GLYCOLYSIS

Where does cellular respiration take place?

PATHWAY	EUKARYOTE	PROKARYOTE
GLYCOLYSIS	CYTOPLASM	CYTOPLASM
INTERMEDIATE STEP	CYTOPLASM	CYTOPLASM
KREB's CYCLE	MITOCHONDRIAL MATRIX	CYTOPLASM
ETC	MITOCHONDRIAL INNER MEMBRANE	PLASMA MEMBRANE

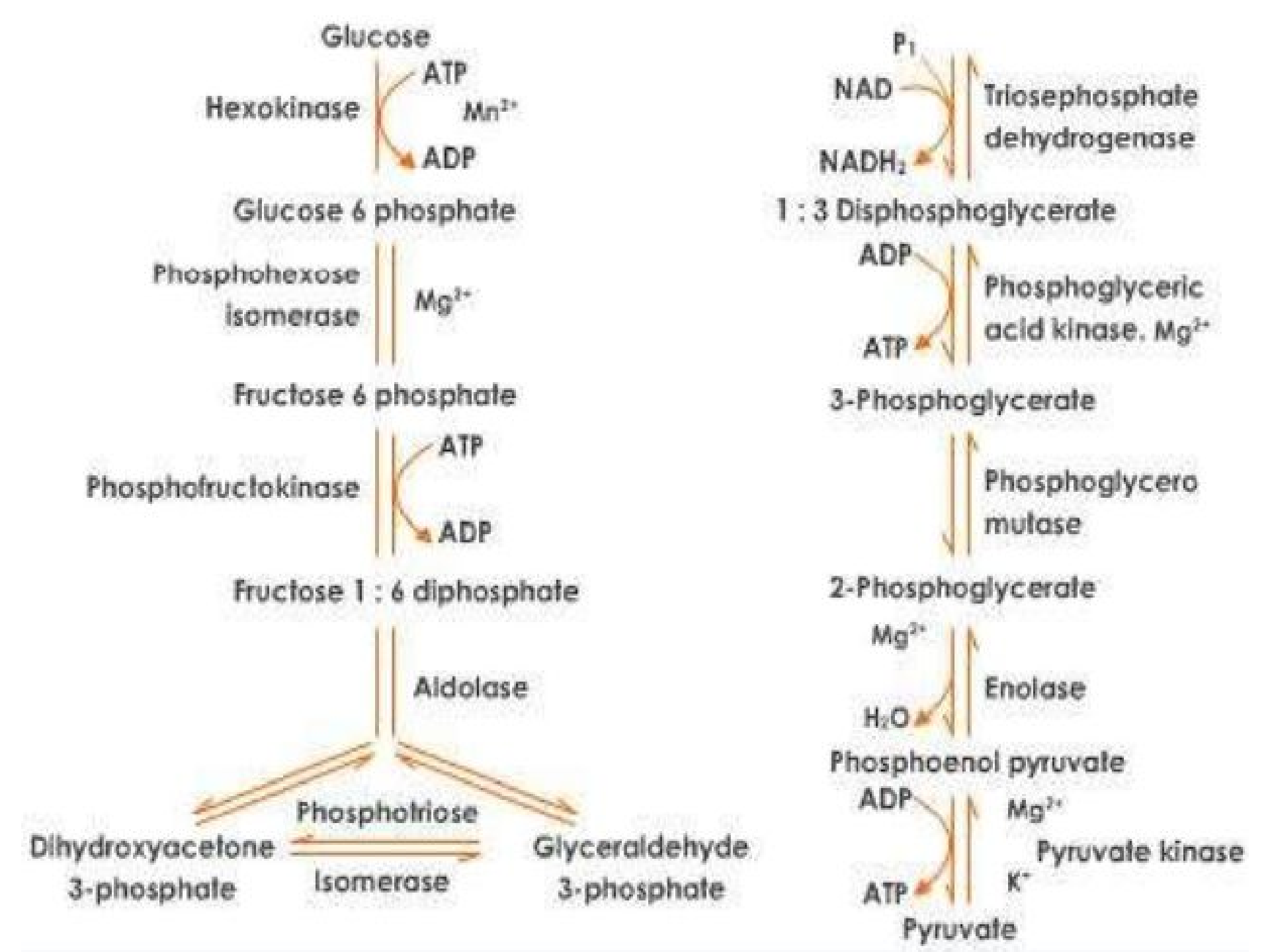
- **Glycolysis** breaks down glucose and forms pyruvate with the production of two molecules of ATP.

- The pyruvate end product of **glycolysis** can be used in either anaerobic respiration if no oxygen is available or in aerobic respiration via the TCA cycle.

- **Phases of glycolysis**

- The pay-off **phase** is where ATP is produced.
- The first five steps of the **glycolysis** reaction is known as the **preparatory or investment phase**.

- This **stage** consumes energy to convert the glucose molecule into two molecules three-carbon sugar molecule.
- The step one in **glycolysis** is phosphorylation.


Glucose
ATP
Hexokinase
Mn^{2+}
ADP
Glucose 6 phosphate
Phosphohexose isomerase
Mg^{2+}
Fructose 6 phosphate
ATP
Phosphofructokinase
ADP
Fructose 1 : 6 diphosphate
Aldolase
Phosphotriose
Isomerase
Dihydroxyacetone 3-phosphate
Glyceraldehyde 3-phosphate
P_i
NAD
$NADH_2$
Triosephosphate dehydrogenase
1 : 3 Disphosphoglycerate
ADP
ATP
Phosphoglyceric acid kinase. Mg^{2+}
3-Phosphoglycerate
Phosphoglycero mutase
2-Phosphoglycerate
Mg^{2+}
H_2O
Enolase
Phosphoenol pyruvate
ADP
ATP
Mg^{2+}
Pyruvate kinase
K^+
Pyruvate

- Glycolysis literally means "splitting sugars" and is the process of releasing energy within sugars.
- In glycolysis, glucose (a six carbon sugar) is split into two molecules of the three-carbon sugar pyruvate.

- Glycolysis can occur with or without oxygen.

- In the presence of oxygen, glycolysis is the first stage of cellular respiration.
- In the abscence of oxygen, glycolysis allows cells to make small amounts of ATP through the process of fermentation.
- Glycolysis takes place in the cytosol of the cell's cytoplasm.

- the next stage of cellular respiration known as the citric acid cycle, occurs in the matrix of cell mitochondria.
- Below are the 10 steps of glycolysis:

- **STEP 1**
- The enzyme hexokinase phosphorylates adds a phosphate group to glucose in the cell's cytoplasm. In the process, a phosphate group from ATP is transferred to glucose producing glucose 6-phosphate.
- **Glucose ($C_6H_{12}O_6$) + hexokinase + ATP → ADP + Glucose 6-phosphate ($C_6H_{13}O_9P$)**

- **STEP 2**
- The enzyme phospho gluco isomerase converts glucose 6-phosphate into its isomer fructose 6-phosphate. Isomers have the same molecular formula, but the atoms of each molecule are arranged differently.
- **Glucose 6-phosphate ($C_6H_{13}O_9P$) + Phosphoglucoisomerase → Fructose 6-phosphate ($C_6H_{13}O_9P$)**

- **STEP 3**
- The enzyme phospho fructokinase uses another ATP molecule to transfer a phosphate group to fructose 6-phosphate to form fructose 1, 6-bisphosphate.
- **Fructose 6-phosphate ($C_6H_{13}O_9P$) + phosphofructokinase + ATP → ADP + Fructose 1, 6-bisphosphate ($C_6H_{14}O_{12}P_2$)**

- **STEP 4**
- The enzyme aldolase splits fructose 1, 6-bisphosphate into two sugars that are isomers of each other. These two sugars are dihydroxy acetone phosphate and glyceraldehyde phosphate.

- **Fructose 1, 6-bisphosphate ($C_6H_{14}O_{12}P_2$) + aldolase → Dihydroxyacetone phosphate ($C_3H_7O_6P$) + Glyceraldehyde phosphate ($C_3H_7O_6P$)**

- **STEP 5**
- The enzyme triose phosphate isomerase rapidly inter-converts the molecules dihydroxy acetone phosphate and glyceraldehyde 3-phosphate.
- Glyceraldehyde 3-phosphate is removed as soon as it is formed to be used in the next step of glycolysis.
- **Dihydroxy acetone phosphate ($C_3H_7O_6P$) → Glyceraldehyde 3-phosphate ($C_3H_7O_6P$)**

- **Net result for steps 4 and 5: Fructose 1, 6-bisphosphate ($C_6H_{14}O_{12}P_2$) ↔ 2 molecules of glyceraldehyde 3-phosphate ($C_3H_7O_6P$)**

- **STEP 6**
- The enzyme triose phosphate dehydrogenase serves **two functions** in this step.
- **First the** enzyme transfers a hydrogen (H^-) from glyceraldehyde phosphate to the oxidizing agent nicotinamide adenine dinucleotide (NAD^+) to form NADH.

- **Next triose** phosphate dehydrogenase adds a phosphate (P) from the cytosol to the oxidized glyceraldehyde phosphate to form 1, 3-bisphosphoglycerate. This occurs for both molecules of glyceraldehyde 3-phosphate produced in step 5.

- **A. Triose phosphate dehydrogenase + 2 H^- + 2 NAD^+ → 2 NADH + 2 H^+**
- **B. Triose phosphate dehydrogenase + 2 P + 2 glyceraldehyde 3-phosphate ($C_3H_7O_6P$) → 2 molecules of 1,3-bisphosphoglycerate ($C_3H_8O_{10}P_2$)**

- **STEP 7**
- The enzyme phospho glycerokinase transfers a P from 1,3-bisphosphoglycerate to a molecule of ADP to form ATP. This happens for each molecule of 1,3-bisphosphoglycerate. The process yields two 3-phosphoglycerate molecules and two ATP molecules.
- **2 molecules of 1,3-bisphoshoglycerate ($C_3H_8O_{10}P_2$) + phosphoglycerokinase + 2 ADP → 2 molecules of 3-phosphoglycerate ($C_3H_7O_7P$) + 2 ATP**

- **STEP 8**
- The enzyme phospho glyceromutase relocates the P from 3-phosphoglycerate from the third carbon to the second carbon to form 2-phosphoglycerate.
- **2 molecules of 3-Phosphoglycerate ($C_3H_7O_7P$) + phosphoglyceromutase → 2 molecules of 2-Phosphoglycerate ($C_3H_7O_7P$)**

- **STEP 9**
- The enzyme enolase removes a molecule of water from 2-phosphoglycerate to form phospho enolpyruvate (PEP). This happens for each molecule of 2-phosphoglycerate.
- **2 molecules of 2-Phosphoglycerate ($C_3H_7O_7P$) + enolase → 2 molecules of phospho enolpyruvate (PEP) ($C_3H_5O_6P$)**

- **STEP 10**

- The enzyme pyruvate kinase transfers a P from PEP to ADP to form pyruvate and ATP. This happens for each molecule of phosphoenolpyruvate. This reaction yields 2 molecules of pyruvate and 2 ATP molecules.
- **2 molecules of phosphoenolpyruvate ($C_3H_5O_6P$) + pyruvate kinase + 2 ADP → 2 molecules of pyruvate ($C_3H_3O_3^-$) + 2 ATP**

- a single glucose molecule in glycolysis produces a total of 2 molecules of pyruvate, 2 molecules of ATP, 2 molecules of NADH and 2 molecules of water.

- Although 2 ATP molecules are used in steps 1-3, 2 ATP molecules are generated in step 7 and 2 more in step 10.
-
- This gives a total of 4 ATP molecules produced.

- If subtract the 2 ATP molecules used in steps 1-3 from the 4 generated at the end of step 10, end up with a net total of 2 ATP molecules produced.

