

# BB 422/622

## OUTLINE:

Review of 421  
Goals of 422  
Review of chemical principles  
Thermodynamics  
C/O cycles  
Overview of Metabolism  
ATP cycles  
Energy Coupling  
Chemical Reactivity  
Bioenergetics  
Membrane Transport  
Review of membrane structure, dynamics, and proteins  
Mediated/non-mediated  
Energetics  
Facilitative Diffusion  
ionophores  
GLUT1  
Aquaporins  
Potassium channel  
Active Transport  
Primary  
Na/K pump  
ABC transporters  
Secondary  
Glc import  
Bicarbonate/Cl  
Lactose/H<sup>+</sup>  
Catabolism of Glucose  
Glycogenolysis  
phosphorylase  
debranching enzyme  
phospho-gluco-mutase (PGM)

## Exam 1

Glycolysis  
Introduction & overview;  
Phase I  
hexokinase- phosphotransferase-coupling  
phospho-gluco-isomerase (PGI)- endiol  
phospho-fructo-kinase (PFK-1)-  
Aldolase- Schiff base  
(electron sink to stabilize a carbanion)  
triose-phosphate isomerase (TPI)- endiol  
Phase II  
GAPDH- oxidation  
PG kinase- return on investment-  
substrate-level phosphorylation  
PG mutase- acid/base;  
phospho-enzyme  
Enolase- enolate  
Pyruvate Kinase- phosphotransferase  
Summary: labeling studies, logic, energetics  
Catabolism of Other sugars  
Pasteur: Anaerobic vs Aerobic  
Fermentations- Anaerobic  
Lactate  
lactate dehydrogenase  
Acetoacetate decarboxylase  
Ethanol  
pyruvate decarboxylase  
alcohol dehydrogenase  
Aerobic  
Pyruvate  
pyruvate dehydrogenase complex

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# BB 422/622

## Clinical Correlations "Friday"

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## Clinical Correlations

### Facilitative Diffusion

**Aquaporins** are important in the kidney nephron. Here water reabsorption is critical for maintaining water balance.

- Loss of these aquaporins leads to NDI (**nephrogenic diabetes insipidus**), hypokalemia, and hypercalcemia.

**Fructose malabsorption** arises from several known and unknown causes.

- One known cause is loss of **GLUT-5**, the facilitative diffusion transporter specific for fructose

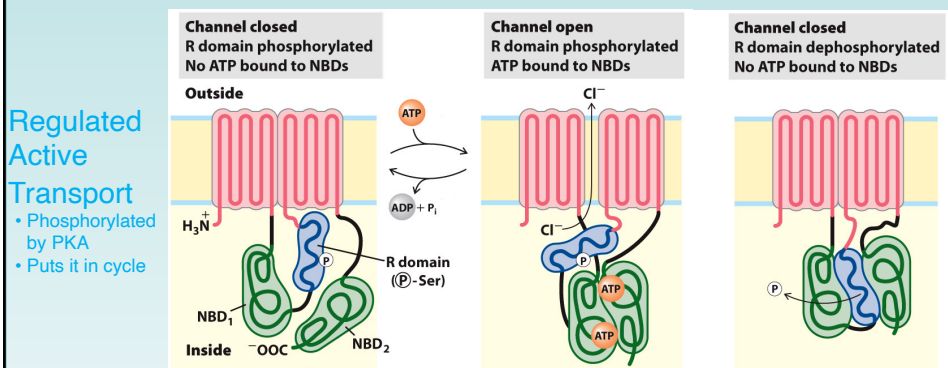
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## Clinical Correlations

### Active Transport

Failure of ABC-Transporters Can Lead to Human Disease

- Mutations in the human CFTR transporter (an ABC-type transporter-like **channel** for chloride ions)(cystic fibrosis transmembrane-conductance regulator) result in the human disease cystic fibrosis.
- Single-amino-acid mutations can render the protein misfolded and/or unable to transport chloride ions, resulting in an imbalance of ions across the membrane.

