Microbial metabolism

Lecture 12

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Metabolism: The Greek metabole, meaning <u>change</u>.

The sum of the biochemical reactions required for energy generation **and** the use of energy to synthesize cell material from small molecules in the environment.

Why do we must know the metabolism of bacteria?

To know how to inhibit or stop bacteria growth and want to control their metabolism.

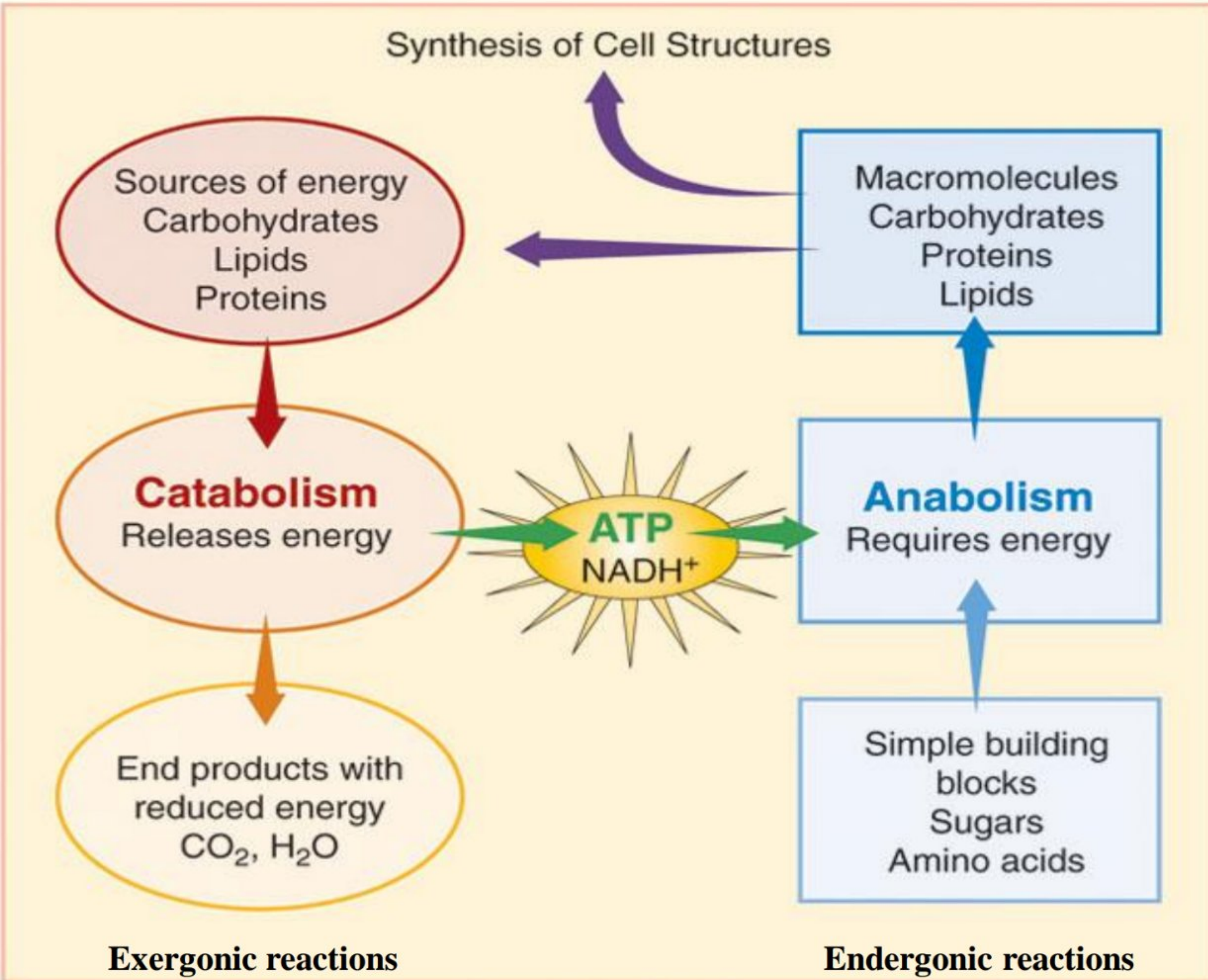
Metabolism

Two components:

- **Anabolism - biosynthesis**
  - building complex molecules from simple ones
  - requires ENERGY (ATP)

- **Catabolism - degradation**
  - breaking down complex molecules into simple ones
  - generates ENERGY (ATP)

- **Three Biochemical Mechanisms Utilized**
  - Aerobic Respiration
  - Anaerobic Respiration
  - Fermentation


Synthesis of Cell Structures
Sources of energy
Carbohydrates
Lipids
Proteins
Macromolecules
Carbohydrates
Proteins
Lipids
Catabolism
Releases energy
ATP
NADH+
Anabolism
Requires energy
End products with
reduced energy
CO_2, H_2O
Simple building
blocks
Sugars
Amino acids
Exergonic reactions
Endergonic reactions

Energy storage

- Molecular energy **stored in the bonds** of complex molecules released in catabolic pathways and harvested in such a way.

- Energy in **cells** is mainly stored and transferred as **high-energy electrons**.

- Electron transfer allows cells to use energy **gradually** instead of in one large, damaging release.

✓ **Oxidation reactions**: Loss of electrons → the molecule becomes more positive (oxidized).

