrow$$

Glucose

$$6CO_2 + 6H_2O + 38ATP$$



Palmitate
Fatty Acyl CoA
Urea Cycle
Ornithine
Citrulline
Serine
Citric Acid Cycle
Isoleucine
Threonine
Valine
Cysteine
Leucine
Glutamine
Proline
Tryptophan
Indole
Chorismate
Prephenate
Anthranilate
Tyrosine
Phenylalanine
Methionine
Alanine
Lysine
Uridine
Uracil
Uric Acid
Dihydrouracil
Guanine
Xanthine
Orotate
OMP
UMP
Cytidine
GMP
XMP
R5P
PRPP
PRA
GAR
FGAR
FGAM
AIR