Microbes as Energy Transducers

- The Metabolic Menu
- Metabolic Strategies
- Respiration & Fermentation
- Chemolithotrophy
- Photoautotrophy
- Biogeochemical Cycles
- Metabolism in Primitive Organisms

All major types of nutrition and metabolism evolved among prokaryotes: they are the ultimate biochemists

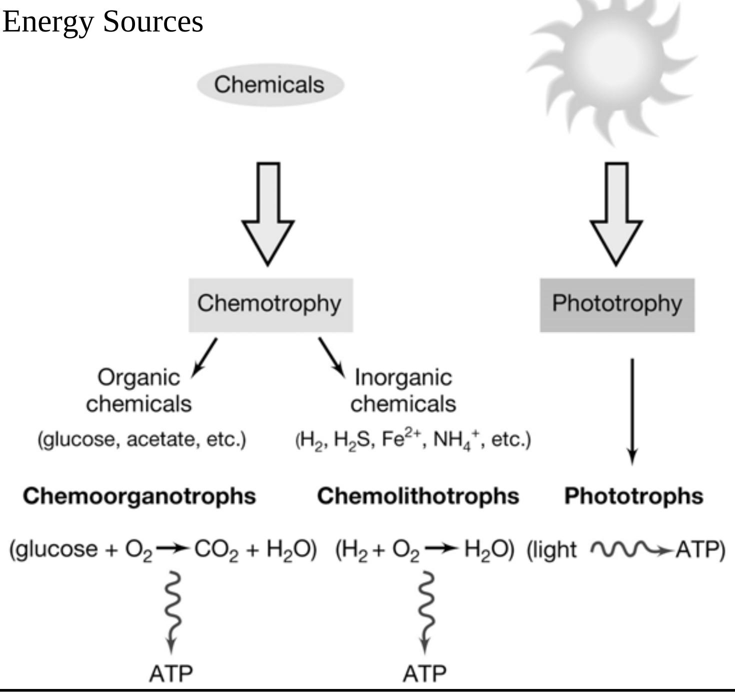
The prokaryotes exhibit some unique modes of nutrition as well as **every type** of nutrition found in eukaryotes.

Major Modes of Nutrition:

Prokaryotes exhibit a great diversity in how they obtain the necessary resources **(energy and carbon)** to synthesize organic compounds.

- Some obtain energy from light **(phototrophs)**, while others use chemicals taken from the environment **(chemotrophs)**.
- Many can utilized CO_2 as a carbon source **(autotrophs)** and others require at least one organic nutrient as a carbon source **(heterotrophs)**.

Energy Sources



Chemicals

Chemotrophy

Phototrophy

Organic chemicals (glucose, acetate, etc.)

Inorganic chemicals (H_2, H_2S, Fe^{2+}, NH_4^+, etc.)

Chemoorganotrophs

Chemolithotrophs

Phototrophs

(glucose + $O_2 \rightarrow CO_2 + H_2O$)

($H_2 + O_2 \rightarrow H_2O$)

(light ∿∿→ ATP)

ATP

ATP

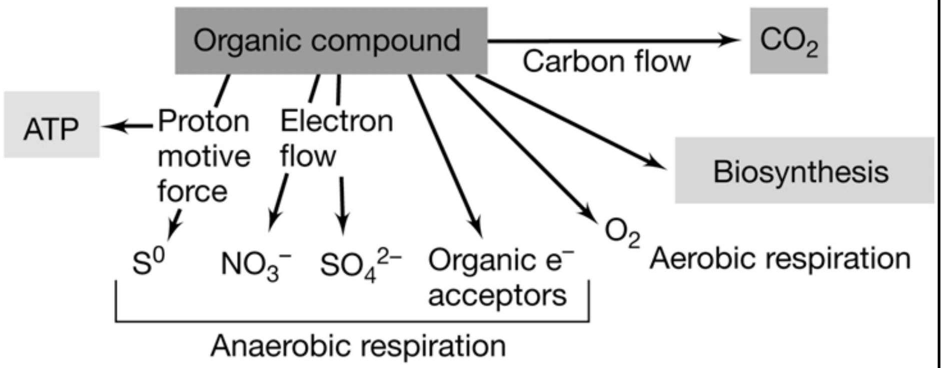
Depending upon the energy source **AND** the carbon source, prokaryotes have **four** possible nutritional modes:

1. Photoautotrophs: Use light energy to synthesize organic compounds from CO_2 – Includes the cyanobacteria. (Actually all photosynthetic eukaryotes fit in this category.)

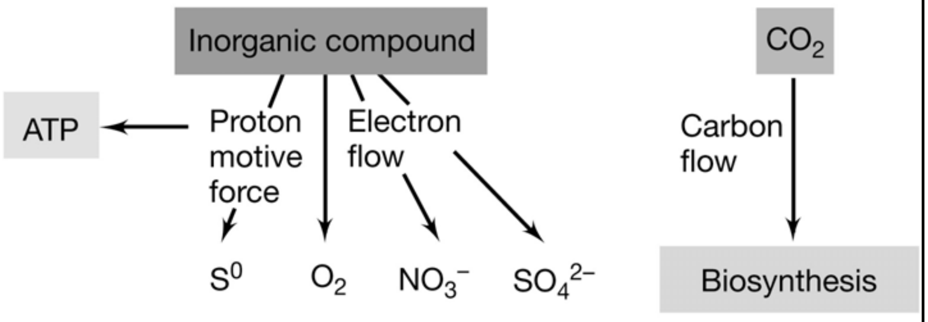
2. Chemoautotrophs: Require only CO_2 as a carbon source and obtain energy by oxidizing inorganic compounds. This mode of nutrition is unique only to certain prokaryotes.

3. Photoheterotrophs: Use light to generate ATP from an organic carbon source. This mode of nutrition is unique only to certain prokaryotes.

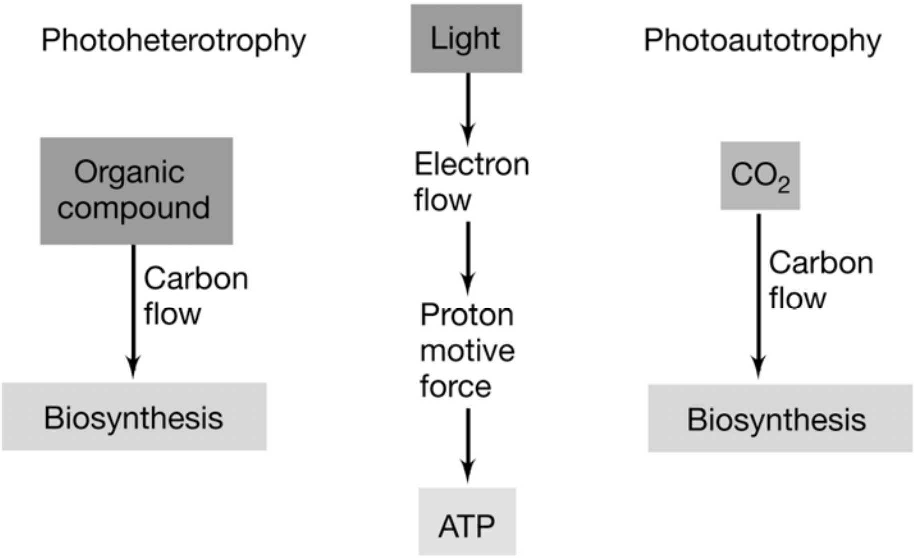
4. Chemoheterotrophs: Must obtain organic molecules for energy and as a source of carbon. Found in many bacteria as well as most eukaryotes.