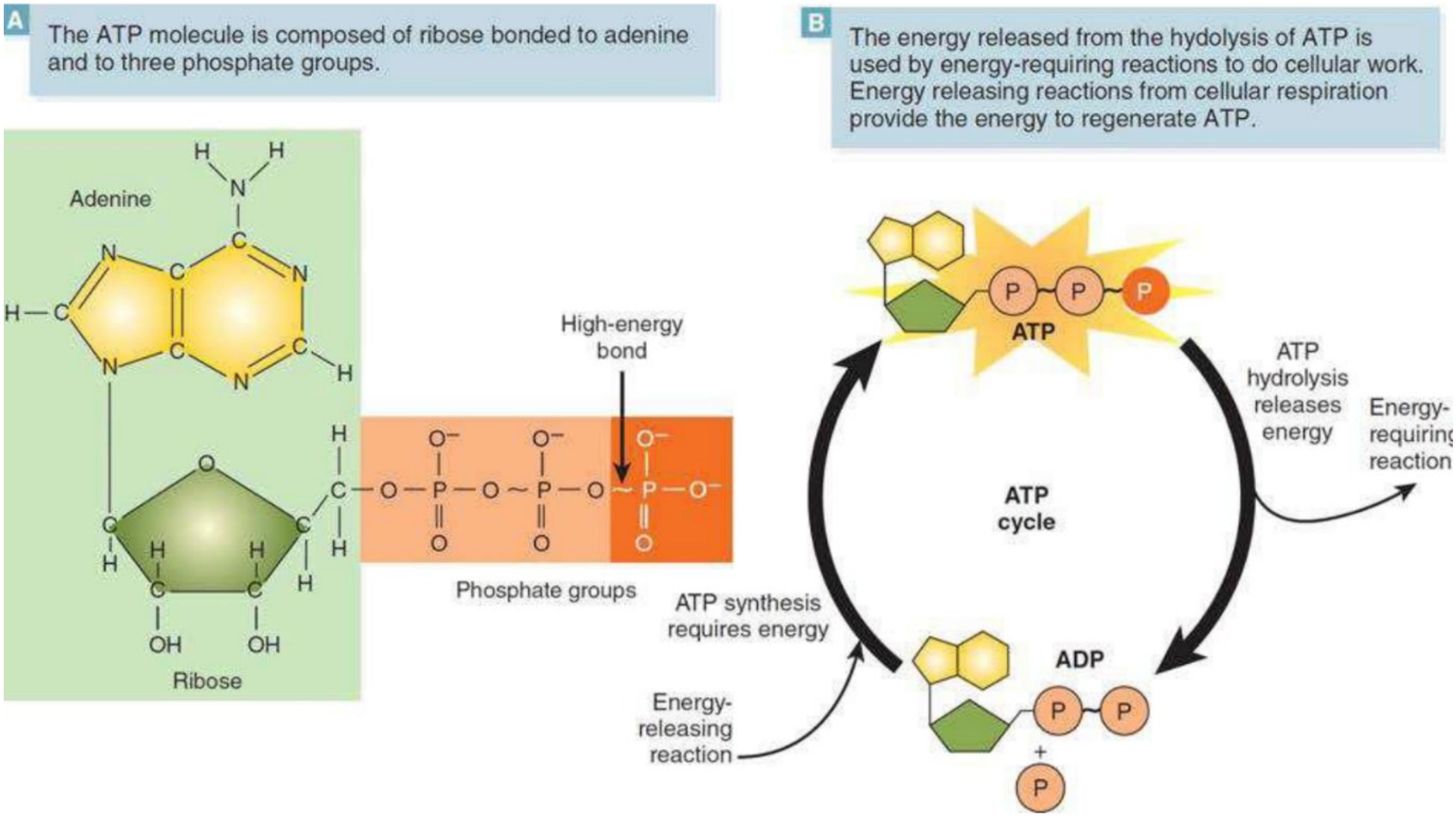
✓ **Reduction reactions**: Gain of electrons → the molecule becomes more negative (reduced)

- Oxidation and reduction always occur **together**.

Energy storage

- **<u>Energy storage</u>** <u>in cells occurs through:</u>
  - ✓ Reduction of **electron carriers**
  - ✓ Formation of **ATP bonds**
- **Mobile electron carriers** transport high-energy electrons between molecules in metabolic pathways.
- These carriers are derived from **B vitamins** and **nucleotides**.
- **Main electron carriers:**
  - **NAD^+ / NADH** – common in **catabolism** (oxidized/reduced forms)
  - **$NADP^+$ / NADPH** – used in **anabolism** and **photosynthesis** (oxidized/reduced forms)
  - **FAD / $FADH_2$** – used in **energy extraction** during catabolism
- **Reducing power**: NADH, NADPH, and $FADH_2$ can **donate electrons** to other reactions.
- **ATP** is the **energy currency** of the cell.

▪ Structure of ATP: **High-energy phosphate bonds** in ATP are unstable due to **repulsion between negative charges**.



$$\mathrm{ATP + H2O \rightleftharpoons ADP + Pi}\,[\Delta G = \mathbf{-30.5}\ \mathrm{KJ/mol}]$$

It has been estimated that a typical bacterial cell must reform about 3 million ATP molecules per second from ADP and phosphate to supply its energy needs.

ATP as a derivative of AMP

- ATP is a derivative of **AMP** (adenosine monophosphate).
- **Two** additional phosphate groups are attached through an **anhydrous linkage (high energy bonds, ~)**.

- These bonds are particularly reactive.

- Reactions and Energy Changes

Adenosine–P~P~P + H_2O → Adenosine–P~P + P

$\Delta G° = -7.3$ Kcal

Adenosine–P~P + H_2O → Adenosine–P + P

$\Delta G° = -7.3$ Kcal

- In **AMP**, adenosine is attached to phosphate by an **ester linkage**, the bond is less free active.
- **Reaction:**

$\Delta G° = -3.4$ Kcal

Aerobic Respiration

- **Oxygen (O_2) serves as the final e^- acceptor of the ETC**
  - O_2 is reduced to H_2O
  - Energy-generating mode used by aerobic **chemoheterotrophs**
  - **General term applied to most human pathogens**

- **3 Coupled Pathways Utilized**
  1. Glycolysis
  2. Kreb's Cycle or Tricarboxylic Acid Cycle or Citric Acid Cycle
  3. Respiratory Chain or Electron Transport Chain (**ETC**)