- Glycolysis:

- Energy balance sheet

- Hexokinase: - 1 ATP
- Phosphofructokinase: -1 ATP
- GAPDH: +2 NADH

- Phsophoglycerate kinase: +2 ATP
- Pyruvate kinase: +2 ATP
- Total/ molecule of glucose: +2 ATP, +2 NADH

- Lactate Fermentation

- Formation of lactate catalyzed by lactate dehydrogenase:

- In highly active muscle, there is anaerobic glycolysis because the supply of O2 cannot keep up with the demand for ATP.
- Lactate builds up causing a drop in pH
- which inactivates glycolytic enzymes.
- End result is energy deprivation and cell death; the symptoms being pain and fatigue of the muscle.
- Lactate is transported to the liver where it can be reconverted to pyruvate by the LDH reverse reaction

- Ethanol fermentation
- Formation of ethanol catalyzed by 2 enzymes

- Pyruvate decarboxylase catalyzes the first irreversible reaction to form acetaldehyde

- Acetaldehyde is reduced by alcohol dehydogenase is a reversible reaction: CH3-CHO + NADH + H+ E + CH3CH2OH + NAD+
- Ethanol fermentation is used during wine-making

Kreb’s CYCLE

- The **Krebs cycle** (or **citric acid cycle**) is a part of cellular respiration.
- Named after Hans **Krebs**, it is a series of chemical reactions used by all aerobic organisms to generate energy.
- Its importance to many biochemical pathways.
- it was one of the earliest parts of cellular metabolism to evolve.
- **Definition of Krebs** cycle. :
- a sequence of reactions in the living organism in which oxidation of acetic acid or acetyl equivalent provides energy for storage in phosphate bonds (as in ATP) —called also **citric acid** cycle, tricarboxylic acid cycle.

- The **Krebs cycle** occurs in the mitochondrial matrix and generates chemical energy (ATP, NADH, and $FADH_2$) from the oxidation of pyruvate, the end product of glycolysis.

- Pyruvate is transported into the mitochondria and loses carbon dioxide to form acetyl-CoA, a 2-carbon molecule.

- **CITRIC ACID CYCLE STEPS**

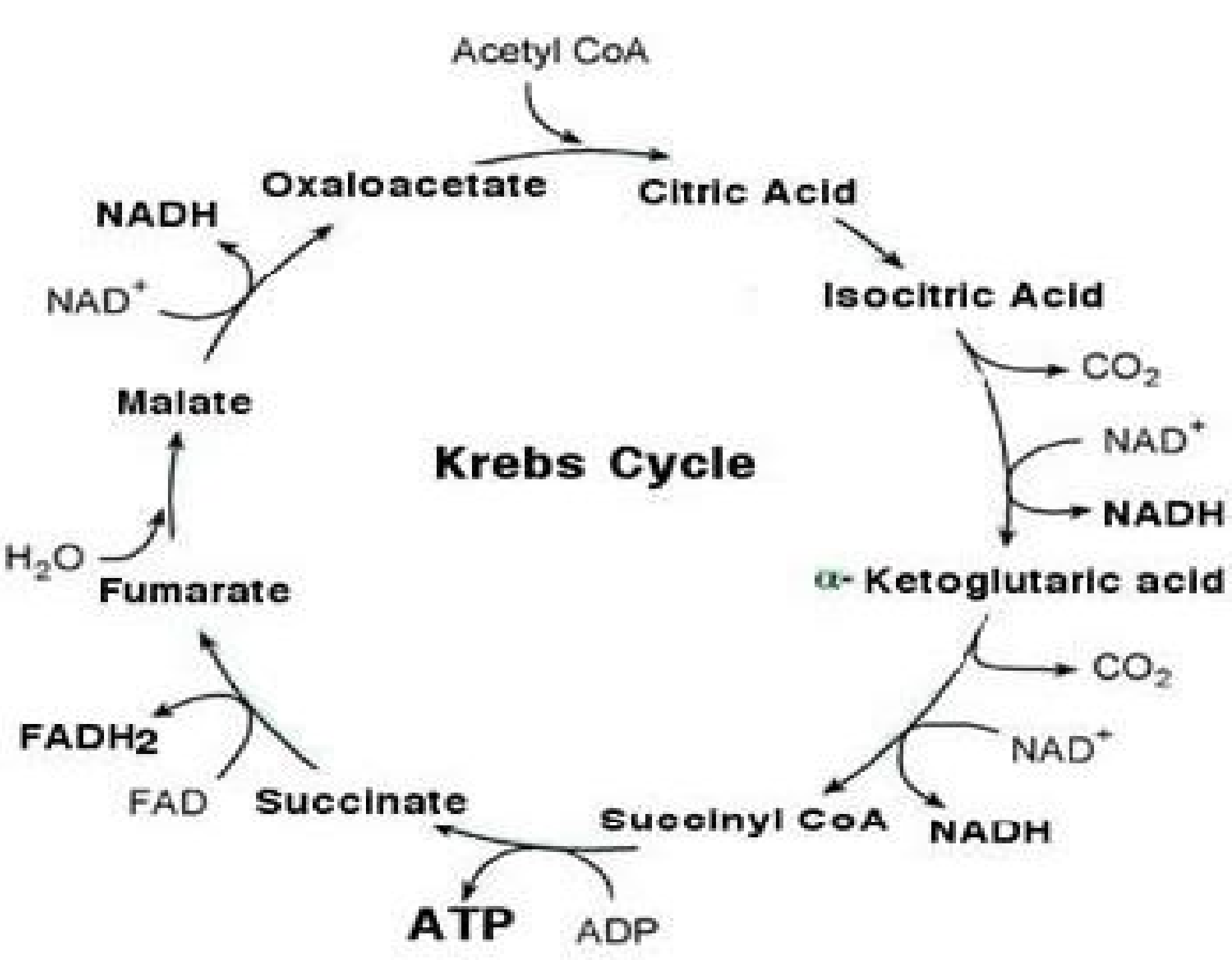
- The first phase of cellular respiration called glycolysis takes place in the cytosol of the cell's cytoplasm.

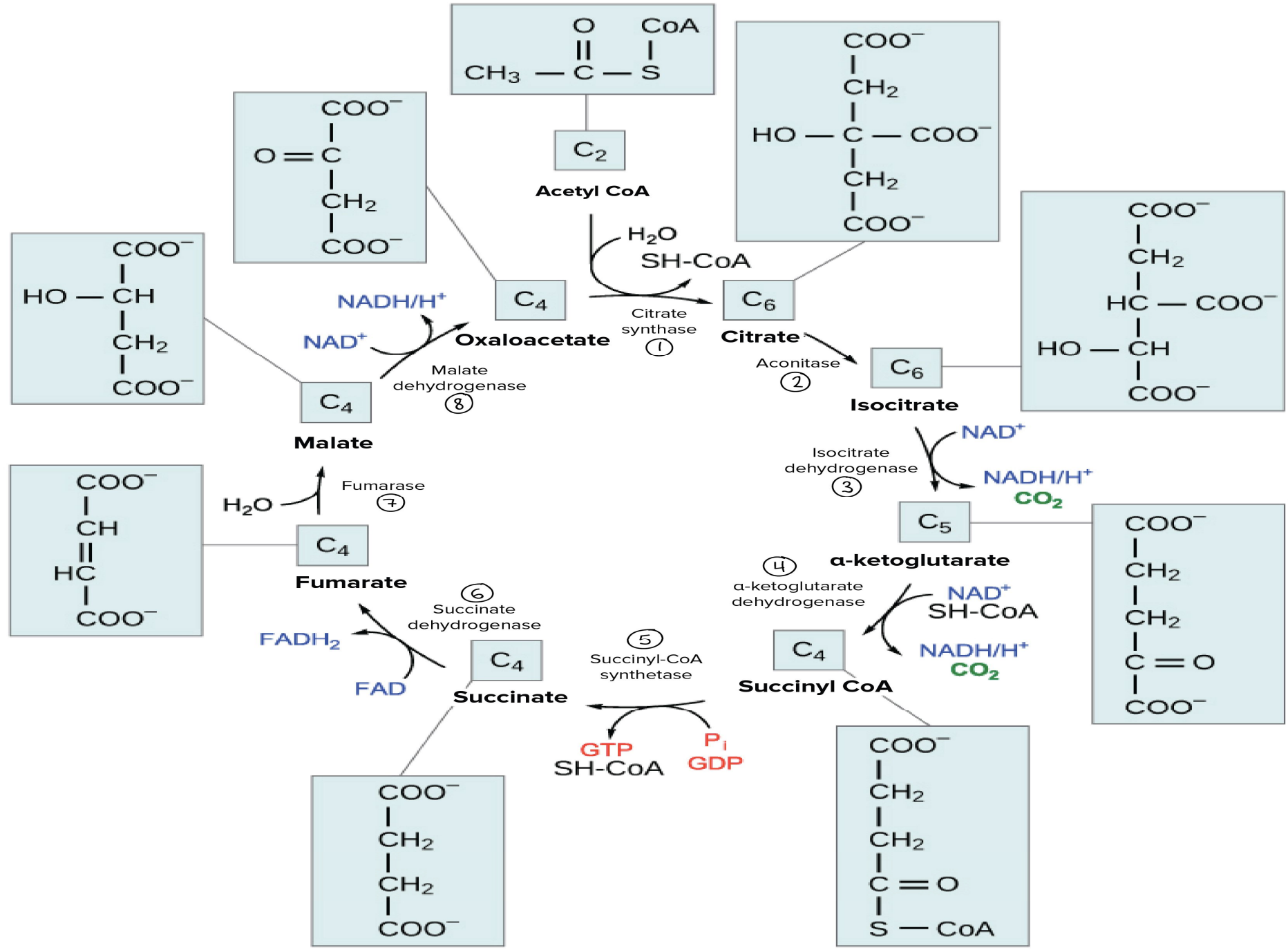
- The citric acid cycle, occurs in the matrix of cell mitochondria.

- Prior to the beginning of the citric acid cycle, pyruvic acid generated in glycolysis crosses the mitochondrial membrane and is used to form **acetyl coenzyme A (acetyl CoA)**.

- Acetyl CoA is then used in the first step of the citric acid cycle. Each step in the cycle is catalyzed by a specific enzyme.

- Acetyl-CoA, main product of the Pyruvate Dehydrogenase Complex in aerobic respiration
- The mechanism of the synthesis of acetyl coenzyme A from pyruvate requires five coenzymes and three enzymes.


Acetyl CoA
Citric Acid
Isocitric Acid
CO_2
NAD^+
NADH
α- Ketoglutaric acid
CO_2
NAD^+
NADH
Succinyl CoA
ATP
ADP
Succinate
FAD
$FADH_2$
Fumarate
H_2O
Malate
NAD^+
NADH
Oxaloacetate
Krebs Cycle


Acetyl CoA
C_2
H_2O
SH-CoA
Citrate synthase
1
C_6
Citrate
Aconitase
2
C_6
Isocitrate
Isocitrate dehydrogenase
3
NAD^+
$NADH/H^+$
CO_2
C_5
α-ketoglutarate
4
α-ketoglutarate dehydrogenase
NAD^+
SH-CoA
$NADH/H^+$
CO_2
C_4
Succinyl CoA
5
Succinyl-CoA synthetase
GTP
SH-CoA
P_i
GDP
C_4
Succinate
6
Succinate dehydrogenase
$FADH_2$
FAD
C_4
Fumarate
H_2O
7
Fumarase
C_4
Malate
$NADH/H^+$
NAD^+
Malate dehydrogenase
8
C_4
Oxaloacetate

- **Step 1** -
- The two-carbon acetyl group of acetyl CoA is added to the four-carbon **oxalo acetate** to form the six-carbon citrate.
- The conjugate acid of citrate is citric acid, hence the name citric acid cycle.
- Oxaloacetate is regenerated at the end of the cycle so that the cycle may continue. Enzyme: citrate synthase.
- **Step 2** -
- **Citrate** loses a molecule of water and another is added. In the process, citric acid is converted to its isomer isocitrate. Enzyme: aconitase.
- **Step 3** -

- **Isocitrate** loses a molecule of carbon dioxide (CO_2) and is oxidized forming the five-carbon alpha keto glutarate. Nicotinamide adenine dinucleotide (NAD^+) is reduced to NADH + H^+ in the process. Enzyme: isocitrate dehydrogenase.
- **Step 4** -
- **Alpha ketoglutarate** is converted to the 4-carbon succinyl CoA. A molecule of CO_2 is removed and NAD^+ is reduced to NADH + H^+ in the process. Enzyme: alpha ketoglutarate dehydrogenase.