(a) **Chemoorganotrophic metabolism**

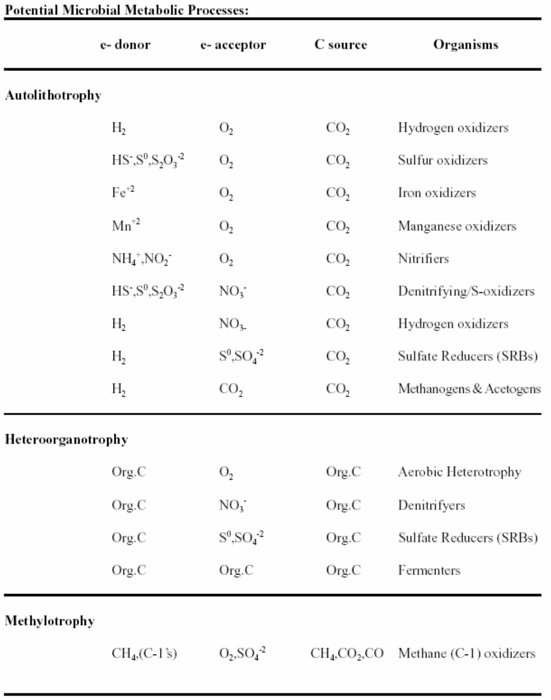


(b) **Chemolithotrophic metabolism**



(c) **Phototrophic metabolism**

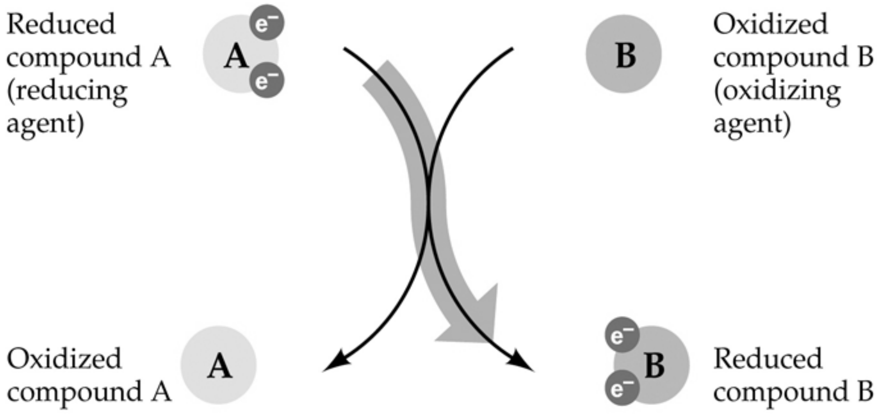
Metabolic Menu For Chemotrophs



Potential Microbial Metabolic Processes:

	e- donor	e- acceptor	C source	Organisms
Autolithotrophy				
	H_2	O_2	CO_2	Hydrogen oxidizers
	$HS^-, S^0, S_2O_3^{-2}$	O_2	CO_2	Sulfur oxidizers
	Fe^{+2}	O_2	CO_2	Iron oxidizers
	Mn^{+2}	O_2	CO_2	Manganese oxidizers
	NH_4^+, NO_2^-	O_2	CO_2	Nitrifiers
	$HS^-, S^0, S_2O_3^{-2}$	NO_3^-	CO_2	Denitrifying/S-oxidizers
	H_2	NO_{3-}	CO_2	Hydrogen oxidizers
	H_2	S^0, SO_4^{-2}	CO_2	Sulfate Reducers (SRBs)
	H_2	CO_2	CO_2	Methanogens & Acetogens
Heteroorganotrophy				
	Org.C	O_2	Org.C	Aerobic Heterotrophy
	Org.C	NO_3^-	Org.C	Denitrifyers
	Org.C	S^0, SO_4^{-2}	Org.C	Sulfate Reducers (SRBs)
	Org.C	Org.C	Org.C	Fermenters
Methylotrophy				
	CH_4,(C-1's)	O_2, SO_4^{-2}	CH_4, CO_2, CO	Methane (C-1) oxidizers

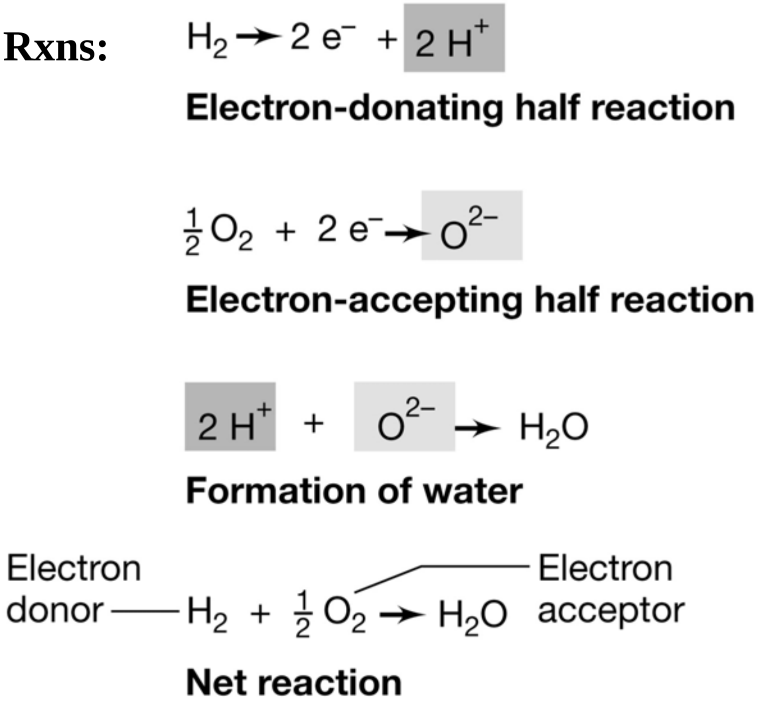
Oxidation and Reduction are Coupled Reactions



A is oxidized,
losing electrons

B is reduced,
gaining electrons

Redox Rxns:



$H_2 \rightarrow 2\ e^- + \boxed{2\ H^+}$

Electron-donating half reaction

$\frac{1}{2} O_2 + 2\ e^- \rightarrow \boxed{O^{2-}}$

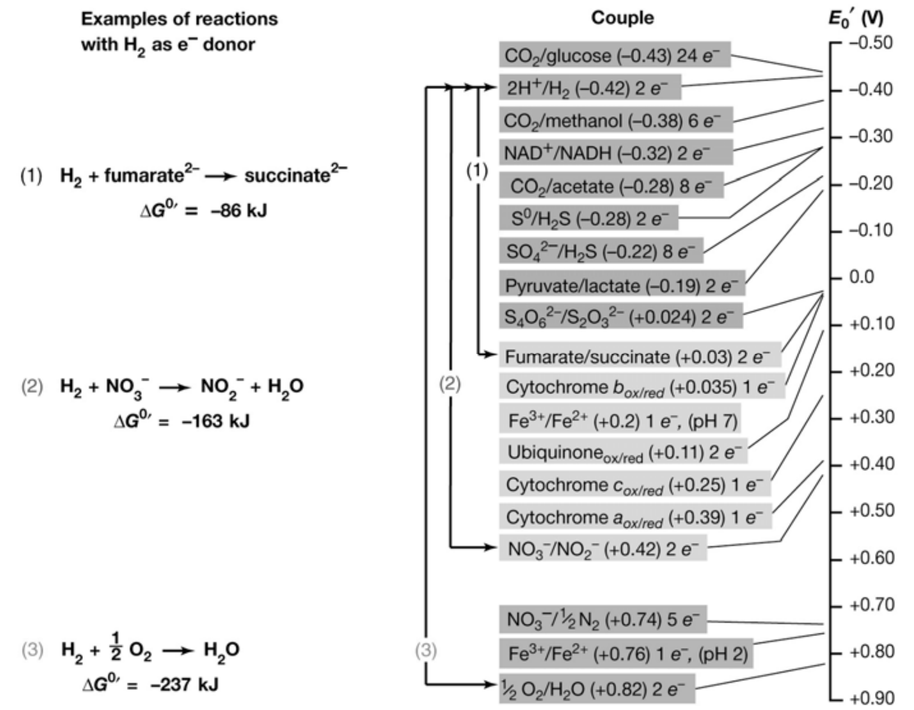
Electron-accepting half reaction

$\boxed{2\ H^+} + \boxed{O^{2-}} \rightarrow H_2O$

Formation of water

Electron donor — $H_2 + \frac{1}{2} O_2 \rightarrow H_2O$ — Electron acceptor

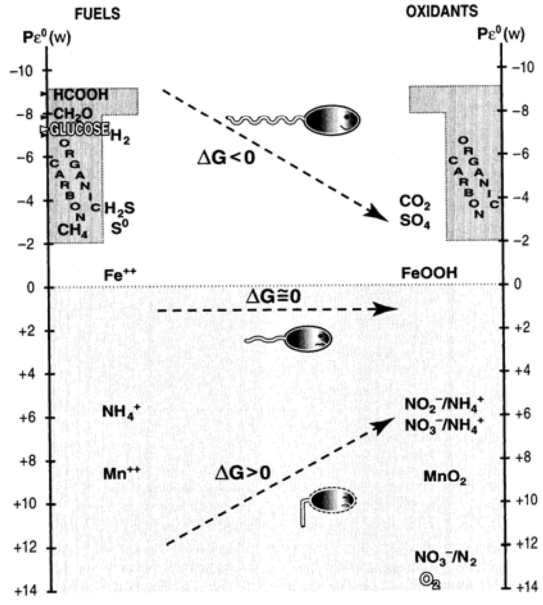
Net reaction



Thermodynamics: The Chemical Fuels and Oxidants of Life

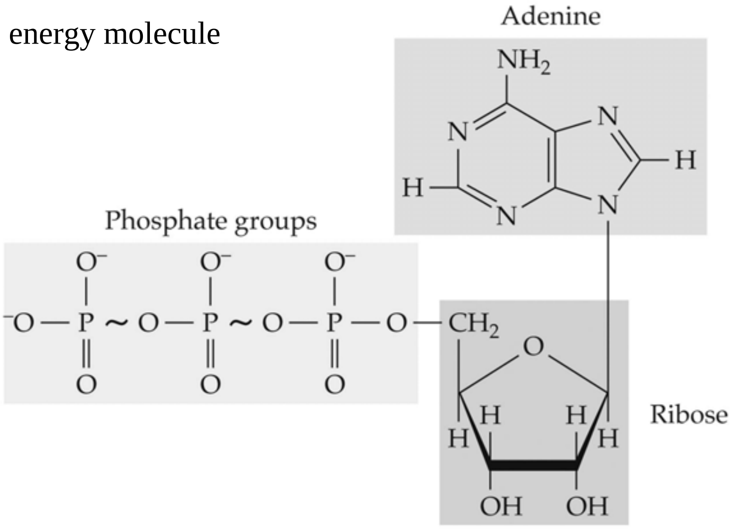
e- source

e- sink



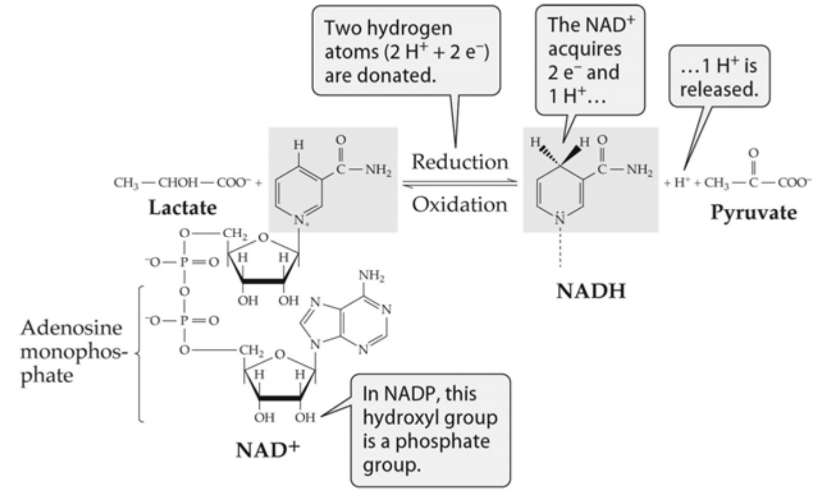
Adenosine-5’-triphosphate (ATP)

The energy molecule



Adenosine triphosphate

Nicotinamide adenine dinucleotide (NAD)



Two hydrogen atoms ($2\ H^+ + 2\ e^-$) are donated.

The NAD^+ acquires $2\ e^-$ and $1\ H^+$…

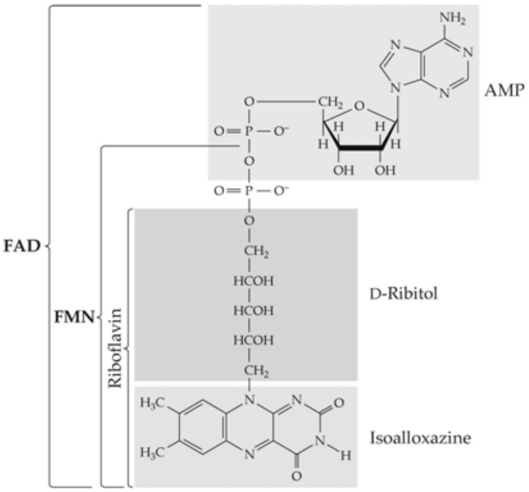
…$1\ H^+$ is released.

In NADP, this hydroxyl group is a phosphate group.

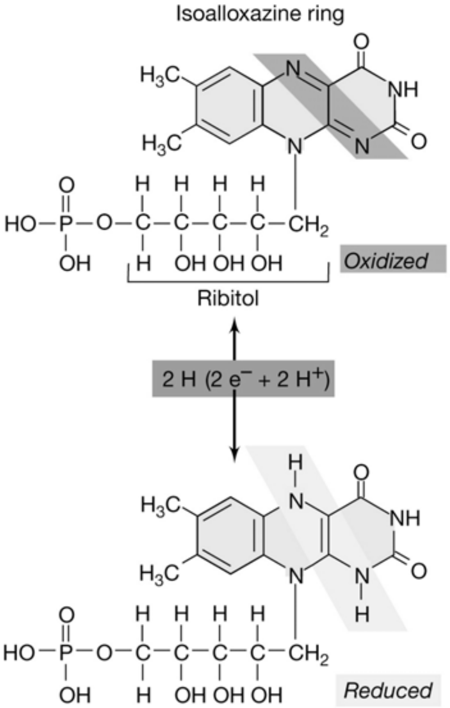
The redox molecule

Flavin nucleotides, components of flavoproteins

Specialty redox molecule

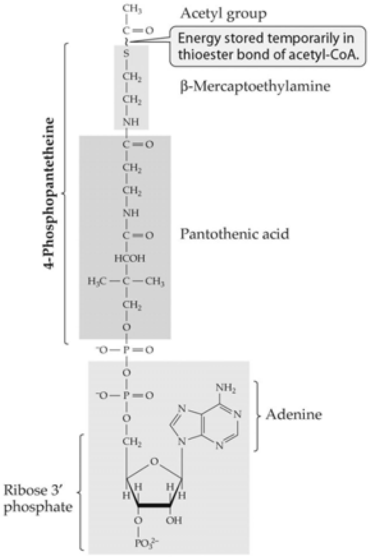


Flavin nucleotides

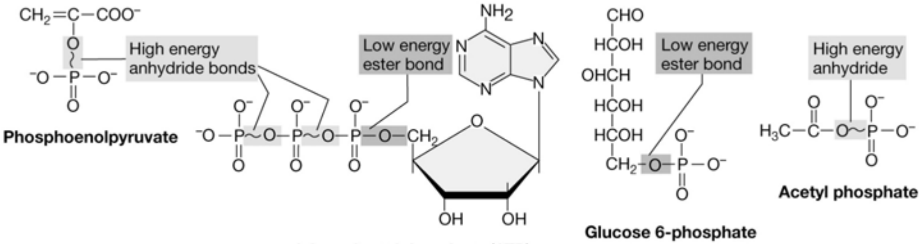


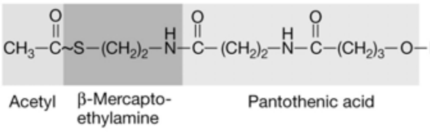
Acetyl-coenzyme A (acetyl-CoA)