1. Glycolysis (splitting of sugar)

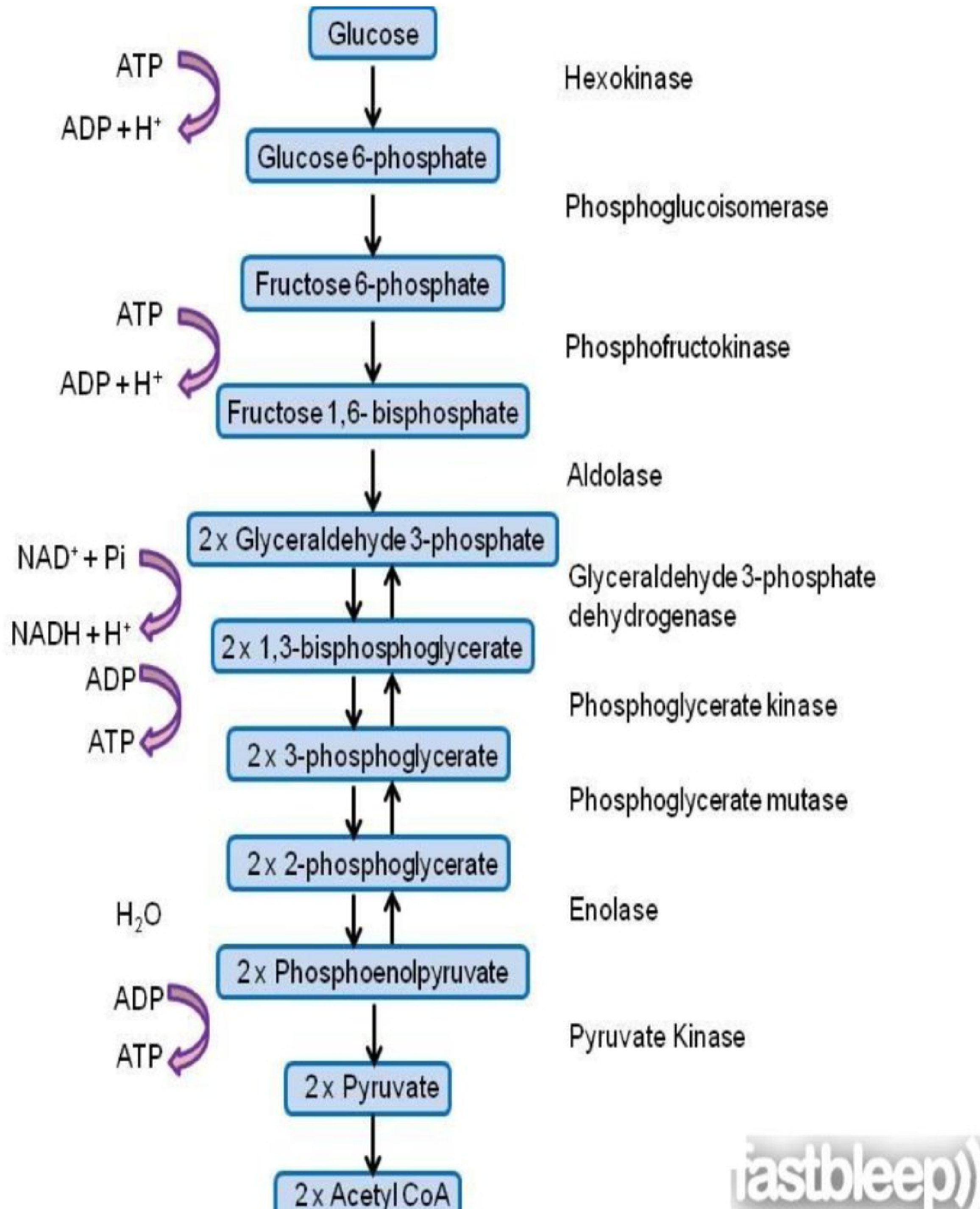
- The most common pathways for glycolysis:
  1. **Embden-Meyerhof-Parnas (EMP) pathway**
  2. **Entner-Doudoroff pathway**.
  3. **Phosphoketolase pathway;** used by some anaerobic bacteria and yeasts.

1. Embden-Meyerhof-Parnas (EMP) Pathway

- The **most common glycolysis pathway** found in animals, plants, and a large number of microorganisms.
- This pathway is used by both anaerobic and aerobic organisms.
- The process occurs in the cytoplasm of both prokaryotes and eukaryotes.

- Has **two main phases**:
  - **Energy Investment Phase**
    - Uses **2 ATP** to modify glucose.
    - Splits glucose into **two G3P (glyceraldehyde-3-phosphate)** molecules.
  - **Energy Payoff Phase**
    - **Oxidizes G3P → pyruvate**.
    - Produces **4 ATP** and **2 NADH**.
    - Electrons come from glucose oxidation.

Glycolysis



The result of the transfer of a phosphate from a substrate to ADP called **Substrate level phosphorylation**.

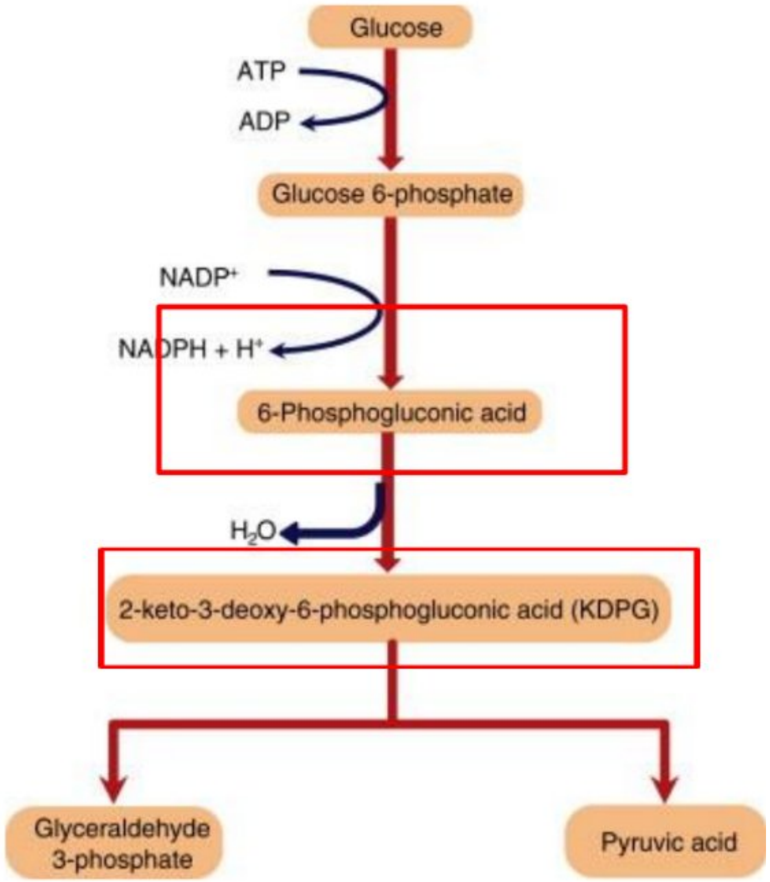
2. Entner-Doudoroff pathway

- ❖ An alternative to the EMP pathway.
- ❖ Key Enzymes of the ED Pathway

1. 6-Phosphogluconate dehydratase
2. 2-Keto-3-deoxy-6-phosphogluconate (KDPG) aldolase.