- **Step 5** -
- CoA is removed from the **succinyl CoA** molecule and is replaced by a phosphate group.
- The phosphate group is then removed and attached to guanosine diphosphate (GDP) thereby forming guanosine triphosphate (GTP).
- Like ATP, GTP is an energy-yielding molecule and is used to generate ATP when it donates a phosphate group to ADP. The final product from the removal of CoA from succinyl CoA is **succinate**. Enzyme: succinyl-CoA synthetase.

- **Step 6** -
- Succinate is oxidized and **fumarate** is formed. Flavin adenine dinucleotide (FAD) is reduced and forms $FADH_2$ in the process. Enzyme: succinate dehydrogenase.

- **Step 7** -
- A water molecule is added and bonds between the carbons in fumarate are rearranged forming **malate**. Enzyme: fumarase.

- **Step 8** -
- Malate is oxidized forming **oxaloacetate**, the beginning substrate in the cycle. NAD^+ is reduced to NADH + H^+ in the process. Enzyme: malate dehydrogenase.

The cycle is a series of enzyme controlled reactions:

In Step 1 - Citrate (6C) is formed from acetyl CoA + oxaloacetate(4C)

In Steps 2-8 Citrate is
decarboxylated to yield CO_2 gas
and dehydrogenated to release Hydrogen ions

these will reduce NAD and FAD

Oxaloacetate is regenerated
One ATP is formed by phosphorylation

The Krebs Cycle will go around twice for each molecule of glucose

- The maximum **yield** of ATP through oxidation of one molecule of glucose in glycolysis, **citric acid cycle**, and oxidative phosphorylation is 38 (assuming 3 molar equivalents of ATP per equivalent NADH and 2 ATP per $FADH_2$).

- The **Krebs cycle produces** two molecules of **ATP** for every molecule of glucose.

- The **Krebs cycle** also **produces** eight molecules of NADH and two molecules of FADH2 per molecule of glucose.

Reactions	No.of ATP formed
1. 2 isocitrate → 2 α-ketoglutarate (2 NADH + 2H⁺) (2 × 3)	6
2. 2 α-ketoglutarate → 2 succinyl CoA (2 NADH + 2H⁺) (2 × 3)	6
3. 2 succinyl CoA → 2 succinate (2 GTP = 2ATP)	2
4. 2 succinate → 2 Fumarate ($2 FADH_2$) (2 × 2)	4
5. 2 malate → 2 oxaloacetate (2 NADH + 2H⁺) (2 × 3)	6
Total No.of ATP formed	24

Sl. No.	Stages of respiration	Number of molecules of			Total number of ATP obtained
		ATP	NADH	FADH	
A.	Glycolysis	2	2	–	8
B.	Oxidative decarboxylation of pyruvic acid	–	2	–	6
C.	Krebs cycle	2	6	2	24
	Total	4	30 ATP	4 ATP	38

Energy Yield

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

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.

†This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19–30 and 19–31.

Table 16-1

Lehninger Principles of Biochemistry, Fifth Edition

Electron transport chain

- An **electron transport** chain (ETC) is a series of complexes that transfer **electrons** from **electron** donors to **electron** acceptors via redox (both reduction and oxidation occurring simultaneously) reactions, and couples this **electron** transfer with the transfer of protons (H^+ ions) across a membrane.

- This creates an electrochemical proton gradient that drives the synthesis of adenosine triphosphate (ATP), a molecule that stores energy chemically .

- The electron transport chain uses the electrons from electron carriers to create a chemical gradient that can be used to power oxidative phosphorylation.

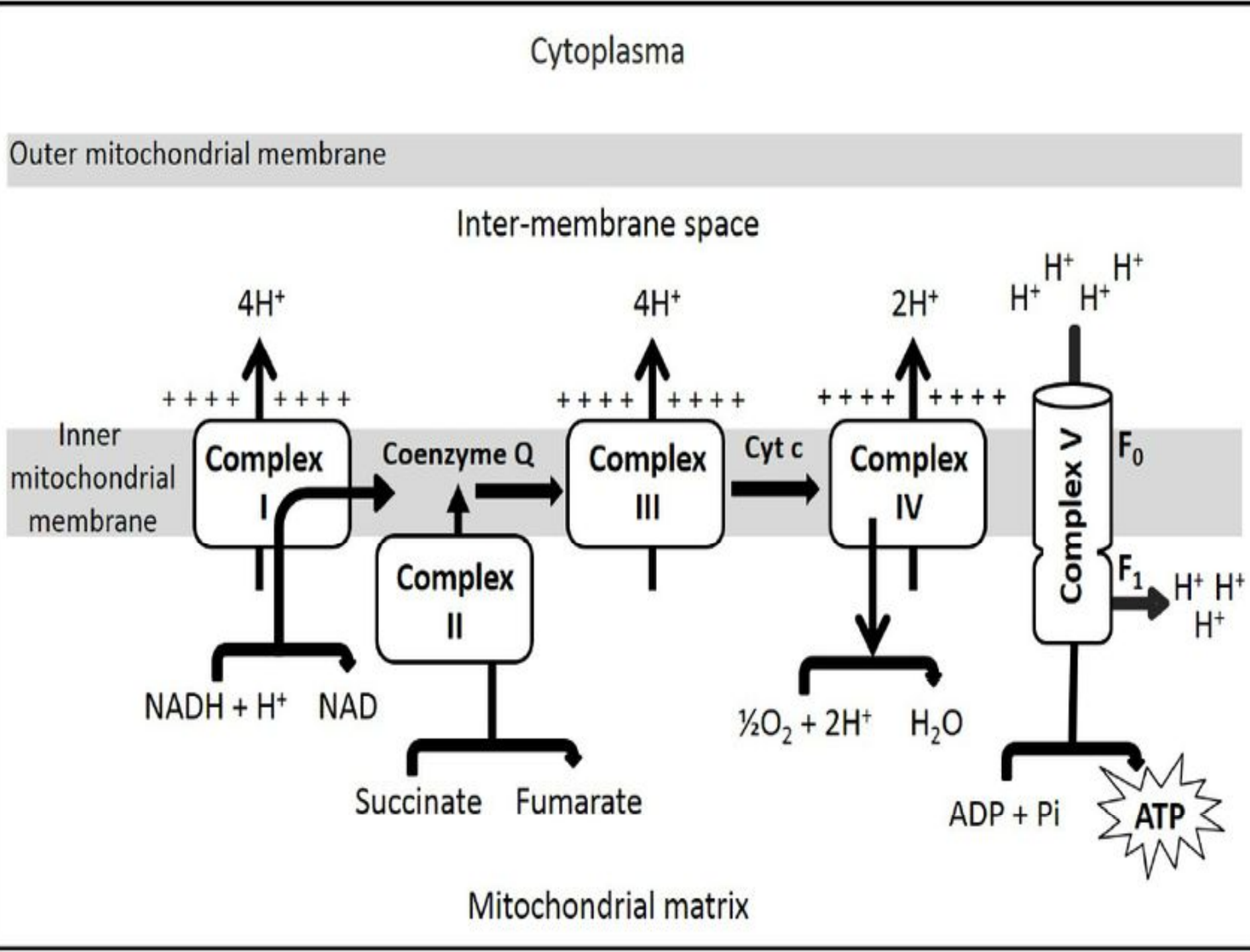
Electron Transport Chain

- Occurs in mitochondria cristae

1. NAD and FAD are reduced.
2. They then donate electrons to the first molecule in the electron transport chain.
3. This releases protons which are then actively transported across the inner mitochondrial membrane.
4. Meanwhile, the electrons pass along the chain of electron transport carrier molecules in a series of oxidation-reduction reactions. The electrons lose energy as they travel down the chain and some of this energy is used to combine ADP and Pi to form ATP. The rest is released as heat.
5. Protons accumulate in intermembrane space before they diffuse back into the mitochondrial matrix through special channel proteins.
6. At the end of the chain, the electrons combine with the protons and oxygen to make water. O2 is the final acceptor of electrons in ETC.

ATP + Respiration

Photosynthesis


Cytoplasma
Outer mitochondrial membrane
Inter-membrane space
4H+
4H+
2H+
H+ H+ H+ H+
Inner mitochondrial membrane
Complex I
Coenzyme Q
Complex III
Cyt c
Complex IV
Complex V
F0
F1
H+ H+ H+
Complex II
NADH + H+
NAD
½O2 + 2H+
H2O
Succinate
Fumarate
ADP + Pi
ATP
Mitochondrial matrix

- The overall reaction involves the oxidation of NADH or FADH2 cofactors, and results in the reduction of oxygen to water.
- This process is the major reason for the requirement for oxygen in most organisms.
- The electron transport pathway is often called the “respiratory chain”, because this pathway is the major reason for respiration (= breathing in animals).

- **NADH dehydrogenase (Complex I)**
- The first complex contains a iron-sulfurs center and an FMN. Complex I accepts electrons from NADH to regenerate NAD. Complex I also pumps protons: each pair of electrons results in the movement of about 4 H+ from the matrix to the intermembrane space. Complex I donates electrons to Coenzyme Q.
- Coenzyme Q
- Coenzyme Q is a non-protein electron carrier located in the inner mitochondrial membrane
- Coenzyme Q can transfer one or two electrons; to the fully oxidized form and the fully reduced form of the molecule. Coenzyme Q can accept electrons from Complex I and II (and from other proteins); it donates the electrons to Complex III.

- **Succinate Dehydrogenase (Complex II)**
- Succinate dehydrogenase is one of the enzymes in the TCA cycle . It is the only TCA cycle enzyme embedded in the mitochondrial inner membrane.
- The conversion of succinate to fumarate results in reduction of the enzyme-bound FAD. The oxidation of the reduced flavin requires the donation of electrons to Coenzyme Q;
- Succinate dehydrogenase does not pump protons

- **Coenzyme Q-dependent cytochrome c reductase (Complex III)** Complex III accepts electrons from Coenzyme Q

- Like Complex I, Complex III is also a proton pump. Complex III contains several heme prosthetic groups.

- referred to as cytochrome b and cytochrome c1; these are all part of the same protein complex.

- The electrons that Complex III receives are donated one at a time to a soluble heme containing electron carrier protein called cytochrome c. Cytochrome c is located in the intermembrane space.

- **Cytochrome c oxidase (Complex IV)** Cytochrome c oxidase, accepts electrons from cytochrome c. Complex IV is the terminal part of the electron chain and transfers electrons directly to oxygen.

- Like Complexes I and III, Complex IV is a proton pump. Cytochrome c oxidase is sometimes referred to as the cytochrome a-a3 complex.

- The electron transport chain uses a number of heme prosthetic groups; cytochrome c oxidase is the only one that is capable of binding oxygen; all of the others have both of the axial heme-iron binding sites occupied by amino acid side-chains.

- **F1F0-ATPase = ATP Synthase (Complex V)**

- The final complex required for the synthesis of ATP is the ATP synthase enzyme complex .
- Complex V uses the proton gradient generated by the proton pumps to synthesize ATP.
- 4 H+ must move down the gradient for each ATP produced.

- **Oxidative Phosphorylation**
- It means coupling of the electron transport in respiratory chain with phosphorylation of ADP to form ATP.

- It is a process by which the energy of biological oxidation is converted to the chemical energy of ATP.