Specialty energy molecule & organic C carrier

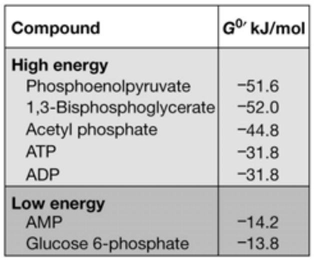


Acetyl-CoA



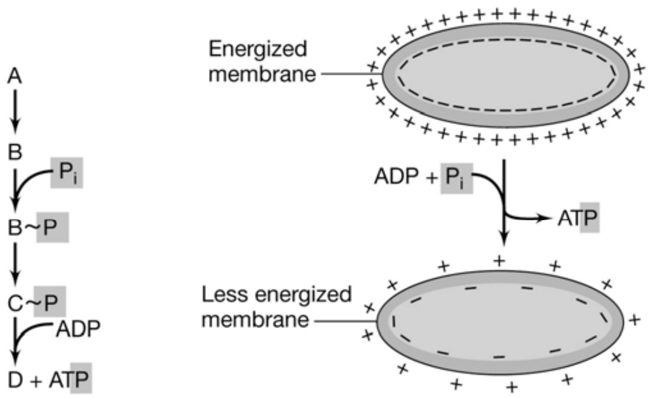


Acetyl-CoA

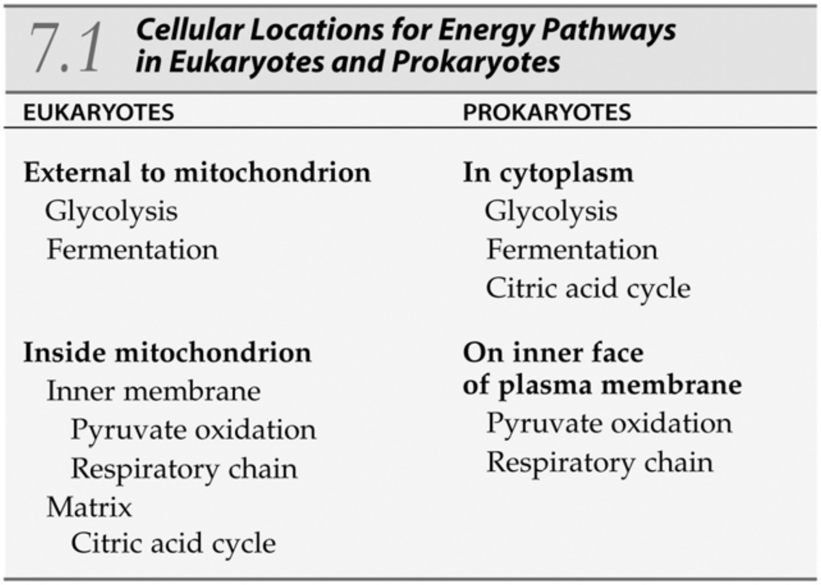


Compound	$G^{0\prime}$ kJ/mol
High energy	
Phosphoenolpyruvate	−51.6
1,3-Bisphosphoglycerate	−52.0
Acetyl phosphate	−44.8
ATP	−31.8
ADP	−31.8
Low energy	
AMP	−14.2
Glucose 6-phosphate	−13.8

Two Ways to Make ATP: Quick & Dirty or Turbo-Charged

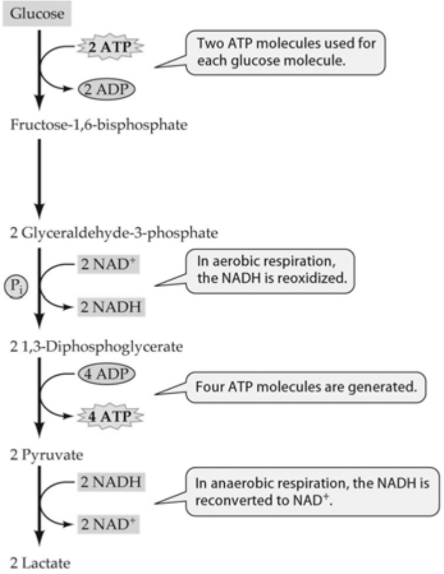


(a) Substrate-level phosphorylation **(b) Oxidative phosphorylation**



7.1 Cellular Locations for Energy Pathways in Eukaryotes and Prokaryotes

EUKARYOTES	PROKARYOTES
External to mitochondrion	**In cytoplasm**
Glycolysis	Glycolysis
Fermentation	Fermentation
	Citric acid cycle
Inside mitochondrion	**On inner face of plasma membrane**
Inner membrane	
Pyruvate oxidation	Pyruvate oxidation
Respiratory chain	Respiratory chain
Matrix	
Citric acid cycle	



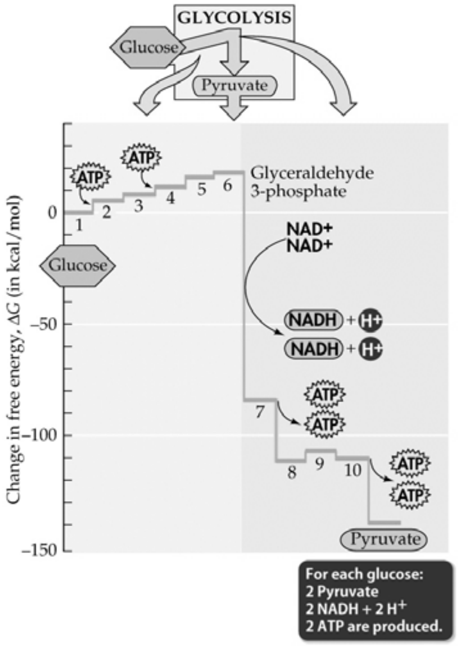
Two ATP molecules used for each glucose molecule.

In aerobic respiration, the NADH is reoxidized.

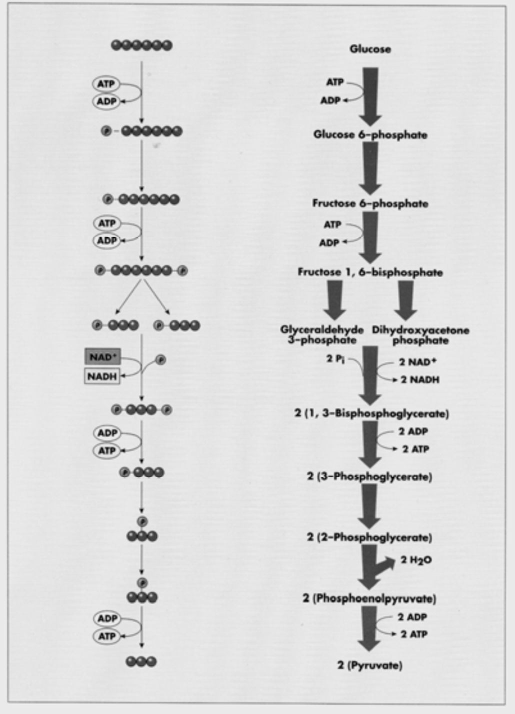
Four ATP molecules are generated.

In anaerobic respiration, the NADH is reconverted to NAD^+.

Glycolysis: short form



For each glucose:
2 Pyruvate
2 NADH + 2 H^+
2 ATP are produced.



Glycolysis aka
Embden-Meyerhof

The short form!

4 ATP / 2 Net
2 NADH+H

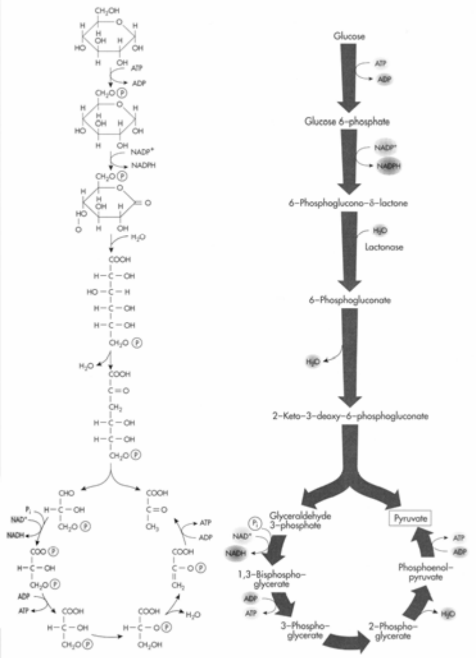


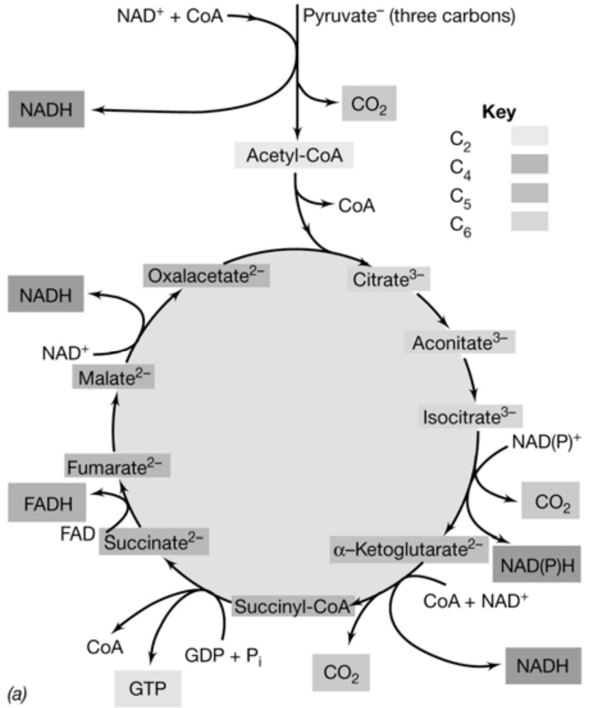
Fig. 4-5 Entner-Doudoroff Pathway of Glycolysis. The Entner-Doudoroff pathway is one of several types of glycolysis. Compared to the Embden-Myerhof pathway, less ATP is generated when this metabolic pathway is used.