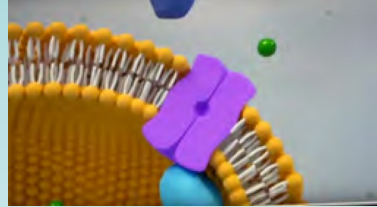


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## Clinical Correlations

### Failure of ABC-Transporters Can Lead to Human Disease

Diseases such as Tangier's (ABC-A1), Juvenile macular degeneration and retinitis pigmentosa (ABC-R), Progressive familial intrahepatic cholestasis (BSEP), and Celiac disease (TAP1) have all been associated with ABC transporters (named in parenthesis).



Nearly all these transports named above are transporting membrane lipids.

While there are about 50 genes for ABC transporters in humans, there are thousands in bacteria and plants where they are involved in transport of many other things such as amino acids, sugars, peptides and even entire proteins.

One of the most clinically relevant ones is the MRP1 transporter, which leads to multi-drug resistance



Dr. Kornberg's "Giraffe" story

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## Clinical Correlations

### Glycogen Storage Diseases: Inborn errors in metabolism

**Pompe** is from a lack of **maltase** ( $\alpha$ -1-4 glucosidase) in liver lysosomes, where glycogen will accumulate. Leads to death in first months after birth.

**Cori** is from lack of **debranching enzyme**. Glycogen accumulates and liver enlargens (hepatomegaly). Also have fasting hypoglycemia.

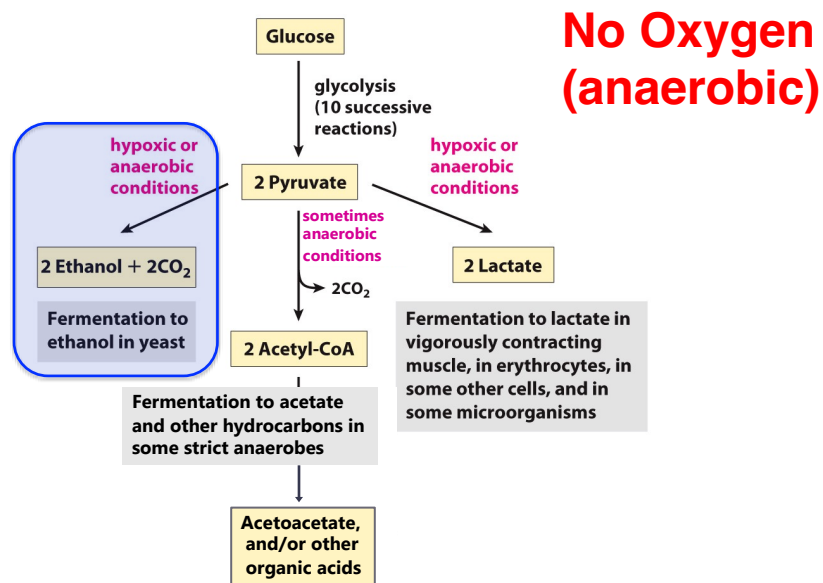
**McArdle** is from the absence of **muscle phosphorylase**. Leads to difficulty in muscle use; cramps, fatigue. Less muscle glycolysis leads to lower levels of lactic acid in the blood. Muscles are damaged and patients become debilitated.

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# Clinical Correlations "Friday"