- There are 3 sites of the chain give enough energy for ATP synthesis.

- These sites are:

- Site I between FMN and Coenzyme Q at enzyme complex I.
- Site II between cyt b and cyt C1 at enzyme complex III
- Site III between cyt a and cyt a3 at enzyme complex IV .

- The number of ATP generated depends on the site at which the substrate is linked to the respiratory chain:

- If substrate is linked to the chain through NAD+, 3 ATP are formed for each molecule oxidized.

- If substrate is linked to the chain through FAD, 2 ATP are formed for each molecule oxidized.

- **P/ O ratio**
-
- It is the ratio of the number of molecules of ADP converted to ATP to the number of oxygen atoms utilized by respiratory chain.
- It is a measure to the efficiency of oxidative phosphorylation.

- It is 3/1 if NADH+ H+ is used
- and 2/1 if FADH2 is used

- **Mechanism of oxidative phosphorylation**
- There are **2 theories** explaining this mechanism
- **1. Chemical theory**
- It suggests that there is a direct chemical coupling of oxidation and phosphorylation through high-energy intermediate compounds.

- This theory is not accepted, as the high-energy intermediate compounds were never found.

- **2. Chemiosomotic theory**

- It suggests that the transfer of electrons through the electron transport chain causes protons [pumped out) from the mitochondrial matrix to the inter membrane space at the three sites of ATP production (i.e. it acts as a proton pump)
- it results in an electrochemical potential difference across the inner mitochondrial membrane.
- The electrical potential difference is due to accumulation of the positively charged hydrogen ions outside the membrane and the chemical potential difference is due to the difference in pH, being more acidic outside the membrane.
- This electrochemical potential difference drives (forces) ATP synthase to generate ATP from ADP and inorganic phosphate

- There are many **evidences** that support **the chemiosmotic theory**:

- a- Addition of protons (acid) to the external medium of intact mitochondria leads to ATP generation.

- b- ATP production does not occur in soluble systems as a closed membrane to establish the electrochemical potential difference.

- c- It can explain the action of couplers.

- d- The components of the electron transport chain are organized in the inner mitochondrial membrane by the chemiosmotic theory.

- **Inhibitors of oxidative phosphorylation**
- The inhibitors of oxidative phosphorylation are classified into:
- **A- Specific-site inhibitors** that inhibit oxidation.
- **B- Non specific-site inhibitors** that inhibit phosphorylation
-
- **A- Specific site inhibitors**

- They block the oxidation process at specific sites on the respiratory chain (at one of the 3 sites of ATP production).

- 1- **Site 1 inhibitors**
- These are substances that inhibit electron transport from reduced FMN to coenzyme Q.
- **They include**:
- i- Rotenone (insecticide and fish poisoning)
- ii- Chloropromazine (tranquilizer)
- iii- Barbiturates (hypnotic)
- iv- Alkyl guanidine (hypotensive)

- **2- Site II inhibitors**
- These are substances that inhibit electron transport from reduced cyt b to cyt c1.
- **They include:**
- i- Antimycin A (antibiotic).
- ii- BAL (British Anti lewisite). It is dithioglycerol
- iii- Dimercaprol
- iv- phenformine (hypoglycemic)
- v- Napthoquinone

- **3- Site III inhibitors**

- These are substances that inhibit electron transport from reduced cyt a to cyt a3.
- **They include**:

- i- Cyanide
- ii- Carbon monoxide (CO)
- iii- Hydrogen sulphide (H_2S)
- iv- Sodium azide

- **B- Non specific-site inhibitors**
-
- They function primarily by blocking phosphorylation, but they prevent the whole process of oxidative phosphorylation
- e.g.

- i- Oligomycin (antibiotic) that inhibits ATP synthase enzyme

- ii- Atractyloside (herbicide) that inhibits ADP/ ATP transporter which is responsible for the transport of ADP into the mitochondria and the transport ATP out of the mitochondria

alcoholic fermentation

- **Ethanol fermentation**, also called **alcoholic fermentation**, is a biological process which converts sugars such as glucose, fructose, and sucrose into cellular energy, producing ethanol and carbon dioxide .

- Because yeasts perform this conversion in the absence of oxygen, alcoholic fermentation is considered an anaerobic process.

- The chemical equations below summarize the fermentation of sucrose ($C_{12}H_{22}O_{11}$) into ethanol (C_2H_5OH).

- Alcoholic fermentation converts one mole of glucose into two moles of ethanol and two moles of carbon dioxide, producing two moles of ATP in the process.

- The overall chemical formula for alcoholic fermentation is:

- **$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$**

- Sucrose is a dimer of glucose and fructose molecules.
- In the first step of alcoholic fermentation, the enzyme invertase cleaves the glycosidic linkage between the glucose and fructose molecules.

- **$C_{12}H_{22}O_{11} + H_2O + \text{invertase} \rightarrow 2\ C_6H_{12}O_6$**

- Next, each glucose molecule is broken down into two pyruvate molecules in a process known as glycolysis.
- Glycolysis is summarized by the equation:

- $C_6H_{12}O_6 + 2\ ADP + 2\ P_i + 2\ NAD^+ \rightarrow 2\ CH_3COCOO^- + 2\ ATP + 2\ NADH + 2\ H_2O + 2\ H^+$

- The chemical formula of pyruvate is CH_3COCOO^-.
- P_i stands for the inorganic phosphate.

- Finally, **pyruvate is converted to ethanol and CO_2 in** two steps, regenerating oxidized NAD+ needed for glycolysis:

- 1. $CH_3COCOO^- + H^+ \rightarrow CH_3CHO + CO_2$ catalyzed by pyruvate decarboxylase

- 2. $CH_3CHO + NADH + H^+ \rightarrow C_2H_5OH + NAD^+$

- This reaction is catalyzed by alcohol dehydrogenase (ADH1 in baker's yeast]

- As by the reaction equation, glycolysis causes the reduction of two molecules of NAD^+ to NADH.

- Two ADP molecules are also converted to two ATP and two water molecules via substrate-level phosphorylation.

Pyruvate Oxidation

In the presence of oxygen, pyruvate is oxidized.

-occurs in the mitochondria in eukaryotes

-occurs at the plasma membrane in prokaryotes

-in mitochondria, a multienzyme complex called **pyruvate dehydrogenase** catalyzes the reaction

- **Pyruvate oxidation**, which follows glycolysis, is essentially the conversion of **pyruvate** molecules to carbon dioxide, acetyl coenzyme A, and NADH.

- The last step of glycolysis produces two **pyruvate** molecules in the cytosol, which are then brought to the mitochondrial matrix via active transport.

- In humans, **aerobic** conditions produce **pyruvate** and **anaerobic** conditions produce lactate.

- In **aerobic** conditions, the process converts one molecule of glucose into two molecules of **pyruvate** (**pyruvic** acid), generating energy in the form of two net molecules of ATP.

- The products of pyruvate oxidation include:

-1 CO_2
-1 NADH
-1 **acetyl-CoA** which consists of 2 carbons from pyruvate attached to coenzyme A

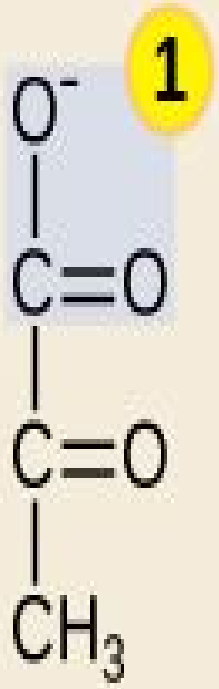
Acetyl-CoA proceeds to the Krebs cycle**.**

- **pyruvate oxidation** happens in the mitochondrial matrix of eukaryotic cells.

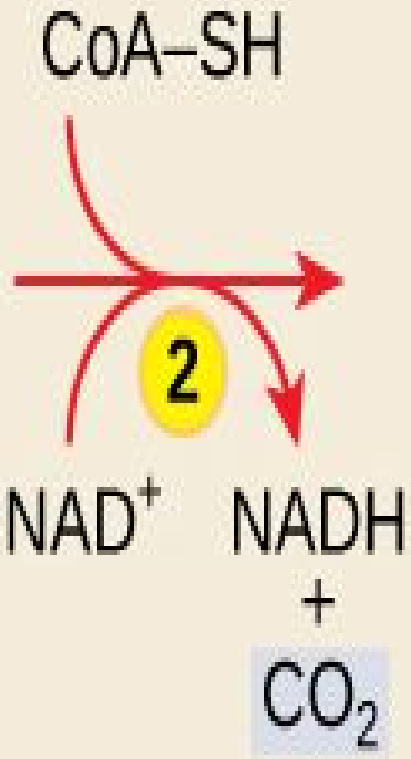
- As soon as **pyruvate** enters the mitochondrial matrix in eukaryotes, it is oxidatively decarboxylated (with the help of the enzyme **Pyruvate** DeHydrogenase, PDH) to form Acetyl CoA

- which is then free to act as a substrate in the Krebs cycle.

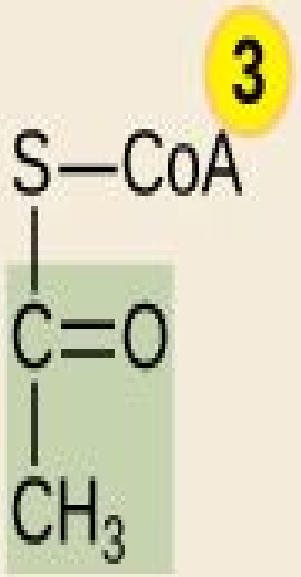
Oxidation of Pyruvate



Pyruvate



Oxidation reaction



Acetyl CoA

- **Hexose MonoPhosphate Shunt**

- The **Hexose MonoPhosphate Shunt** is also known as "***Pentose phosphate Pathway***" (PPP).
- This is *alternative Glucose oxidation pathway*.

- The *hexose monophosphate pathway* is used for production of *NADPH from NADP*.