Entner-Doudoroff:

2 ATP / only 1 Net
2 NADH+H

No PFK!!!

Many Gram negatives
use this pathway



Overall reaction: Pyruvate$^-$ + 4 NAD$^+$ + FAD → 3 CO_2 + 4 NADH + FADH

(1) Substrate-level phosphorylation

$GDP + P_i \rightarrow GTP$

$GTP + ADP \rightarrow GDP + ATP$

(2) Electron transport phosphorylation

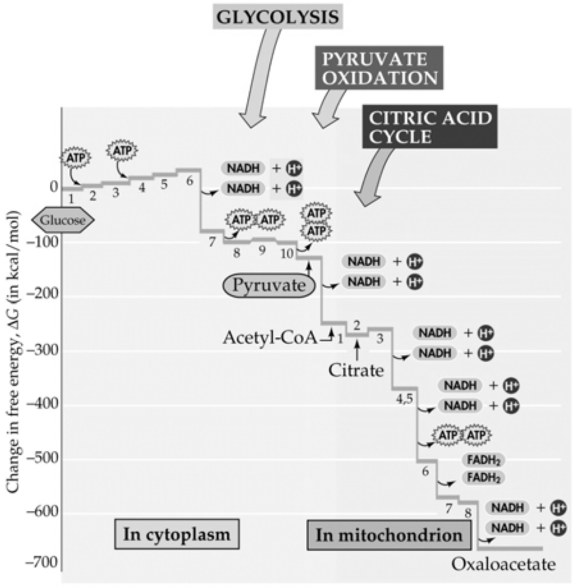
4 NADH ≡ 12 ATP

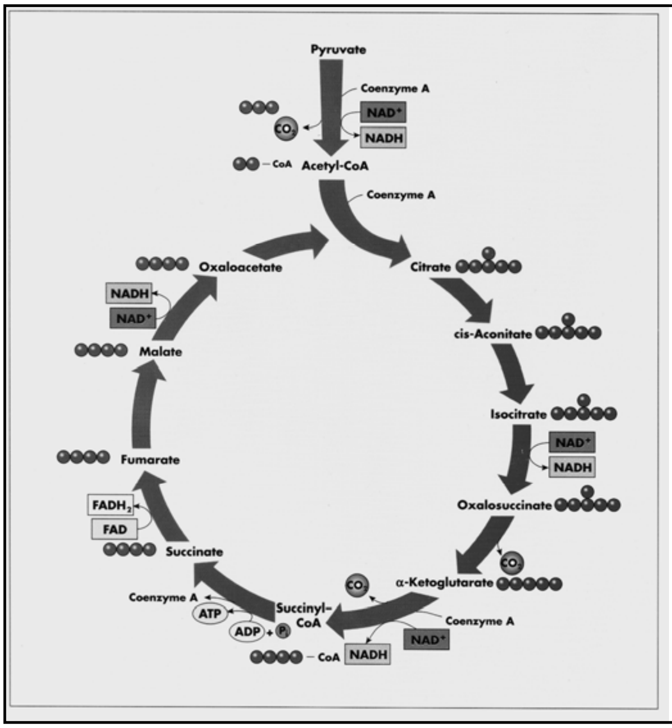
FADH ≡ 2 ATP

15 ATP

(3) Sum: CAC plus glycolysis → 38 ATP per glucose

(b)





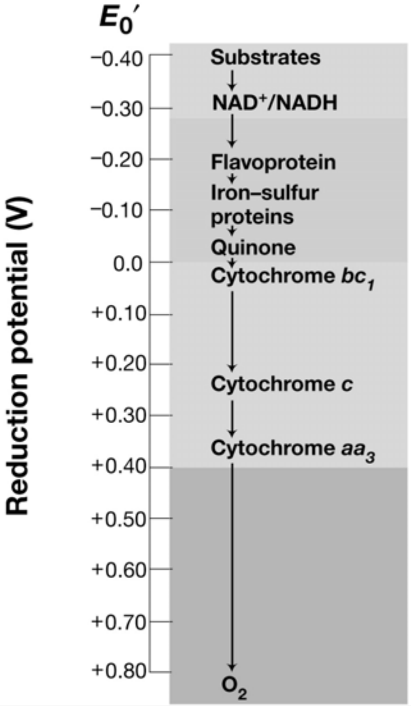
Citric Acid Cycle
aka TCA cycle

The short form!

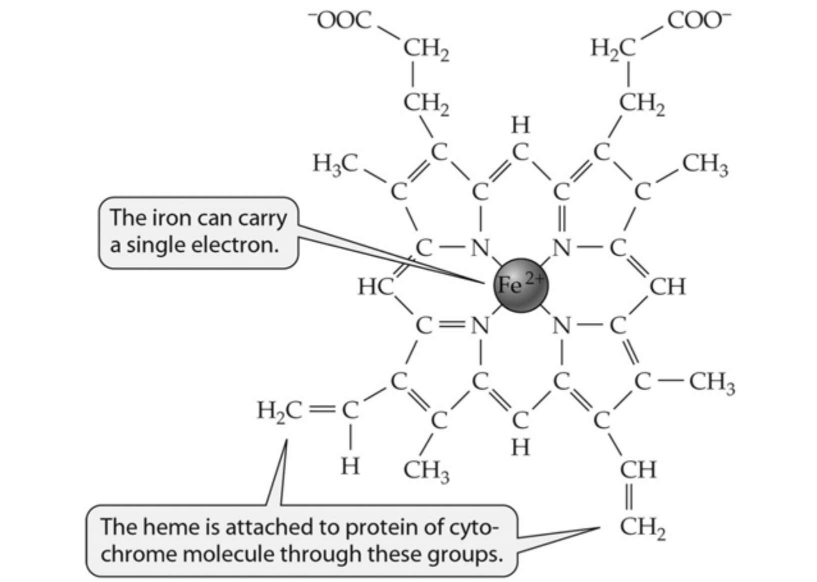
2 ATP (via GTP)
8 NADH+H
2 $FADH_2$

All Carbon to CO_2

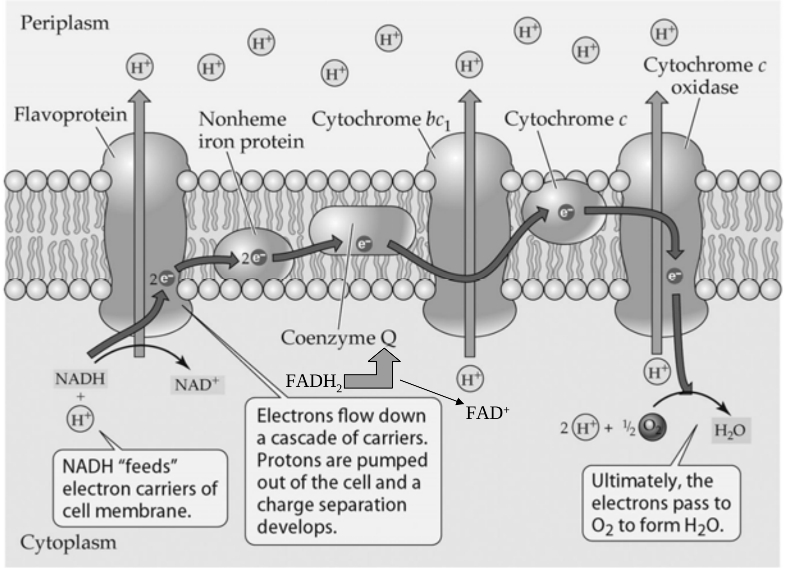
Electron Transport Chains and their Relative Potential



The heme part of a cytochrome, the elegant porphyrin ring!



Electron transport chain in aerobic bacterium

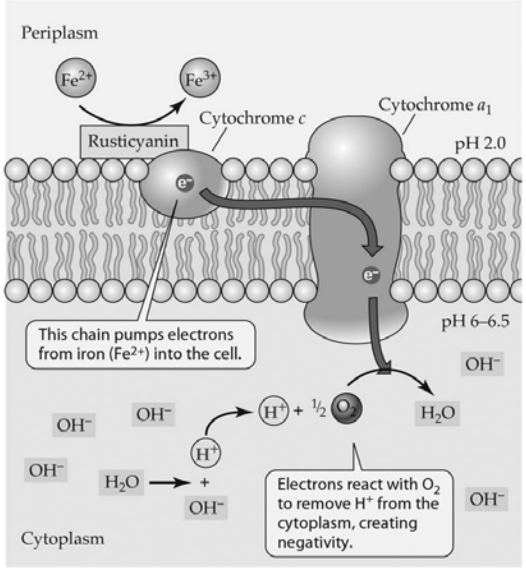


NADH "feeds" electron carriers of cell membrane.