- ❖ Widespread among **Gram-negative bacteria**: ***Pseudomonas aeruginosa*** (uses only the ED pathway).
- ❖ Primary route for metabolizing specific sugars.
- ❖ For example, it is the only way for *Vibrio cholerae* to metabolize **gluconate**.

- *E. coli* (can use either ED or EMP pathways).
- **Products**
  - 1 ATP,
  - 1 NADH
  - 1 NADPH per glucose molecule.



3. Phosphoketolase pathway (Pentose phosphate pathway)

- The pathway begins with the oxidation of **glucose-6-phosphate (G-6-P) to 6-phosphogluconate**.
- **6-phosphogluconate is then oxidized to ribulose-5-phosphate** (**Ru-5-P**) **and CO_2**.
- **NADPH** is produced during these oxidation reactions.
- Ribulose-5-phosphate is converted to a mixture of sugar phosphates (ranging from 3- to 7-carbon sugars).
- Key Enzymes:

1. Transketolase

Catalyzes the transfer of **2-carbon keto groups**.

2. Transaldolase

Transfers a 3-carbon group from sedoheptulose-7-phosphate to G-3-P

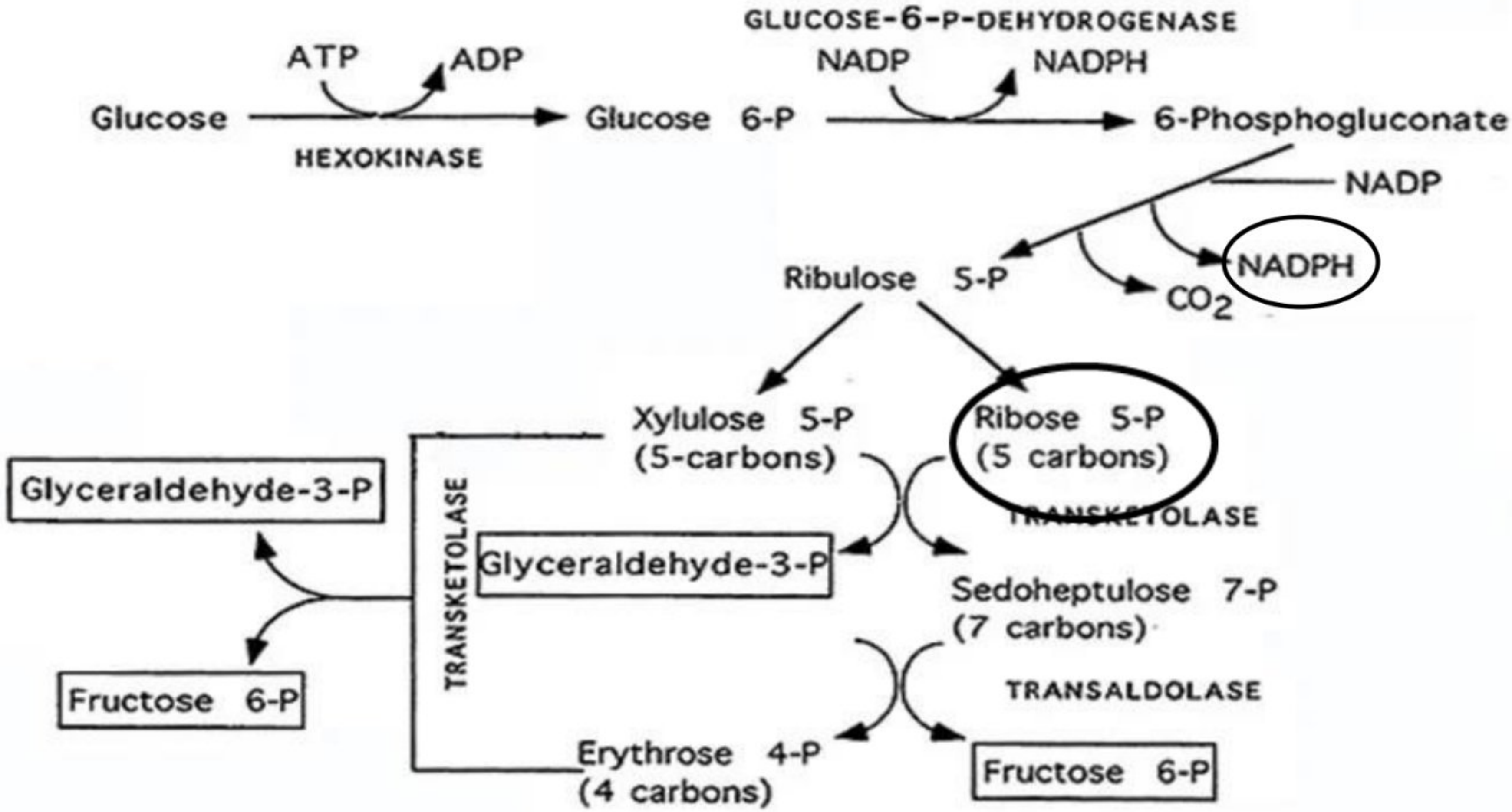
Three molecules of **G-6-P** are converted to:

- 2 fructose-6-phosphate

- 1 glyceraldehyde-3-phosphate (G3P)