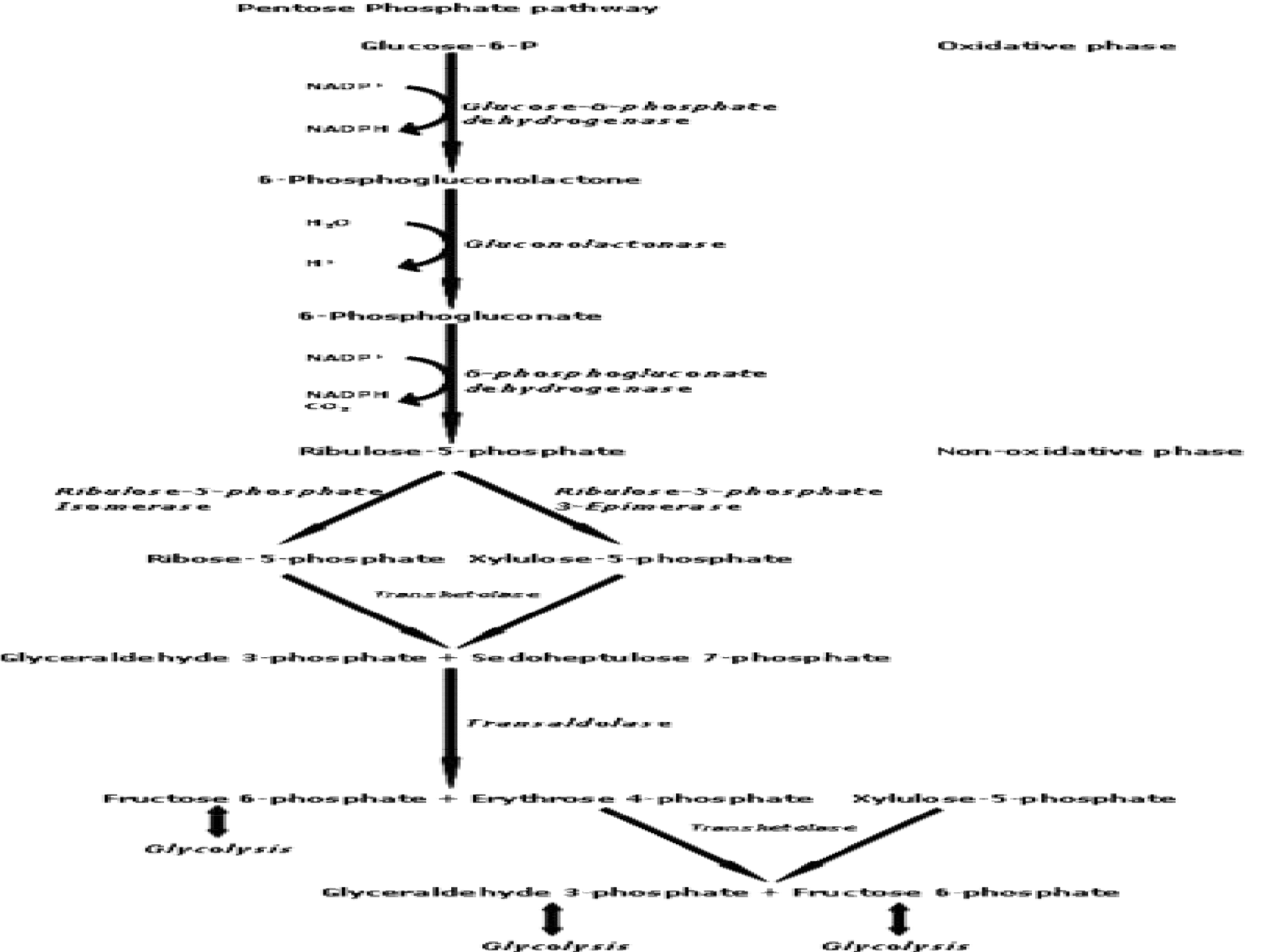
- Steroidogenic tissues, red blood cells, and the liver are the major sites of hexose monophosphate pathway.

- Muscle has small amounts of some of the **Hexose MonoPhosphate Shunt** enzymes, because it has little need for NADPH.

Overview of the shunt pathway

- It has two phases
- Oxidative phase
- Glucose 6 phosphate is oxidised to generate two molecules of NADPH with liberation of one CO_2

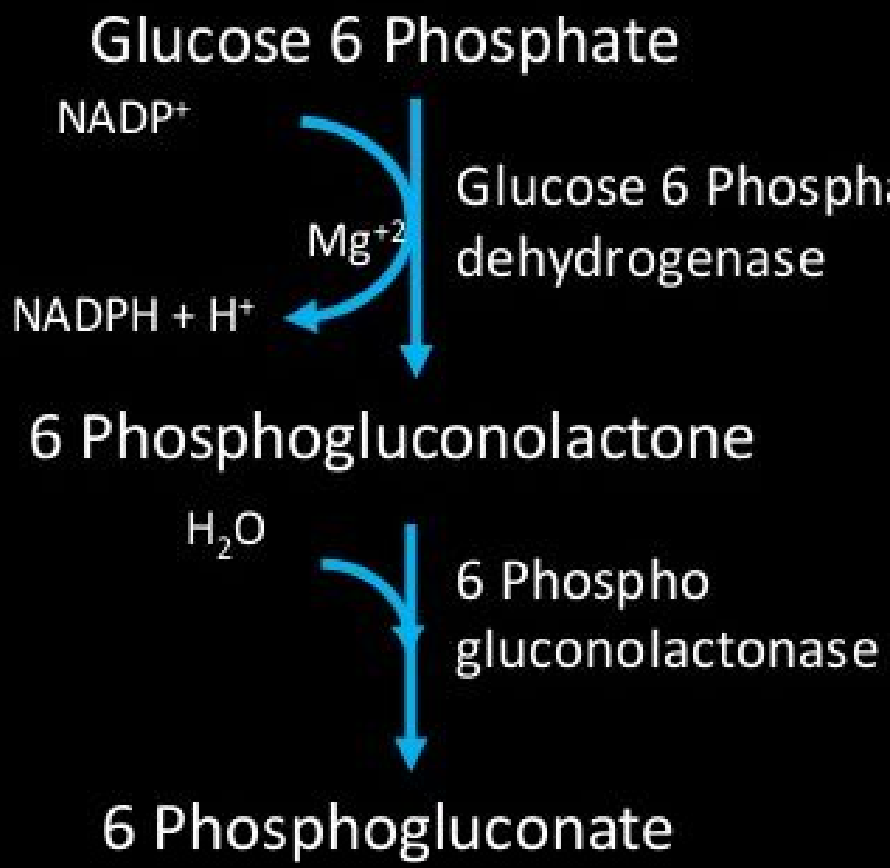
- Non oxidative phase
- Pentose phosphate is converted to intermediates of glycolysis


Pentose Phosphate pathway
Glucose-6-P
Oxidative phase
NADP+
NADPH
Glucose-6-phosphate dehydrogenase
6-Phosphogluconolactone
H2O
H+
Gluconolactonase
6-Phosphogluconate
NADP+
NADPH
CO2
6-phosphogluconate dehydrogenase
Ribulose-5-phosphate
Non-oxidative phase
Ribulose-5-phosphate Isomerase
Ribulose-5-phosphate 3-Epimerase
Ribose-5-phosphate
Xylulose-5-phosphate
Transketolase
Glyceraldehyde 3-phosphate + Sedoheptulose 7-phosphate
Transaldolase
Fructose 6-phosphate + Erythrose 4-phosphate
Xylulose-5-phosphate
Glycolysis
Transketolase
Glyceraldehyde 3-phosphate + Fructose 6-phosphate
Glycolysis
Glycolysis

- **Oxidative phase:**
- The oxidative portion of HMP shunt consists of 3 reactions
- that lead to formation of Ribulose-5-Phosphate, Carbon dioxide and 2 molecules of NADPH, for each molecule of Glucose-6-Phosphate oxidized.

- During oxidative phase, from glucose-6-phosphate obtained by phosphorylation of the free glucose, NADPH finally obtained is formed pentose, ribulose 5-phosphate
-
- So this metabolic process is called “the Pentose Mono phosphate Pathway”.

1 – Oxidative Phase



- Reducing equivalent NADPH are generated
- G6PDH is allosteric enzyme hence this is rate limiting step

- **Step 1: Dehydrogenation of Glucose – 6-Phosphate:**
- *Glucose-6-Phosphate* is converted into "*6-Phospho Gluconate*" in the presence of the enzyme, *Glc-6-Phosphate dehydrogenase*.
- In this reaction NADP+ act as a coenzyme.

- In this first step the C1 group is dehydrogenated to give a group carboxyl, which, next to C5 forms a lactone, i.e. an ester intramolecular.

- two free hydrogen ions (proton) and two electrons are transferred to NADP+ which acts as electron acceptor reduced to form the first molecule of NADPH;.

- **Step 2: Formation of Ribulose-5-Phosphate:**
- *6-Phospho Gluconate* is converted into *Ribulose-5-Phosphate* by eliminating CO_2 from Carbon one of Glucose, in the presence of the enzyme *6-Phosphogluconate dehydrogenase*.
- Then, it produces Lactone by hydrolysis and by the action of the lactonase, the free acid is obtained 6-phosphogluconate.
- Then, the 6- phospho gluconate becomes ribulose-5-phosphate by the action of 6-phosphogluconate dehydrogenase.
- Here NADPH second molecule is obtained, in addition to the release of a molecule of CO_2 because of the oxidative decarboxylation.
- Finally, the enzyme pentose-5-phosphate isomerase, isomerizes the ribulose 5-phosphate and converts ribose-5-phosphate to the transformation of the group ketose in aldose.
- it carries out the transition to non-oxidative metabolic phase of the pentose phosphate pathway.

- The **general reaction** of this first phase is:
- **Glucose-6-phosphate + 2 $NADP^+$ + H_2O**
- **→ ribulose-5-phophate + 2 NADPH + 2 H^+ + CO_2**

- **Non-oxidative phase:**

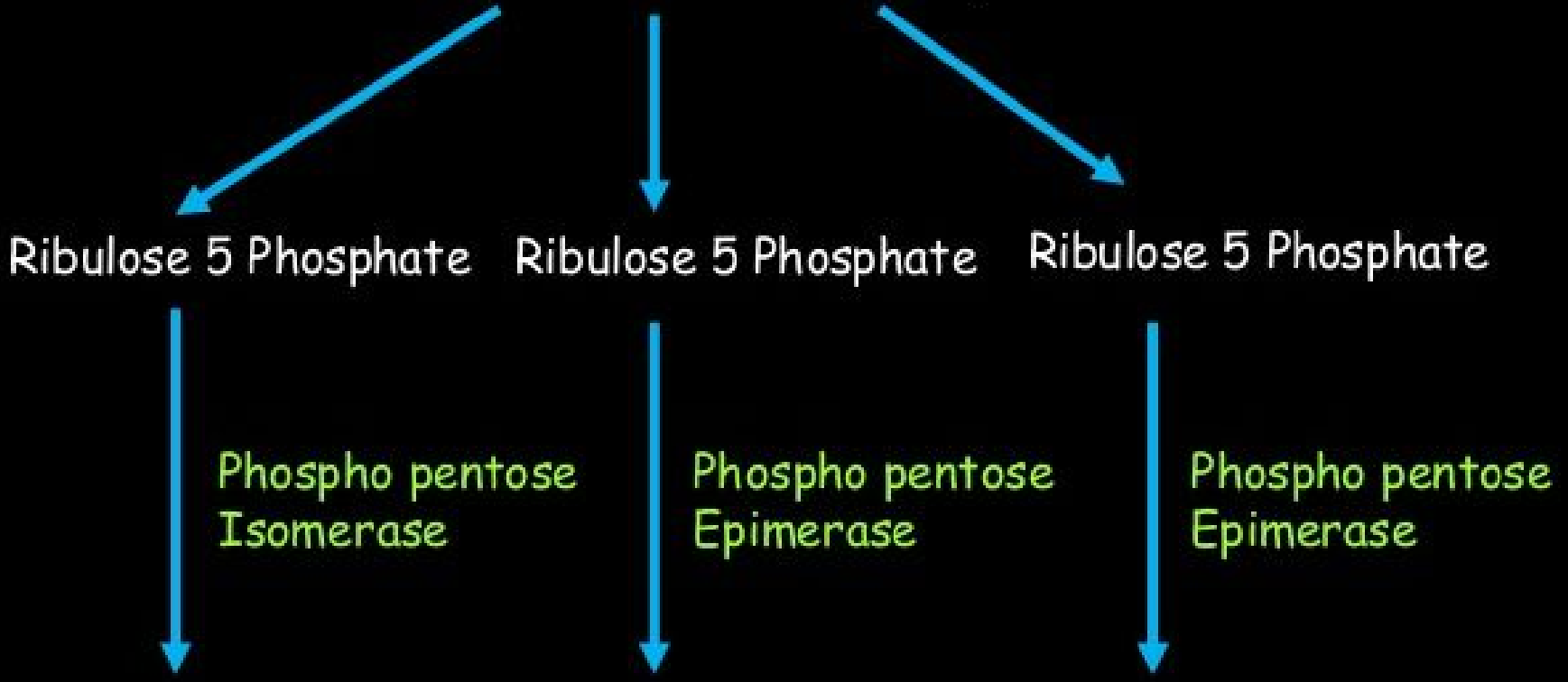
- The non-oxidative reaction of pentose phosphate pathway catalyzes the inter-conversion of 3, 4, 5 and 7- carbon sugars.

- The **non-oxidative phase** of the pentose phosphate pathway is initiated when the cell needs more NADPH than ribose-5-phosphate.