Electrons flow down a cascade of carriers. Protons are pumped out of the cell and a charge separation develops.

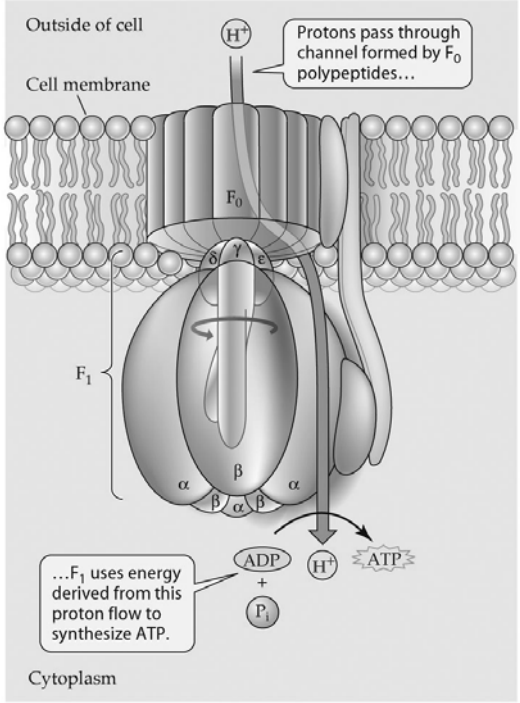
Ultimately, the electrons pass to O_2 to form H_2O.

Abbreviated electron transport chain of an "iron-oxidizing" bacterium



This chain pumps electrons from iron (Fe^{2+}) into the cell.

Electrons react with O_2 to remove H^+ from the cytoplasm, creating negativity.



ATP Synthase
Structure & Function

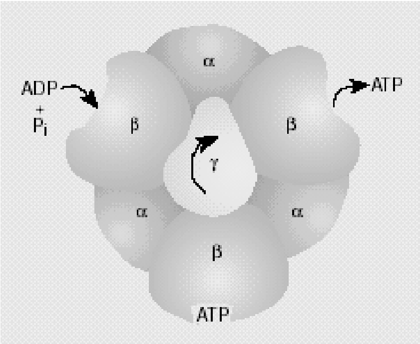


Figure 2 How rotation of the γ subunit drives catalysis. During ATP synthesis, rotation of the γ subunit causes sequential changes in the β subunits. A rotation of 120° changes the β subunit that binds ADP and P_i to a form with tightly bound ATP. The subunit with tightly bound ATP then changes to a form that releases ATP, and the third subunit prepares to bind another ADP and P_i.

F1 Subunit Topview

ATP Synthase acts as a rotary motor turning in 120 degree steps.

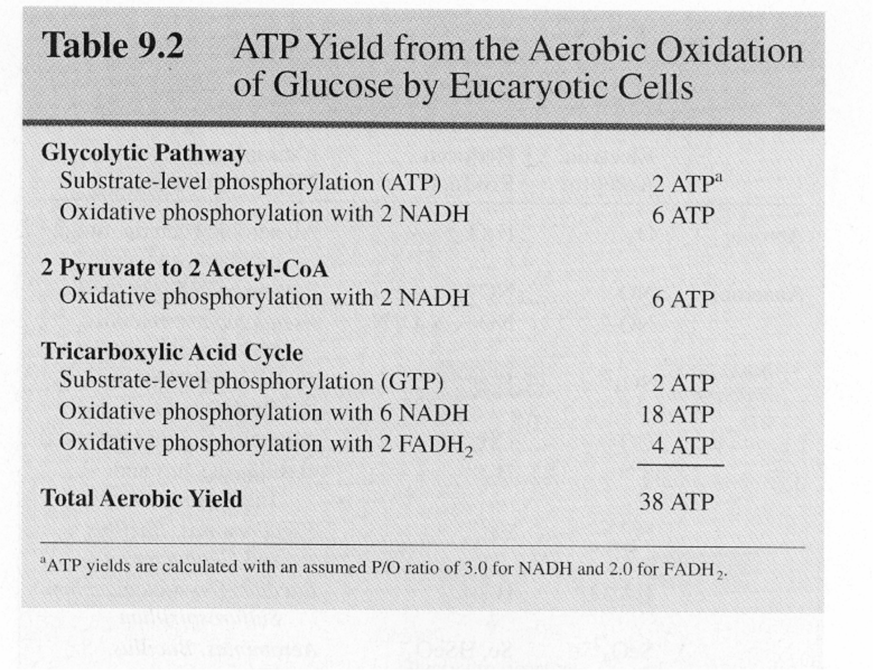


Table 9.2 ATP Yield from the Aerobic Oxidation of Glucose by Eucaryotic Cells

Glycolytic Pathway	
Substrate-level phosphorylation (ATP)	2 ATP[a]
Oxidative phosphorylation with 2 NADH	6 ATP
2 Pyruvate to 2 Acetyl-CoA	
Oxidative phosphorylation with 2 NADH	6 ATP
Tricarboxylic Acid Cycle	
Substrate-level phosphorylation (GTP)	2 ATP
Oxidative phosphorylation with 6 NADH	18 ATP
Oxidative phosphorylation with 2 $FADH_2$	4 ATP
Total Aerobic Yield	38 ATP

[a] ATP yields are calculated with an assumed P/O ratio of 3.0 for NADH and 2.0 for $FADH_2$.

Fermentation – Key Features

(1) Substrate-level phosphorylation is the rule*.

(2) Always anaerobic (even when some O_2 might be around).

(3) No externally supplied terminal electron acceptor.

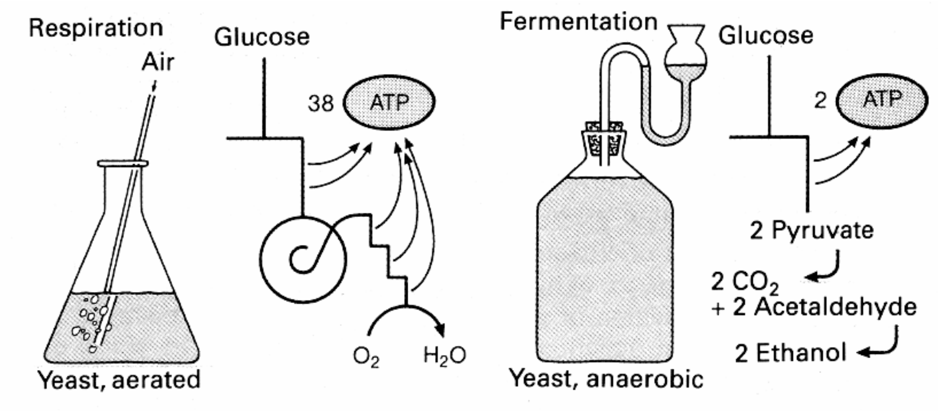
Many types.... 2 major themes

(1) $NADH+H^+$ gets oxidized to NAD^+

(2) Electron acceptor is usually **Pyruvate** or its derivative.

*Rules are always meant to be broken!