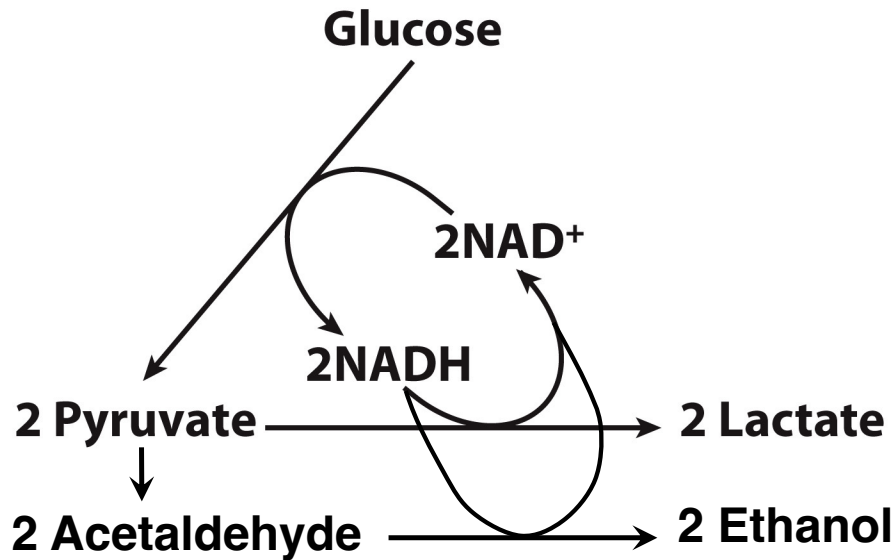
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## Fates of Pyruvate: Fermentation to Ethanol



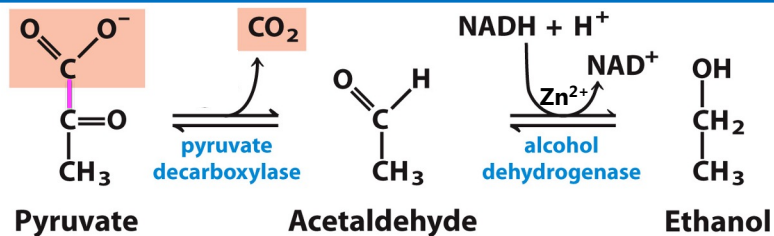
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## Fermentation



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## Fates of Pyruvate: Fermentation to Ethanol



- Two-step reduction of pyruvate to ethanol
- Humans do not have pyruvate decarboxylase.
- Both steps require cofactors.
  - pyruvate decarboxylase: Mg<sup>++</sup> and thiamine pyrophosphate (TPP)
  - alcohol dehydrogenase: Zn<sup>++</sup> and NAD<sup>+</sup>
- CO<sub>2</sub> produced in the first step is responsible for:
  - carbonation in beer
  - dough rising when baking bread

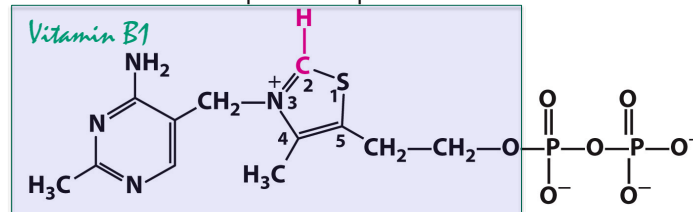
This is cleavage of a C–C bond that is alpha to the carbonyl; which is more difficult and needs help

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# Fermentation: Pyruvate decarboxylase

Here is our helper

thiazolium ring



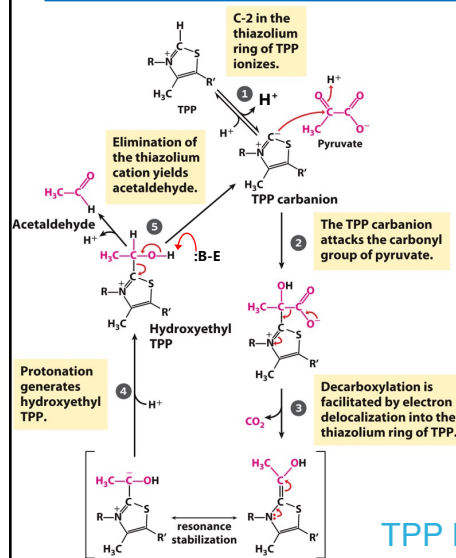
Thiamine pyrophosphate (TPP)

TPP is a Common Acetaldehyde Carrier

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# Fermentation: Pyruvate decarboxylase

Mechanism



- TPP forms a covalent bond with carbonyl carbon, forming an alcohol, and resulting in release of  $\text{CO}_2$ .