- In this second process are a complex sequence of reactions that change the C3, C4, C5, C6 and C7 pentose sugars to form finally **glyceraldehyde-3-phosphate** and **fructose 6-phosphate**, which can go directly to glycolysis.

Phase 2 Non oxidative phase
Inter conversion of pentoses

3 D Ribulose 5 Phosphate



- **Step 1: Epimerization of ribulose-5-P into Xylulose-5-P:**
- *Ribulose-5-Phosphate* is converted into *Xylulose-5-Phosphate*; in the presence of the enzyme "*Phosphopento epimerase*" this reaction is the one of the example to Epimerization.
-
- **Step 2: Isomerization of Ribulose-5-Phosphate to Ribose-5-Phosphate:**
- *Ribulose-5-Phosphate* is isomeriss into *Ribose-5-Phosphate* by the enzyme "*Phospho pentose isomerase*". This eliminate excess ribose-5-phosphate to transforming it into intermediates of glycolysis.

- **Step 3: Epimerization of Ribulose – 5- Phosphate to Xylulose-5-Phosphate :**

- The final product of oxidative reactions *Ribulose-5-Phosphate* is epimerizes into *Xylulose-5-Phosphate*. This reaction proceeds by the utilization of second glucose molecule. This reaction is catalyzed by "*Phospho pentose Epimerase*".
- The reaction is carried out **epimerization**, regulated by the pentose-5-phosphate epimerase enzyme.

- **Step 4: Transketolation:**
- When the *Ribose-5-Phosphate* reacts with Xylulose-5-Phosphate. It gives *Sedoheptulose-7-Phosphate* and *Glyceraldehyde-3-Phosphate* by the enzyme *Transketolase*.
- Here TPP (Thiamine Pyrophosphate) acts as a Co-enzyme. this is a 2 carbon shifting mechanism.
- This will convert *Xylulose 5-phosphate* into *Ribose-5-phosphate* by transferring unit C2 of the aldose to ketose, will produce *glyceraldehyde-3-phosphate* and *sedoheptulose-7-phosphate*.

- **Step 5: Transaldolation:**

- When *Sedoheptulose-7-Phosphate* reacts with *Glyceraldehyde-3-Phosphate*; it gives 4 carbon compound – *Erythrose-4-Phosphate* and 6 carbon compound *Fructose-6-Phosphate*.
- This reaction is catalyzed by the enzyme *Transaldolase*,
- In this reaction first 3 carbons of *Sedoheptulose-7-Phosphate* is shifted to the aldehyde group of the *Glycerldehyde-3-Phosphate*.
- this is a 3 carbon shifting mechanism.

- **Step 6: Transketolation:**

- When the *Erythrose-4-Phosphate* reacts with *Fructose-6-Phosphate* gives *Xylulose-5-Phosphate* and *Glyceraldehyde-3-Phosphate*.

- This reaction is catalyzed by "*Transketolase*".

- This is TPP dependent enzyme.
- again *Transketolase* enzyme transferring a C2 unit, from *Xylulose-5-phosphate* to *Erythrose-4-phosphate*, thus form another molecule of *Fructose 6-phosphate* and *Glyceraldehyde-3-phosphate*, both are intermediates of glycolysis.

- Thus, the phase of this non-oxidative metabolic pathway is closed.

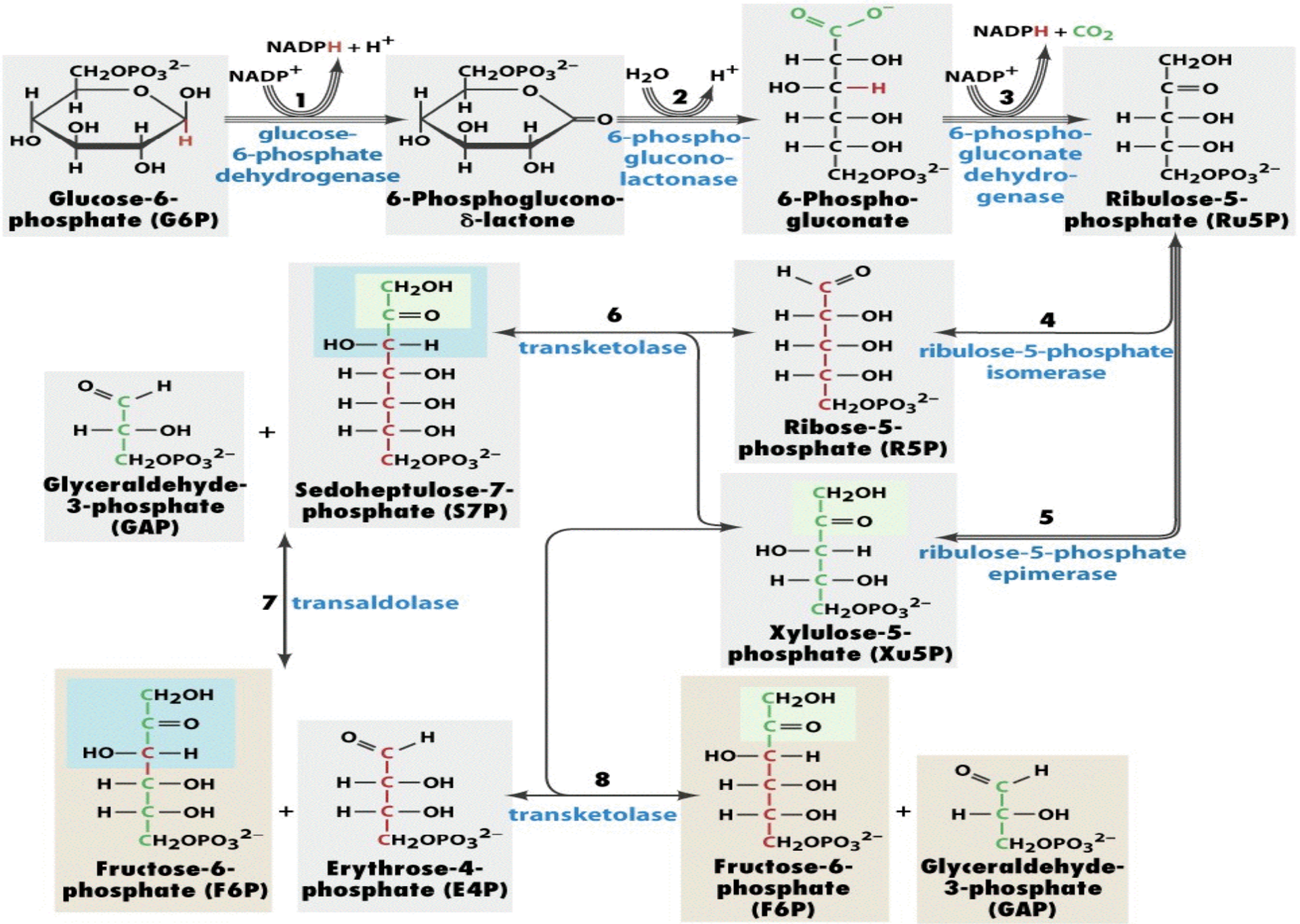
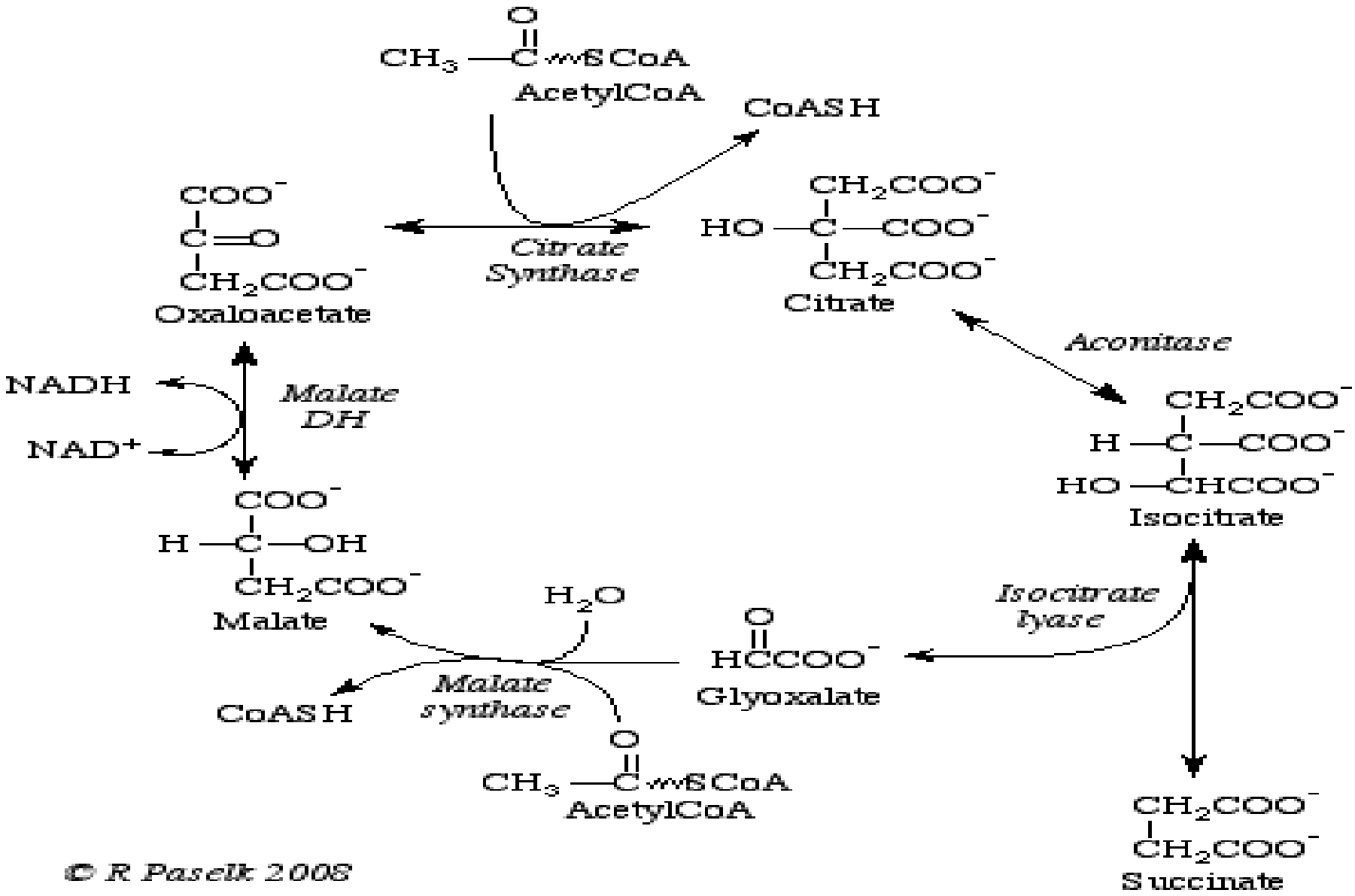


Figure 14-30 Fundamentals of Biochemistry, 2/e

glyoxylate cycle

- The **glyoxylate cycle**, a variation of the tricarboxylic acid **cycle**, is an anabolic pathway occurring in plants, bacteria, protists, and fungi.
- The **glyoxylate cycle** centers on the conversion of acetyl-CoA to succinate for the synthesis of carbohydrates.
- The glyoxylic acid was obtained through the breakdown of Iso-Citric Acid (an inter-mediate of Krebs' Cycle) by the enzyme Isocitratase
- Isocitric acid → glyoxylic acid + succinic acid
- The glyoxylate cycle is occur in many other bacteria, yeasts, molds, and higher plants and is completed in glyoxysomes, mitochondria and cytosol.

Glyoxylate Cycle



Steps Involved in Glyoxylate Cycle:

- **Reactions in Glyoxysome:**
- (i) Acetyl-CoA produced after the β-oxidation of fatty acids (in glyoxysomes) condenses with oxalo acetic acid to form Citric Acid.