- 3 CO_2 molecules

TWO MAIN PRODUCTS



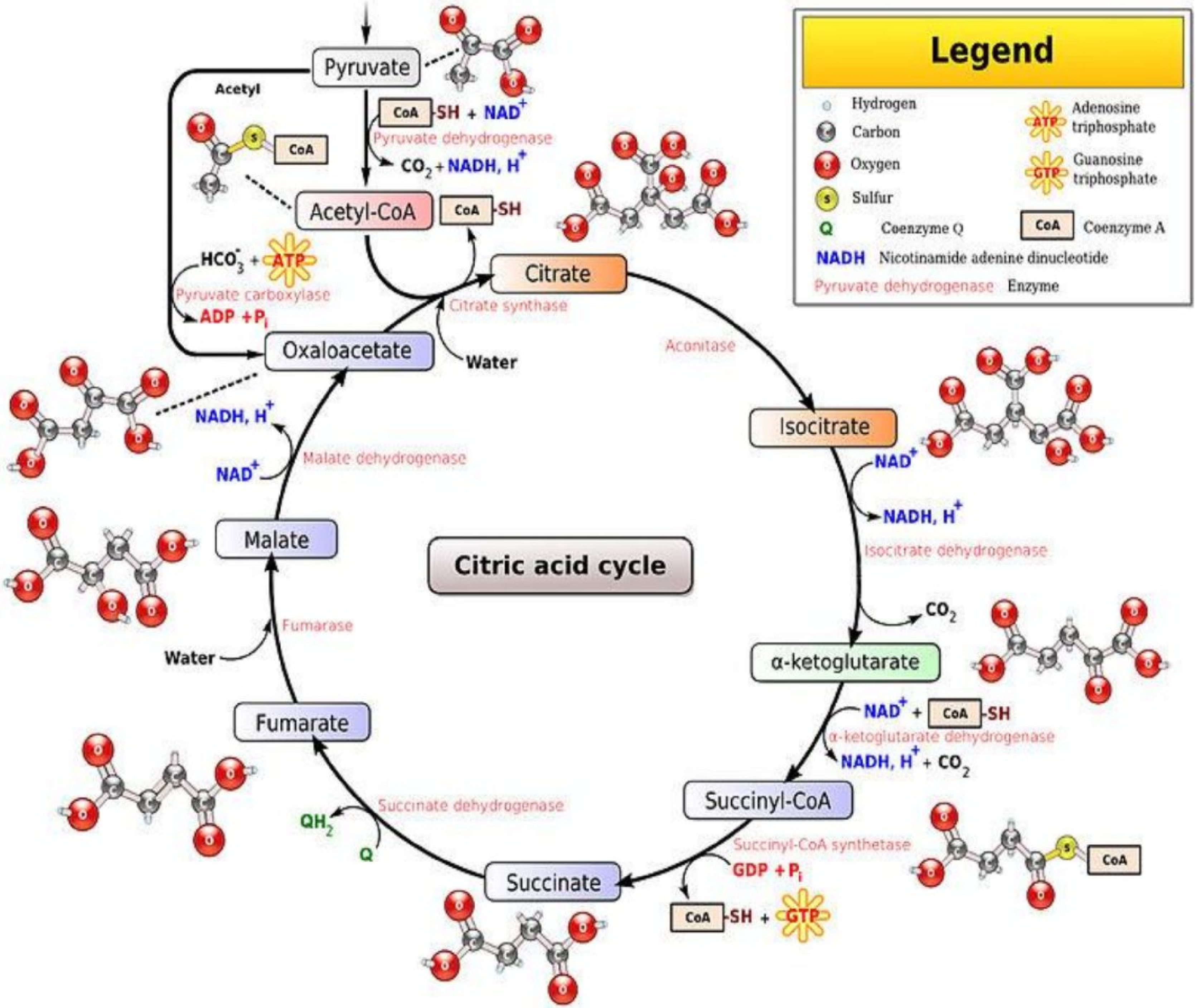
2. Krebs Cycle (Citric Acid Cycle,TCA)

- Series of chemical reactions that begin and end with citric acid and 8 enzymes controlled reactions in this cycle

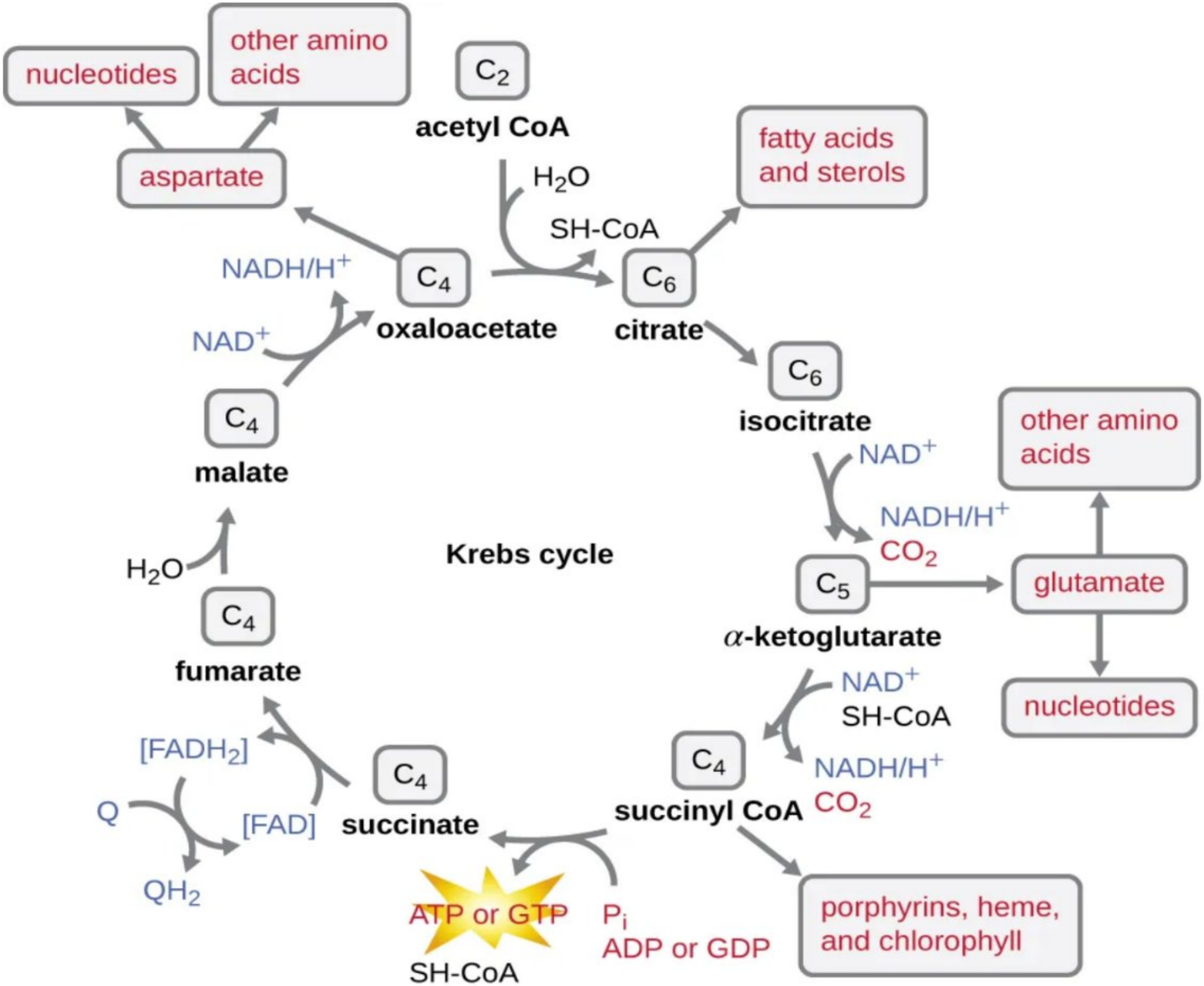
1. **Initial substrate** – modified end product of Glycolysis
   - 2 Pyruvic Acid is modified to 2Acetyl-CoA, which enters the TCA cycle
2. **Circuit of organic acids** – series of oxidations and reductions
   - Eukaryotes and Eukaryotes microbes, including the protists and fungi – Mitochondrial Matrix
   - Prokaryotes – Cytoplasm of bacteria