- TPP then allows rearrangement of and protonation of carbonyl carbon to release from complex.

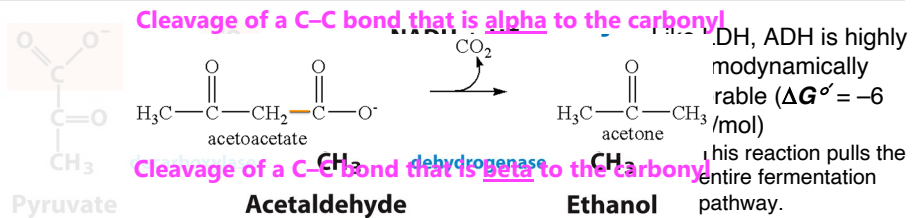
TPP Is a Common Acetaldehyde Carrier

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# Fermentation: Pyruvate decarboxylase

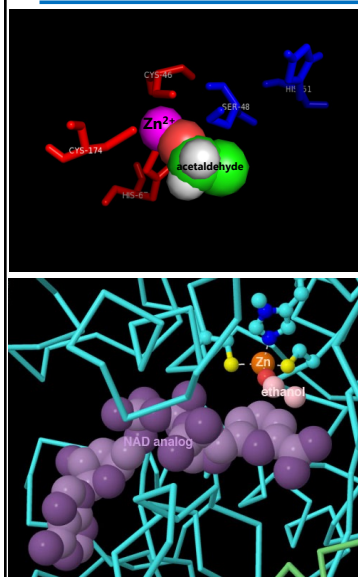
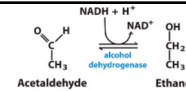
**TABLE 14-1** Some TPP-Dependent Reactions

Enzyme	Pathway(s)	Bond cleaved	Bond formed
Pyruvate decarboxylase	Ethanol fermentation		
Pyruvate dehydrogenase $\alpha$ -Ketoglutarate dehydrogenase	Synthesis of acetyl-CoA Citric acid cycle		
Transketolase	Carbon-assimilation reactions Pentose phosphate pathway		



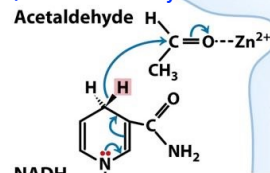
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# Fermentation: Alcohol Dehydrogenase (ADH)



His,2Cys: bind  $\text{Zn}^{2+}$

Ser/His: acid-base catalysis



$\text{Zn}^{2+}$  at the active site polarizes the carbonyl oxygen of acetaldehyde, allowing transfer of a hydride ion (pink) from NADH. The reduced intermediate acquires a proton from the medium (blue) to form ethanol.

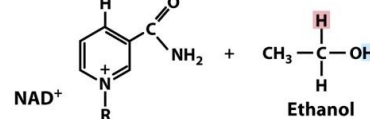
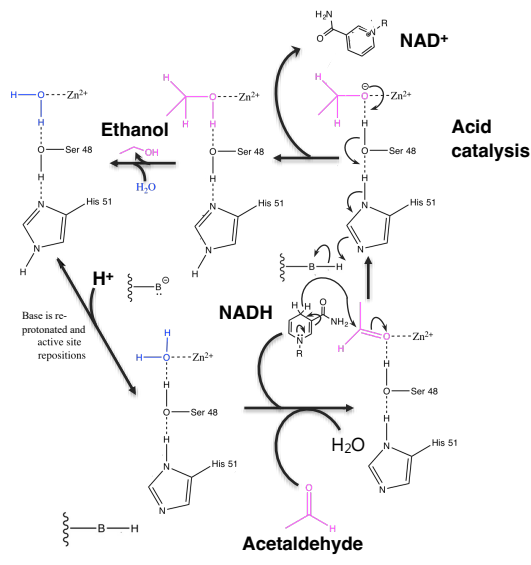
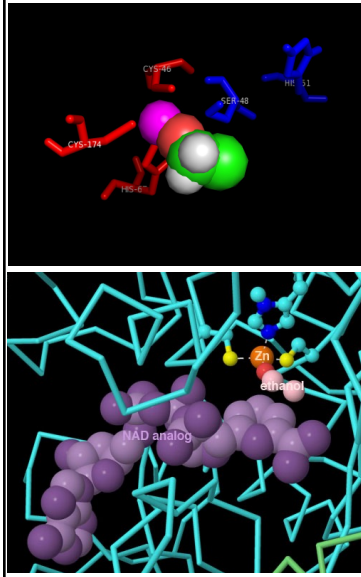
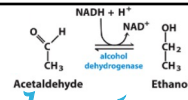