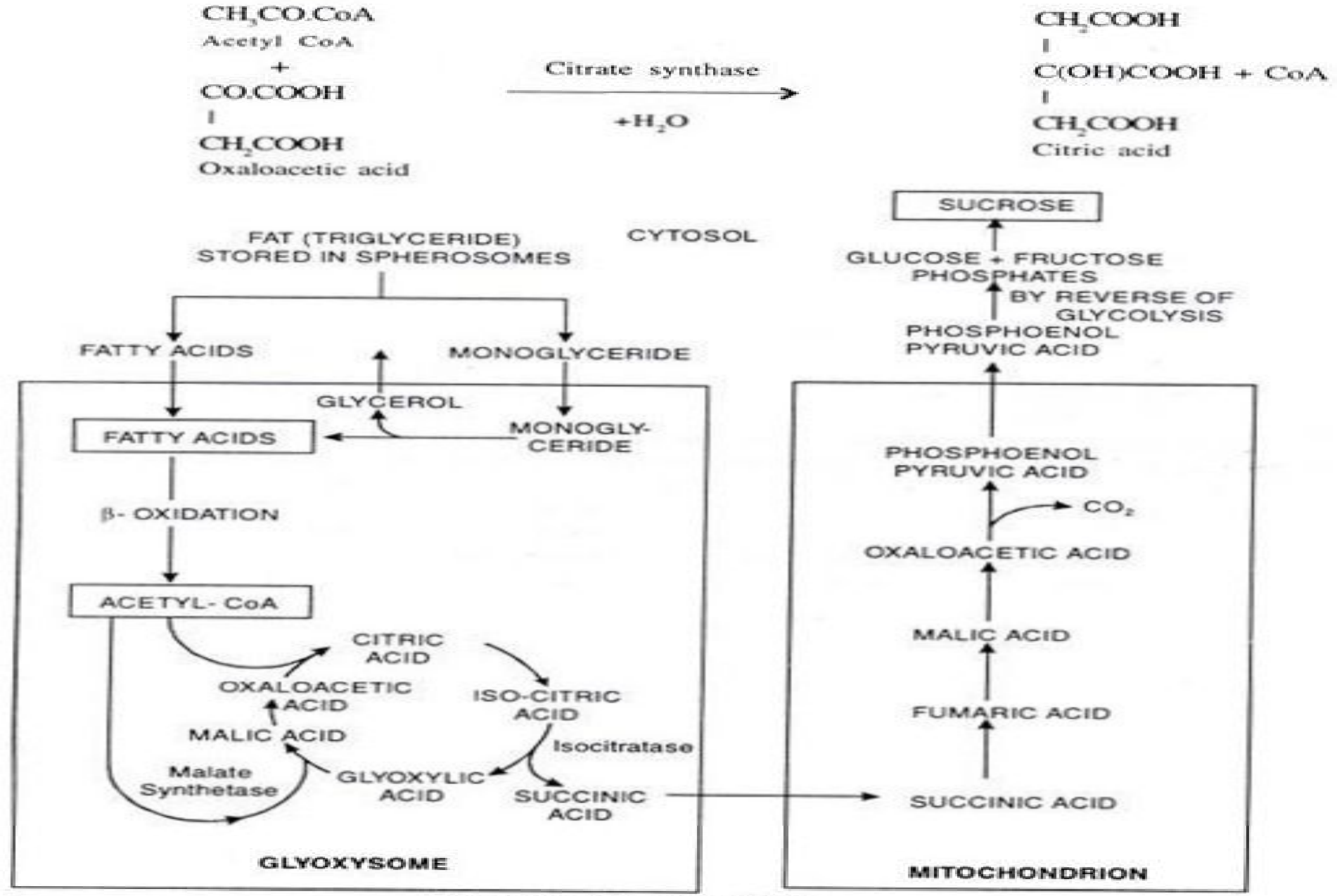
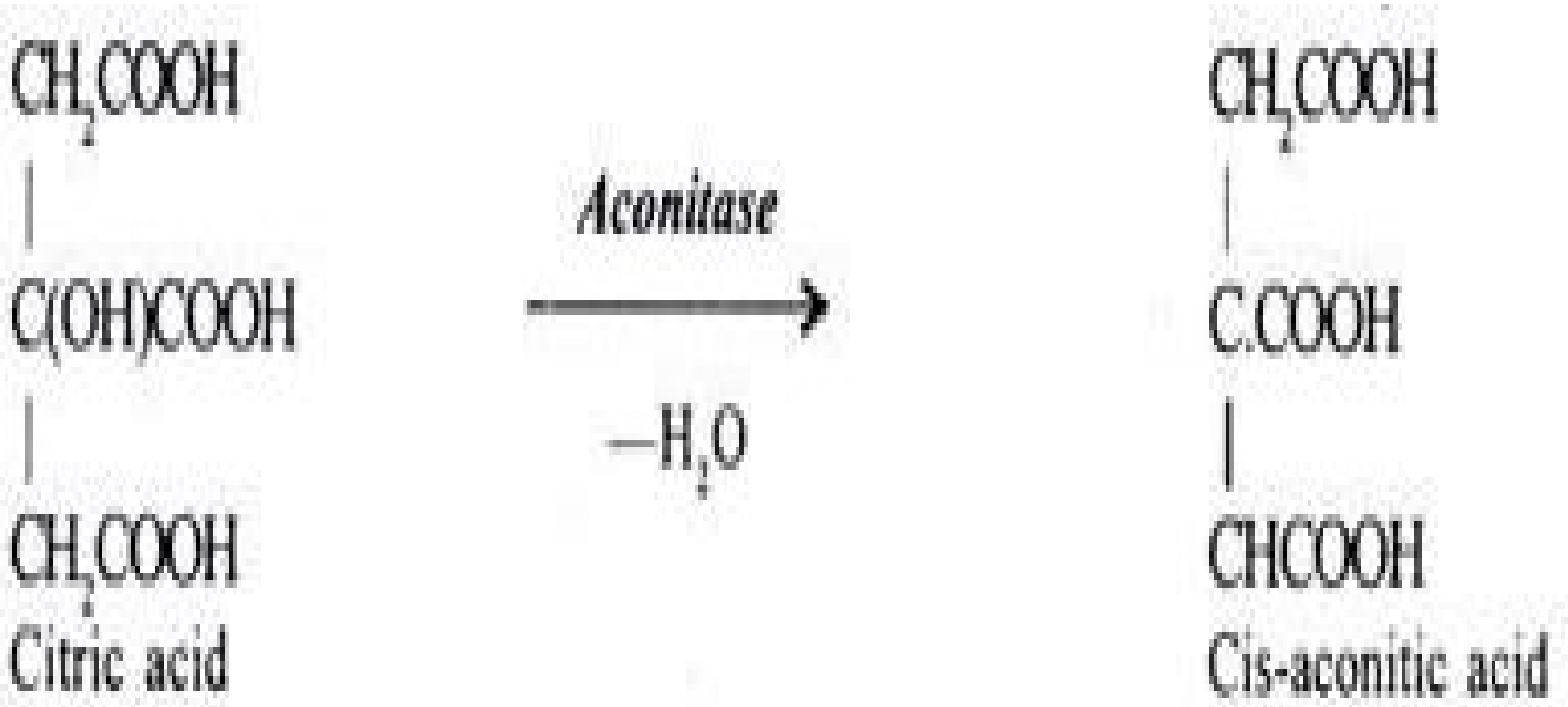
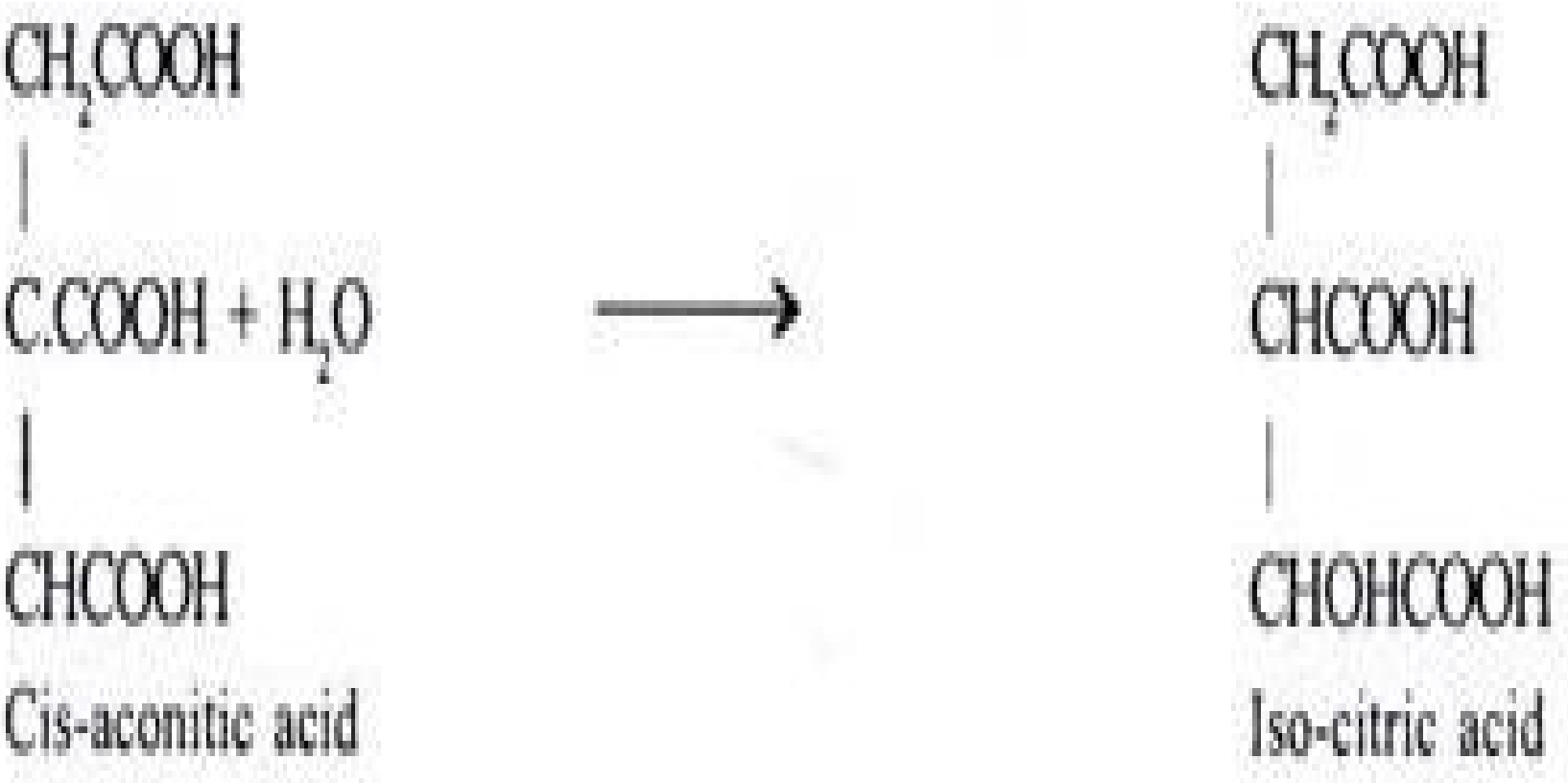


Fig. 14.3. Glyoxylate Cycle.