- **Products:**
  - 2 ATP
  - 6 NADH2
  - 2 FADH2
  - 4 CO2

TCA cycle



Legend
Hydrogen
Carbon
Oxygen
Sulfur
Q Coenzyme Q
NADH Nicotinamide adenine dinucleotide
Pyruvate dehydrogenase Enzyme
ATP Adenosine triphosphate
GTP Guanosine triphosphate
CoA Coenzyme A
Pyruvate
Acetyl
CoA-SH + NAD+
Pyruvate dehydrogenase
CO2 + NADH, H+
Acetyl-CoA
CoA-SH
HCO3- + ATP
Pyruvate carboxylase
ADP + Pi
Oxaloacetate
Citrate
Citrate synthase
Water
Aconitase
Isocitrate
NAD+
NADH, H+
Isocitrate dehydrogenase
CO2
α-ketoglutarate
NAD+ + CoA-SH
α-ketoglutarate dehydrogenase
NADH, H+ + CO2
Succinyl-CoA
Succinyl-CoA synthetase
GDP + Pi
CoA-SH + GTP
Succinate
Succinate dehydrogenase
QH2
Q
Fumarate
Fumarase
Water
Malate
Malate dehydrogenase
NAD+
NADH, H+
Citric acid cycle



Many organisms use intermediates from the Krebs cycle, such as amino acids, fatty acids, and nucleotides, as building blocks for biosynthesis.

Source	Carbon Flow	Molecules of Reduced Coenzymes Produced	Net ATP Molecules Made by Substrate-Level Phosphorylation	Net ATP Molecules Made by Oxidative Phosphorylation	Theoretical Maximum Yield of ATP Molecules
Glycolysis (EMP)	Glucose (6C) → 2 pyruvates (2C)	2 NADH	2 ATP	6 ATP from 2 NADH	8
Transition reaction	2 pyruvates (3C) → 2 acetyl (2C) + 2 CO_2	2 NADH		6 ATP from 2 NADH	6
Krebs cycle	2 acetyl (2C) → 4 CO_2	6 NADH 2 $FADH_2$	2 ATP	18 ATP from 6 NADH 4 ATP from 2 $FADH_2$	24
Total:	glucose (6C) → 6 CO_2	10 NADH 2 $FADH_2$	4 ATP	34 ATP	38 ATP

Actually 34 ATP

The Pathway of Electron Transport

❖ Electron Transfer

- NADH and $FADH_2$ carry pairs of electrons from glycolysis and the citric acid cycle.
- These electrons are transferred to the first cytochrome in a series of iron-containing protein molecules.
- As electrons are transferred, protons (H^+) are released.
- The oxidized coenzymes (NAD^+ and FAD) are recycled back into glycolysis and the citric acid cycle.