Figure 14-13  
Lehninger Principles of Biochemistry, Fifth Edition  
© 2008 W. H. Freeman and Company

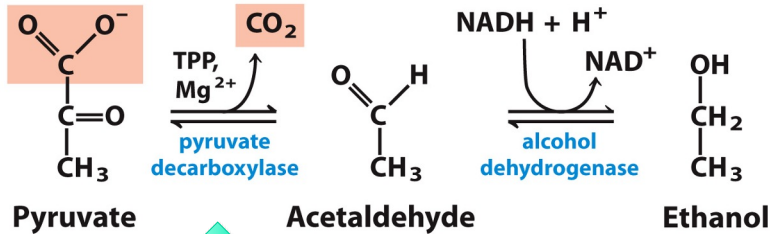
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# Fermentation: Alcohol Dehydrogenase (ADH) Mechanism



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# Fermentation: Ethanol



Humans are missing the gene for this enzyme.

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## Fermentation: Ethanol

$$\begin{array}{c}
 \text{O} \\
 \parallel \\
 \text{C} \\
 | \\
 \text{CH}_3
 \end{array}
 \begin{array}{c}
 \text{O}^- \\
 | \\
 \text{O}^-
 \end{array}
 \xrightarrow[\text{Mg}^{2+}]{\text{TPP, pyruvate decarboxylase}}
 \begin{array}{c}
 \text{O} \\
 \parallel \\
 \text{C} \\
 | \\
 \text{CH}_3
 \end{array}
 \text{H}
 + \text{CO}_2$$

Pyruvate

$$\begin{array}{c}
 \text{O} \\
 \parallel \\
 \text{C} \\
 | \\
 \text{CH}_3
 \end{array}
 \text{H}
 \xrightarrow[\text{NAD}^+]{\text{alcohol dehydrogenase}}
 \begin{array}{c}
 \text{OH} \\
 | \\
 \text{CH}_2 \\
 | \\
 \text{CH}_3
 \end{array}
 + \text{NADH} + \text{H}^+$$

Acetaldehyde

$$\begin{array}{c}
 \text{OH} \\
 | \\
 \text{CH}_2 \\
 | \\
 \text{CH}_3
 \end{array}$$

Ethanol

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## Fates of Pyruvate

**Glucose**

↓ glycolysis (10 successive reactions)

**2 Pyruvate**

↓ sometimes anaerobic conditions → 2CO<sub>2</sub>

**2 Acetyl-CoA**

↓ Fermentation to acetate and other hydrocarbons in some strict anaerobes

**Acetoacetate, and/or other organic acids**

No Oxygen (anaerobic)

Pyruvate Oxidation

Oxygen available (aerobic)