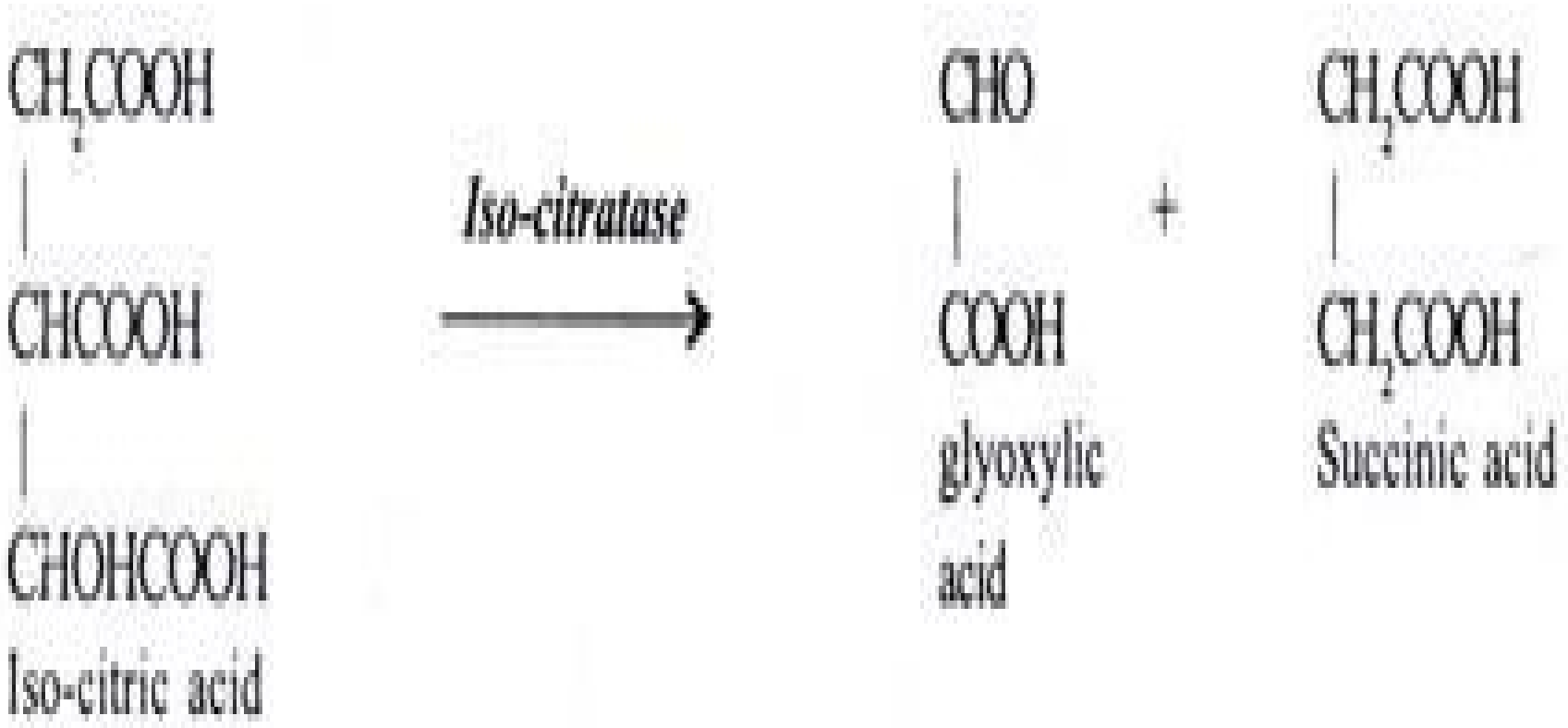
(ii) Citric acid is dehydrated to produce Cis-aconitic Acid in the presence of Aconitase.



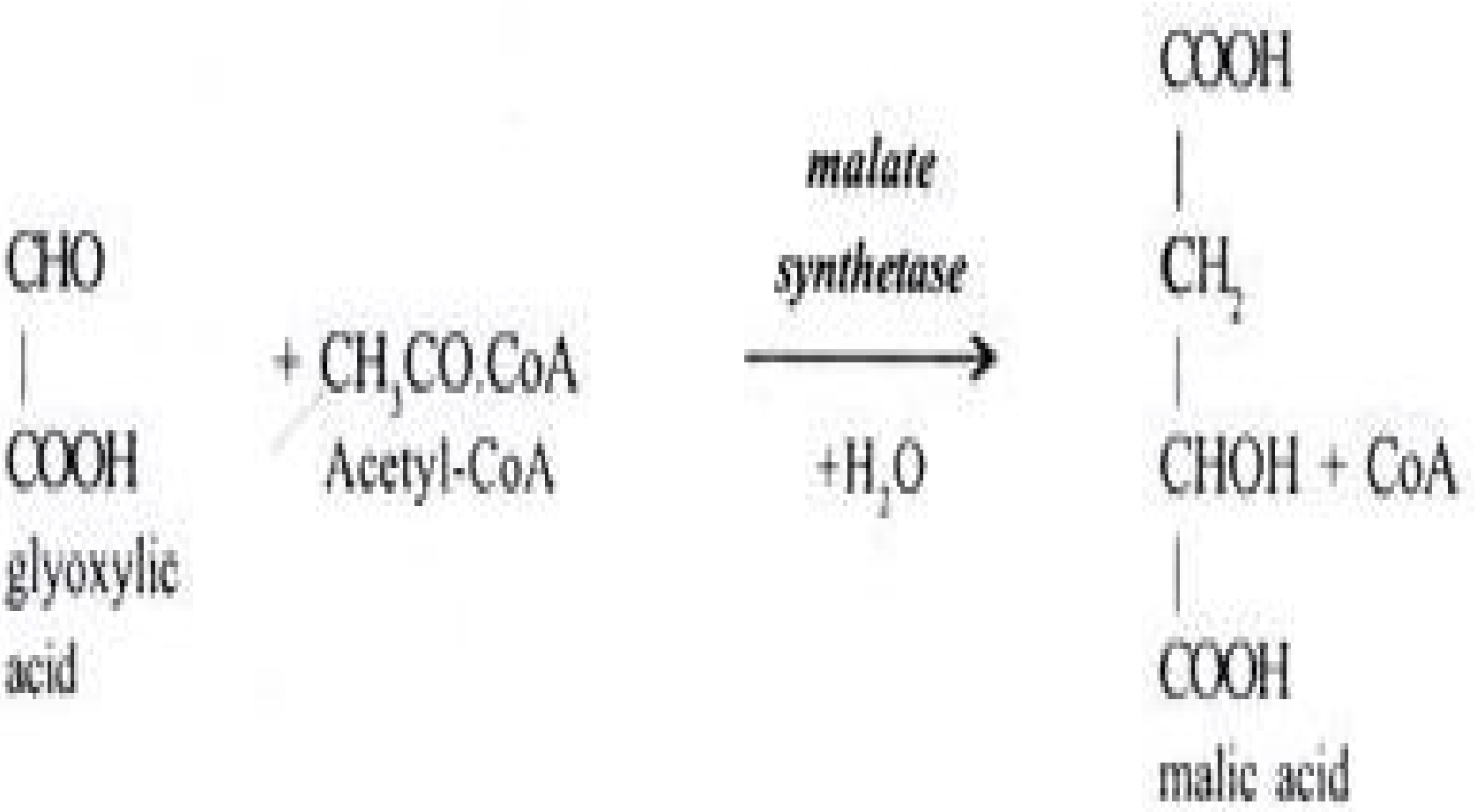
(iii) Cis-aconitic acid reacts with one molecule of H_2O to form Iso-citric acid.



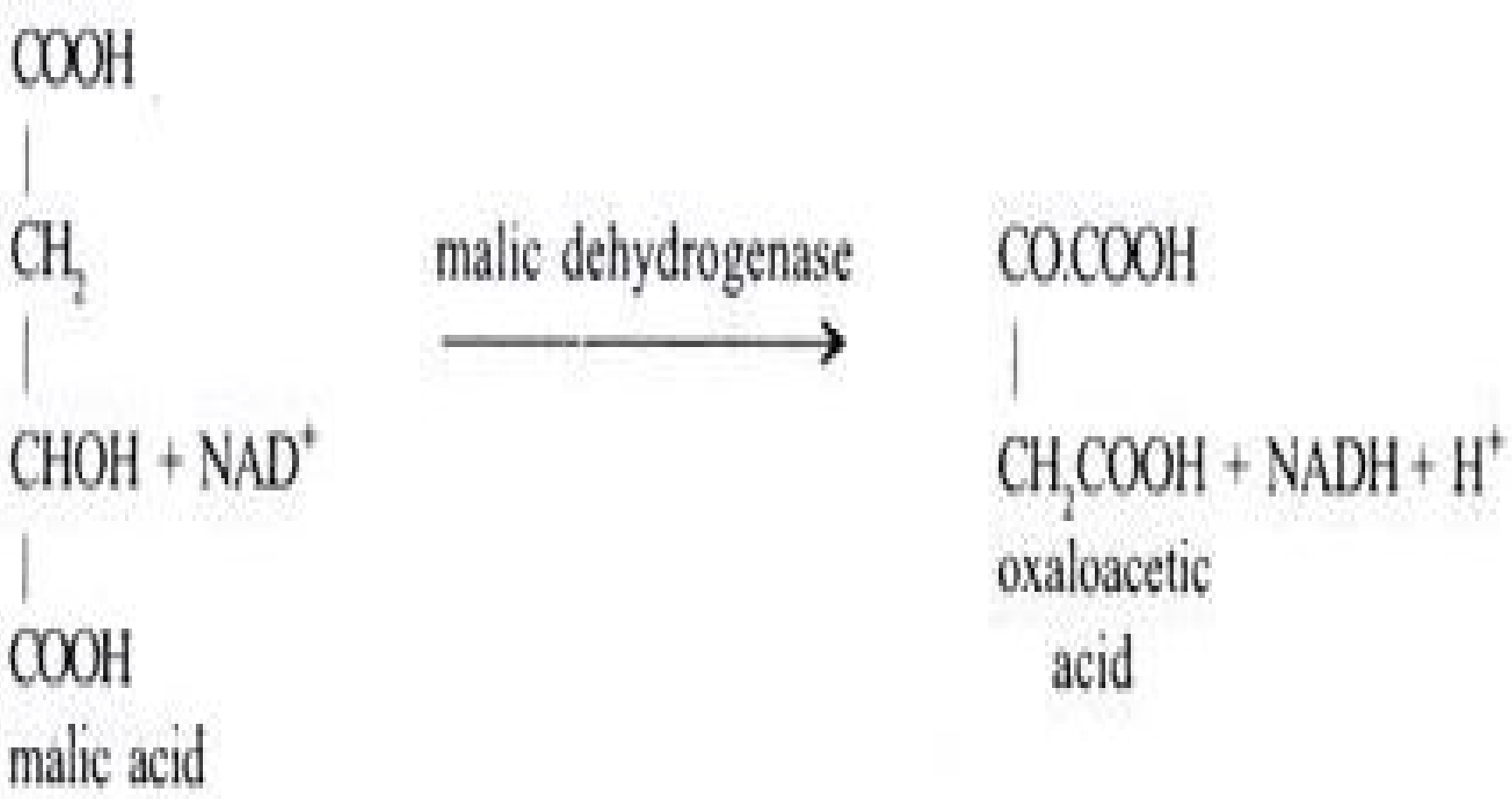
(iv) Iso-citric acid is broken down into glyoxylic acid and succinic acid by the enzyme Isocitratase.



(v) Glyoxylic acid combines with acetyl CoA (produced after the β-oxidation of fatty ac-ids) in the presence of Malate synthetase to produce Malic acid.



(vi) Malic acid is oxidised into oxaloacetic acid in the presence of Malic dehydrogenase and the coenzyme NAD.



- **Significance of Glyoxylate Cycle:**

- (1) During the germination of fatty seeds, the fats which are insoluble are hydrolysed into fatty acids and glycerol.

- Fatty acids after β-oxidation produce acetyl-CoA units which synthesize sucrose (which is soluble) through glyoxylate cycle.

- Soluble sucrose is then supplied to different growing regions of the young germinating seedling till it develops its own photosynthetic system.

- (2) Those micro-organisms which can grow on ethyl alcohol or acetate as a source of energy and carbon make use of this cycle in synthesizing longer carbon chains.
-
- (3) The glyoxylate cycle is an example of gluconeogenesis.