❖ Electron Transport

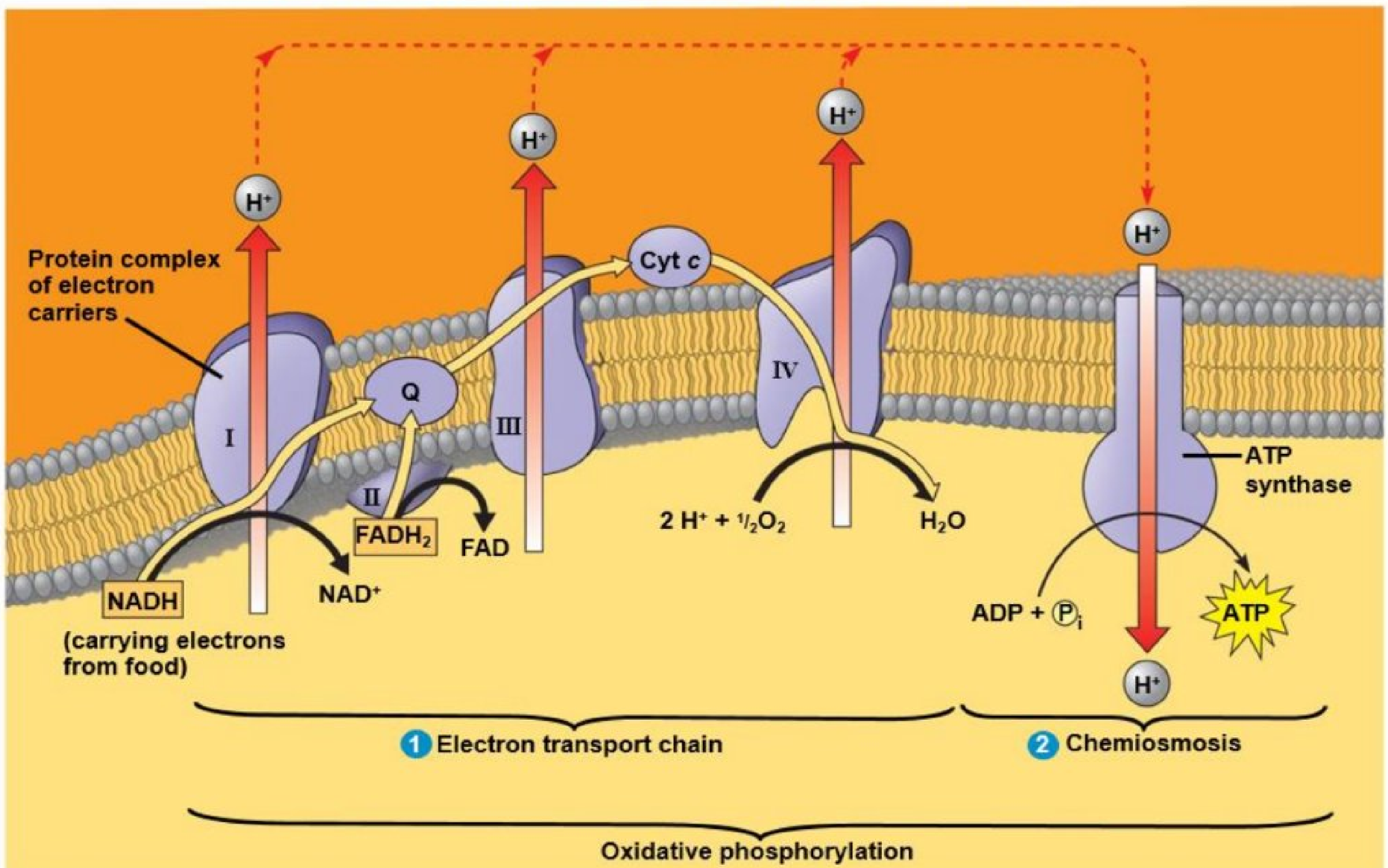
- Electrons are passed stepwise along cytochrome complexes (I–IV) in the **electron transport chain (ETC)**.
- The final electron acceptor is **oxygen (O_2)**, which combines with electrons and protons to form **water (H_2O)**.
- Without oxygen, cytochromes cannot unload electrons, causing the chain to stop functioning.
- The energy released during electron transfer is used to **pump protons (H^+)** across the cell membrane by **active transport**.

❖ Chemiosmosis (ATP Synthesis)

- The pumped protons accumulate outside the membrane, creating a **proton gradient**.
- **ATP synthase** channels allow protons to flow back through the membrane by **facilitated diffusion**.
- As protons move through ATP synthase, their energy drives the formation of **ATP from ADP and phosphate**. **Proton-motive force**
- This energy-coupling process is called **chemiosmosis** or **oxidative phosphorylation**.
- If the cell membrane is damaged, proton flow and ATP synthesis stop, leading to **cell death**.
- Some **antibiotics and disinfectants** kill bacteria by disrupting the membrane and halting chemiosmosis.

- The energy stored in a H^+ gradient across a membrane couples the redox reactions of the electron transport chain to ATP synthesis
- The H^+ gradient is referred to as a **proton-motive force**, emphasizing its capacity to do work.

Chemiosmosis couples the electron transport chain to ATP synthesis



Comparison of energy yields between aerobic and anaerobic respiration				
Type of Metabolism	**Example**	**Final Electron Acceptor**	**Pathways Involved in ATP Synthesis (Type of Phosphorylation)**	**Maximum Yield of ATP Molecules**
Aerobic respiration	*Pseudomonas aeruginosa*	O_2	EMP glycolysis Krebs cycle Electron transport and chemiosmosis:	2 2 34
			Total	**38**
Anaerobic respiration	*Paracoccus denitrificans*	NO_3^-, SO_4^{-2}, Fe^{+3}, CO_2 other inorganics	EMP glycolysis Krebs cycle Electron transport and chemiosmosis:	2 2 1–32
			Total	**5–36**

Based on magnitude of transport chain between donors and acceptors

- Electrons move from low reduction potential to high reduction potential

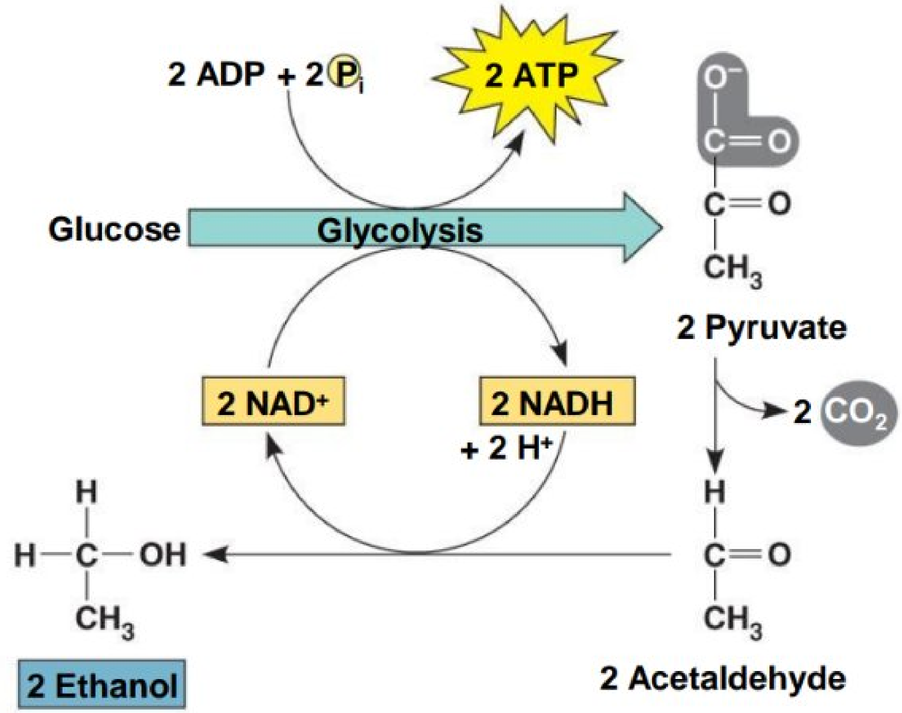
$\Delta G = -nF\Delta E$

n = the number of electrons transferred and
F = Faraday's constant **23.06** kcal/V/mol).
The larger a positive ΔE, the more exergonic a reaction