Instead of reducing pyruvate, we oxidize it further

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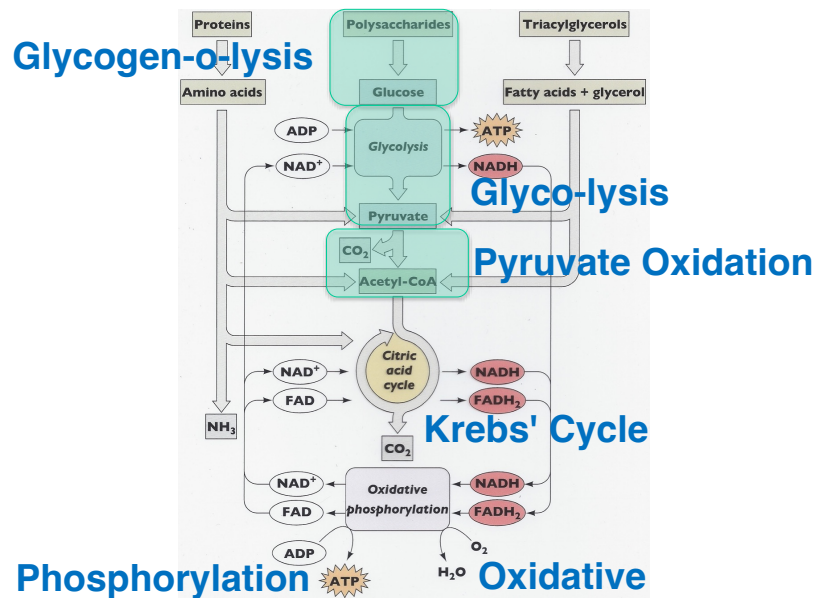
# Pyruvate Oxidation

**ONLY when O<sub>2</sub> is present**

- This is why it is called cellular *respiration*.
- The result will be the COMPLETE oxidation of carbon to CO<sub>2</sub>

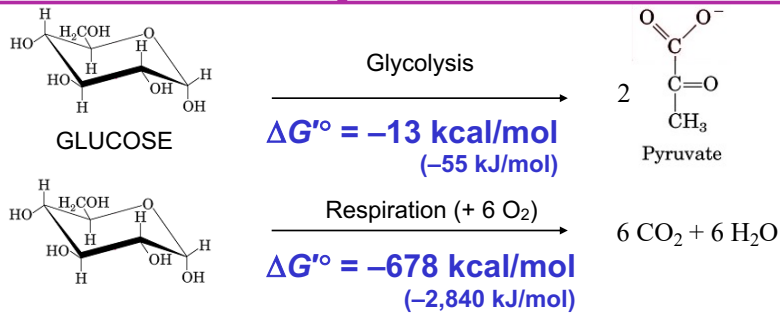
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# Pyruvate Oxidation



2

# Pyruvate Oxidation



- Process in which cells consume O<sub>2</sub> and produce CO<sub>2</sub>
- Provides more energy (ATP) per glucose than glycolysis
- Process is slower than glycolysis
- Evolutionary origin: developed about 2,500 mya
- Used by animals, plants, and many microorganisms
- Other fuel sources (lipids and AA) converge on respiration.

## Respiration occurs in three major stages:

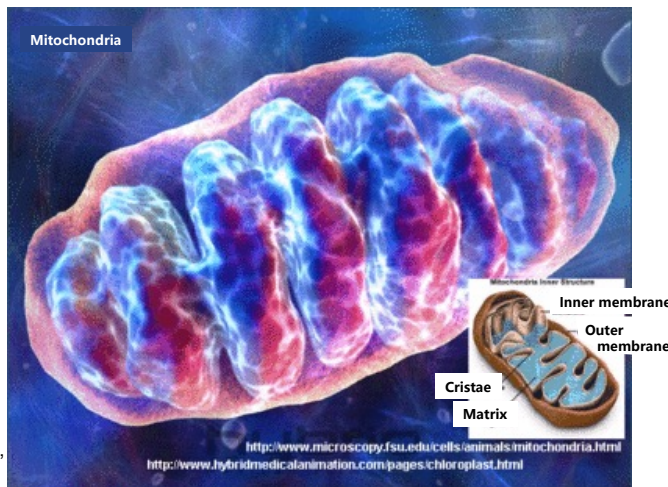
- 1) Pyruvate oxidation (acetyl CoA production)
- 2) acetyl CoA oxidation (CO<sub>2</sub> production)
- 3) electron transfer and oxidative phosphorylation (H<sub>2</sub>O production)

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# Respiration

## Localization: In Eukaryotes, Respiration is Localized to the Mitochondria (Compartmentation)

- Glycolysis occurs in the cytoplasm.
- Pyruvate Oxidation and Citric Acid Cycle occurs in the mitochondrial matrix.†
- Fantastic example of *Compartmentation*.
- Oxidative phosphorylation occurs in the inner membrane.