Pasteur Effect: ~20X more biomass when aerated



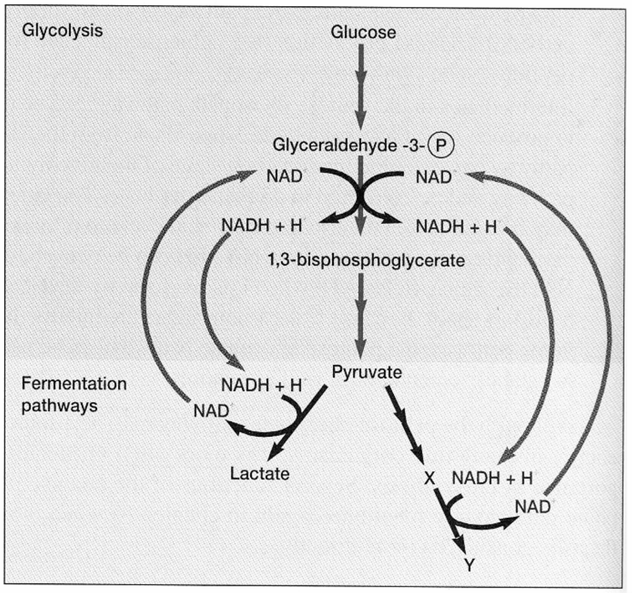
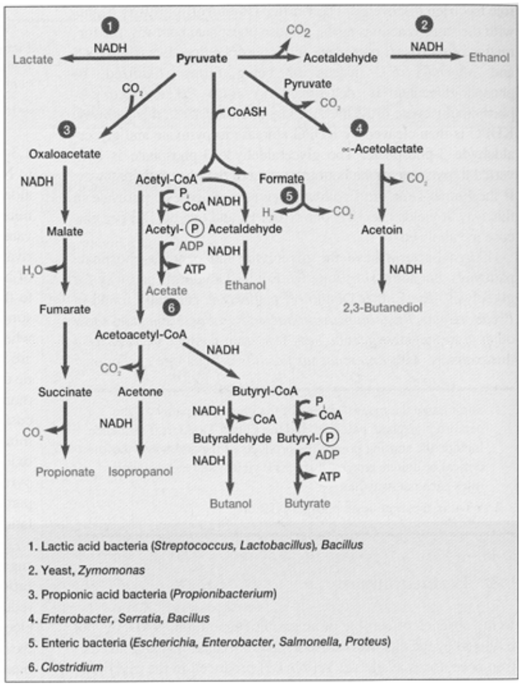
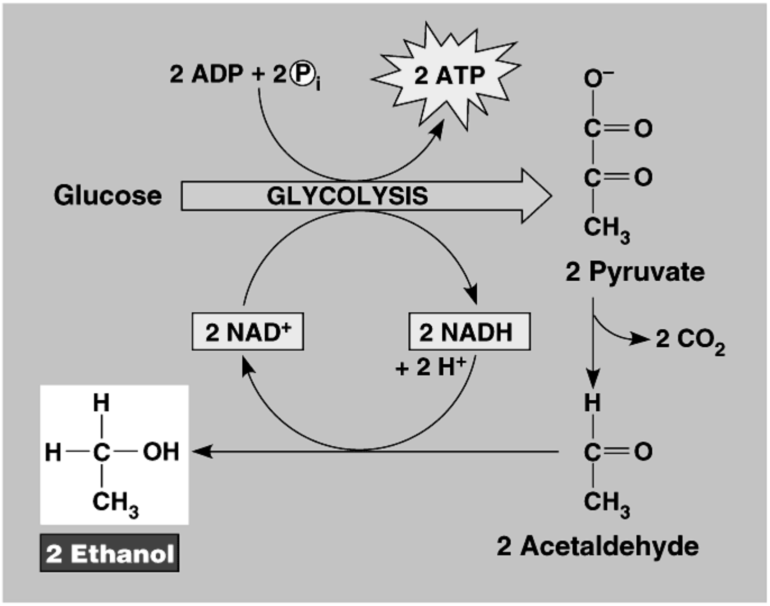


Figure 9.9 Reoxidation of NADH During Fermentation. NADH from glycolysis is reoxidized by being used to reduce pyruvate or a pyruvate derivative (X). Either lactate or reduced product Y result.

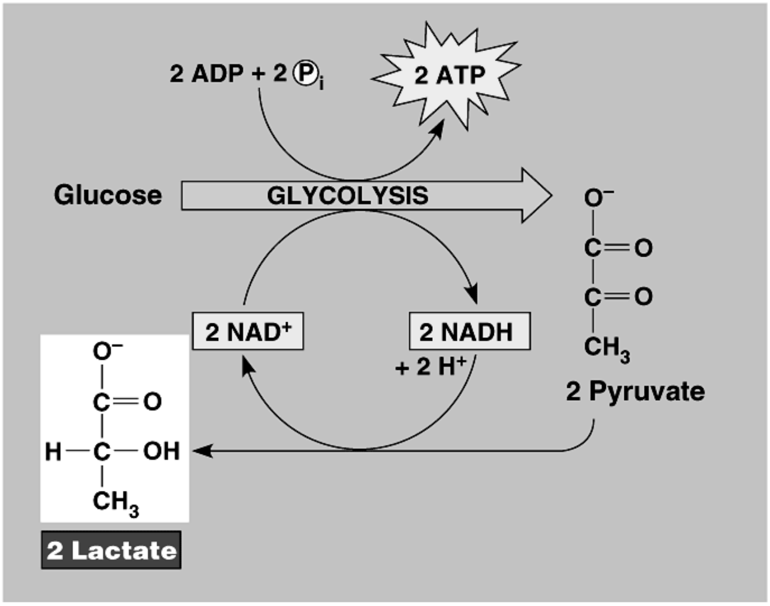
Figure 9.10 Some Common Microbial Fermentations. Only pyruvate fermentations are shown for the sake of simplicity; many other organic molecules can be fermented. Most of these pathways have been simplified by deletion of one or more steps and intermediates. Pyruvate and major end products are shown in color.



1. Lactic acid bacteria (*Streptococcus, Lactobacillus*), *Bacillus*
2. Yeast, *Zymomonas*
3. Propionic acid bacteria (*Propionibacterium*)
4. *Enterobacter, Serratia, Bacillus*
5. Enteric bacteria (*Escherichia, Enterobacter, Salmonella, Proteus*)
6. *Clostridium*



(a) Alcohol fermentation



(b) Lactic acid fermentation

Proprionic Acid Fermentation

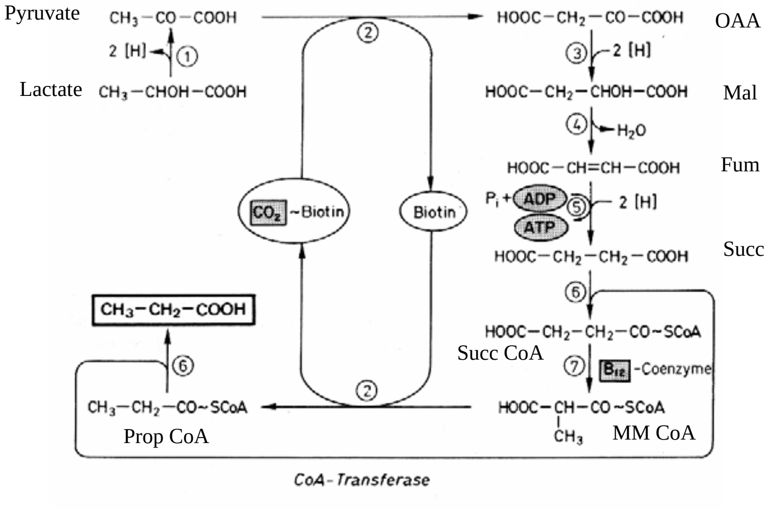
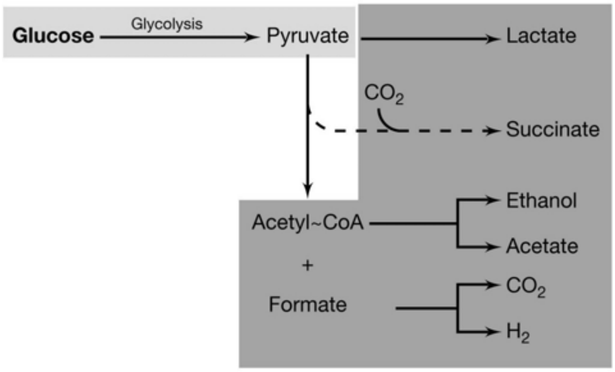


Fig. 8.3. *Methylmalonyl-CoA pathway of propionate formation.*

Enzymes: (1) lactate dehydrogenase; (2) methylmalonyl-CoA carboxytransferase; (3) malate dehydrogenase; (4) fumarase; (5) fumarate reductase (leading to regeneration of ATP by proton translocation); (6) CoA transferase; (7) methylmalonyl-CoA mutase.