Redox Couple	$E^{0\prime}$ (V)
CO_2/glucose	-0.43
$2H^+/H_2$	-0.42
$Ferredoxin_{oxid/red}$	-0.39
CO_2/methanol	-0.38
NAD^+/NADH	-0.32
CO_2/acetate	-0.28
S_0/H_2S	-0.28
CO_2/CH_4	-0.24
SO_4^{2-}/H_2S	-0.22
$FAD/FADH_2$	-0.22
Pyruvate/lactate	-0.19
Fumarate/succinate	+0.03
Cytochrome $b_{oxid/red}$	+0.035
$Ubiquinone_{oxid/red}$	+0.110
Fe^{3+}/Fe^{2+} (pH 7)	+0.20
Cytochrome $c_{oxid/red}$	+0.25
Cytochrome $a_{oxid/red}$	+0.39
NO_3^-/NO_2^-	+0.42
NO_2^-/NH_4^+	+0.44
$NO_3^-/½N_2$	+0.74
Fe^{3+}/Fe^{2+} (pH 2)	+0.77
$½O_2/H_2O$	+0.82

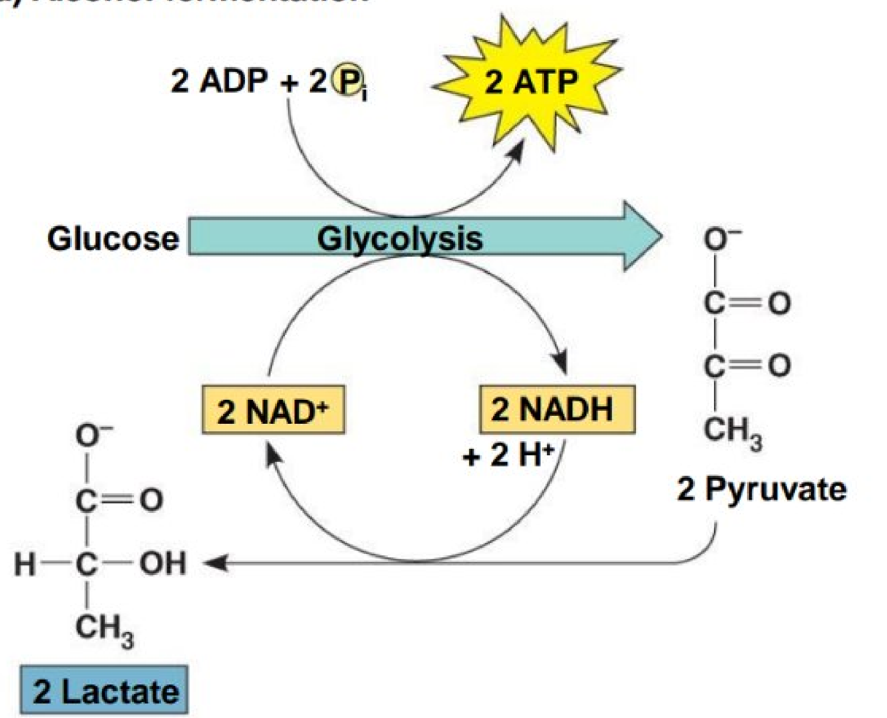
Fermentation and anaerobic respiration enable cells to produce ATP without the use of oxygen

- Glycolysis can produce ATP with or without O_2 (in aerobic or anaerobic conditions)
- In the absence of O_2, glycolysis couples with fermentation or anaerobic respiration to produce ATP
- Many human enteric, facultative species, use nitrate (NO_3^-) to form nitrite (NO_2^-).
- ATP yield is **lower in anaerobic respiration** compared to aerobic respiration. Approximately 2ATP
- Fermentation uses phosphorylation instead of an electron transport chain to generate ATP



(a) Alcohol fermentation

Streptococcus lactis



(b) Lactic acid fermentation

❖ Fats as Energy Sources

- **Fats** are composed of **three fatty acids** attached to a **glycerol** molecule.
- The **carbon–carbon covalent bonds** in fatty acids store **high amounts of chemical energy**.
- The enzyme **lipase** breaks fats into **glycerol** and **fatty acids**.
  - **Glycerol** is converted into an **intermediate of glycolysis** and enters that pathway for ATP production.
  - **Fatty acids** undergo **β-oxidation (beta-oxidation)** — a sequence of reactions that split them into **two-carbon (2C) units**.
- Each 2C unit is transformed into **acetyl-CoA**, which then enters the **citric acid cycle** for further oxidation and ATP generation.
- Because each glucose molecule produces about **20 ATP** in the citric acid cycle, a **16-carbon fatty acid** (producing **eight 2C units**) yields a **much greater total ATP output**, demonstrating fats' high energy content.

Step 1: β-oxidation of the 16-carbon fatty acid

- Each **β-oxidation cycle** removes **2 carbons** as **acetyl-CoA**.
- For a 16-carbon chain:
  - Number of **acetyl-CoA** molecules = 16 ÷ 2 = **8**
  - Number of **β-oxidation cycles** = 8 − 1 = **7**
- Each β-oxidation cycle yields:

  1 NADH → 2.5 ATP (in the electron transport chain)

  1 $FADH_2$ → 1.5 ATP

→ So, **7 NADH × 2.5 = 17.5 ATP**

→ **7 $FADH_2$ × 1.5 = 10.5 ATP**

Total from β-oxidation itself = 17.5+ 10.5 = 28 ATP

Step 2: ATP from oxidation of acetyl-CoA in the citric acid cycle

- Each **acetyl-CoA** yields approximately:

•3 NADH → 7.5 ATP

•1 $FADH_2$ → 1.5 ATP

•1 GTP → 1 ATP * *******

→ **Total = 10 ATP per acetyl-CoA**

For **8 acetyl-CoA**:

→ 8 × 10 = **80 ATP**

Activation of the fatty acid (to form fatty acyl-CoA) costs **2 ATP**. Net ATP= 106

Deamination