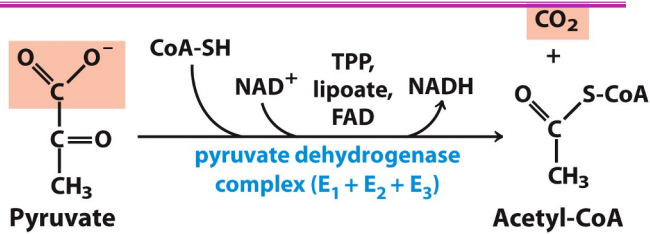


†Except succinate dehydrogenase, which is located in the inner membrane

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# Pyruvate Oxidation

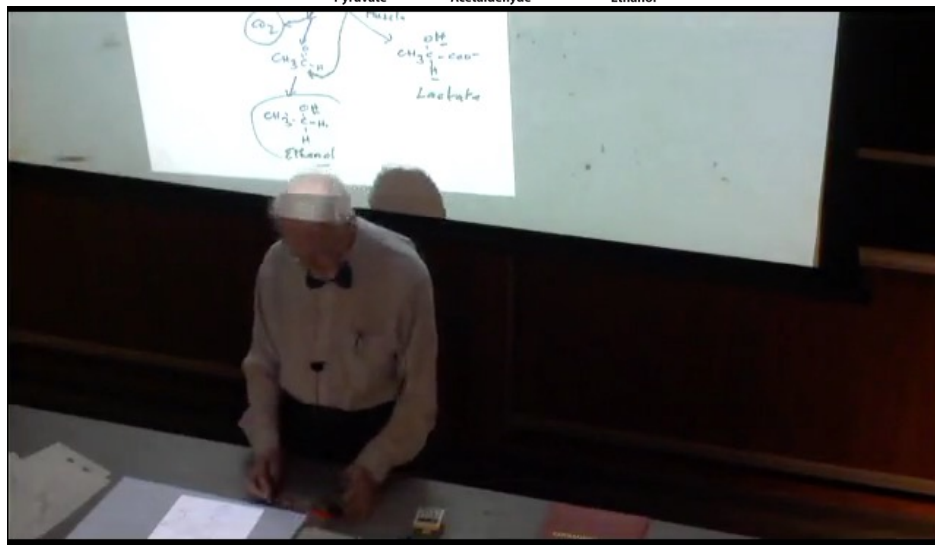
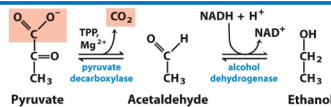
## Conversion of Pyruvate to Acetyl-CoA



- Net reaction: **Pyruvate**
  - oxidative decarboxylation of pyruvate
    - Means pyruvate will get oxidized as the carboxylate leaves (as  $\text{CO}_2$ )
  - first carbons of glucose to be fully oxidized (C3 & C4)
- Fairly simple reaction done by a complicated process.
- Highly thermodynamically favorable/irreversible ( $\Delta G^\circ = -8$  kcal/mol); mostly due to the loss of  $\text{CO}_2$
- Catalyzed by the **Pyruvate Dehydrogenase Complex (PDC)**
  - Three main enzyme, each with multiple subunits: **E1, E2, E3**
  - Regulatory subunits: PD kinase & PD phosphatase
  - Overall structure of **E1<sub>96</sub>, E2<sub>24</sub>, E3<sub>24</sub>**
  - requires 5 coenzymes
  - **TPP, lipoic acid, and FAD** are prosthetic groups.
  - **NAD<sup>+</sup>** and **CoA-SH** are co-substrates.

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## Dr. Kornberg's Final Word on Fermentation: Ethanol



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