(a) **Mixed acid fermentation** (for example, *Escherichia coli*)



Glucose $\xrightarrow{\text{Glycolysis}}$ Pyruvate → Lactate

Pyruvate + CO_2 ⇢ Succinate

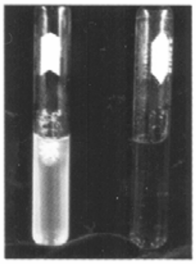
Pyruvate → Acetyl~CoA + Formate

Acetyl~CoA → Ethanol, Acetate

Formate → CO_2, H_2

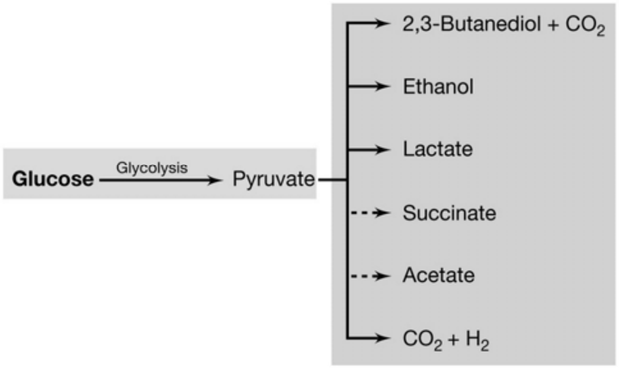
Typical products (molar amounts)

Acidic : neutral
4 : 1
$CO_2 : H_2$
1 : 1



Methyl Red Test

(b) **Butanediol fermentation** (for example, *Enterobacter*)

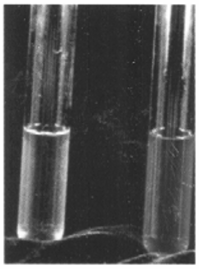


Glucose $\xrightarrow{\text{Glycolysis}}$ Pyruvate →

- 2,3-Butanediol + CO_2
- Ethanol
- Lactate
- Succinate
- Acetate
- $CO_2 + H_2$

Typical products (molar amounts)

Acidic : neutral
1 : 6
$CO_2 : H_2$
5 : 1



Voges-Proskauer Test

Clostridial Fermentations

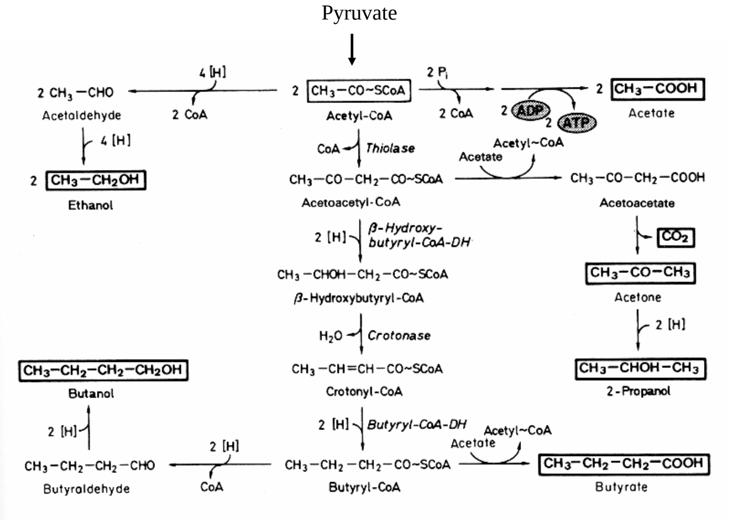
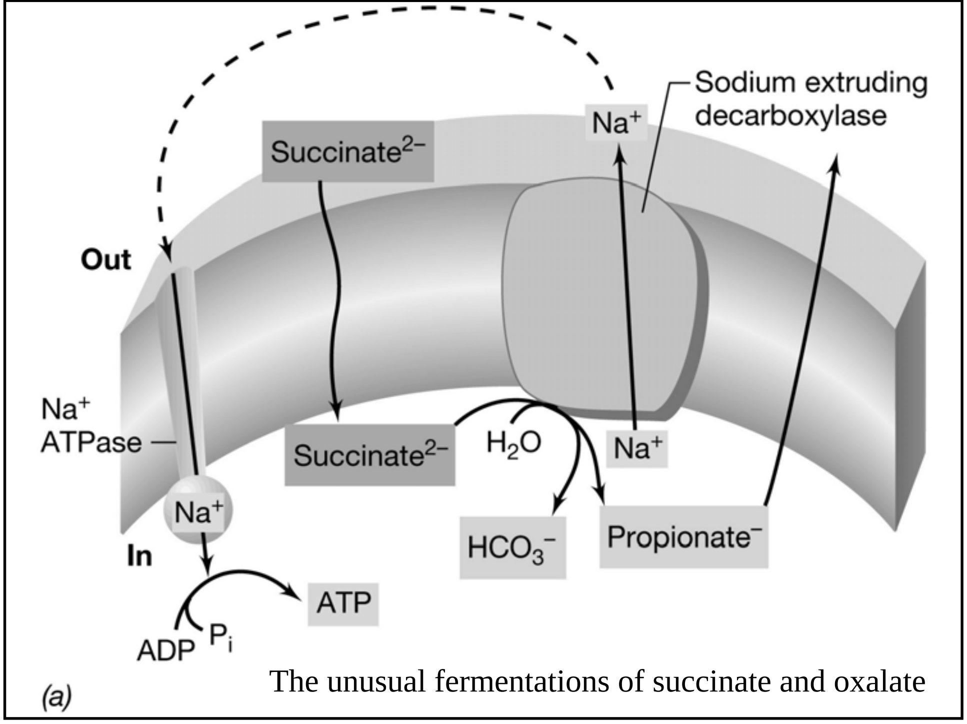
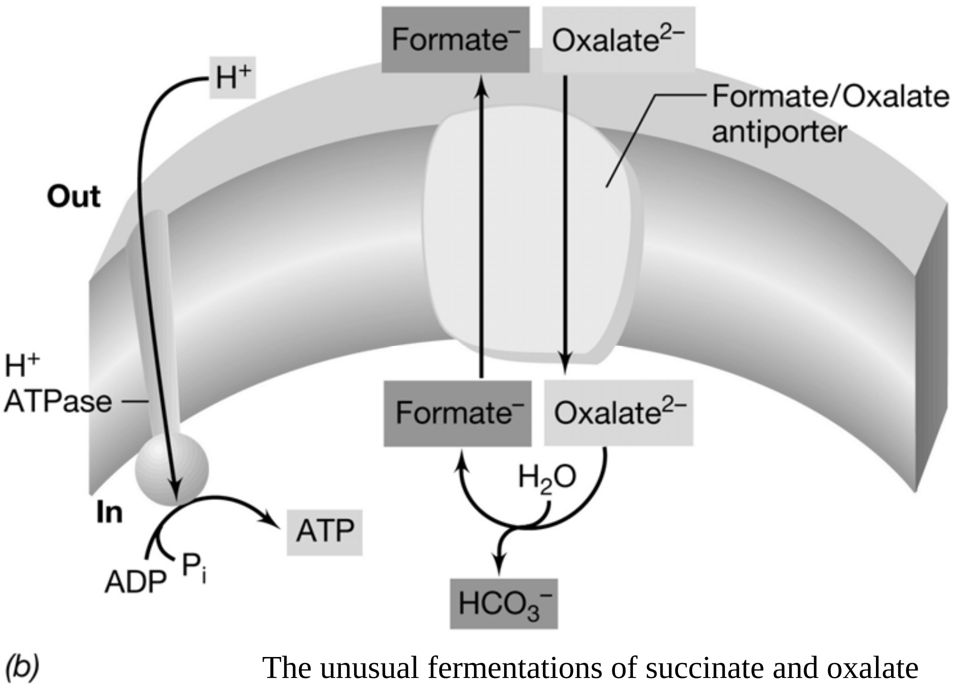


Fig. 8.4. *The formation of acetate, ethanol,* n-*butanol, butyrate, acetone, and 2-propanol during clostridial fermentations.*

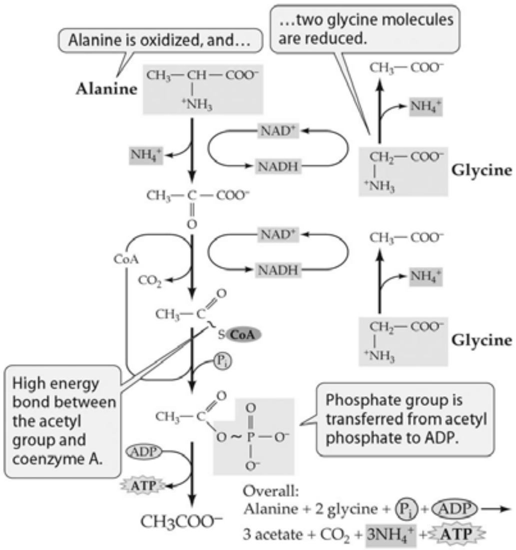


The unusual fermentations of succinate and oxalate



The unusual fermentations of succinate and oxalate

Stickland reaction: *Clostridium* using amino acids for substrate-level phosphorylation



Alanine is oxidized, and…

…two glycine molecules are reduced.

High energy bond between the acetyl group and coenzyme A.

Phosphate group is transferred from acetyl phosphate to ADP.

Overall:
Alanine + 2 glycine + P_i + ADP ⟶
3 acetate + CO_2 + $3NH_4^+$ + ATP