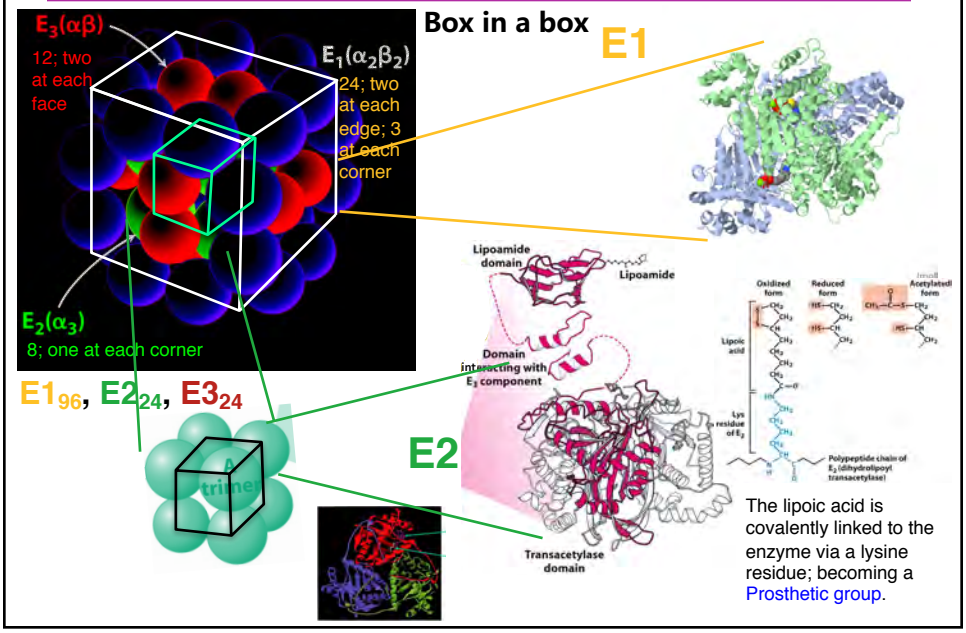
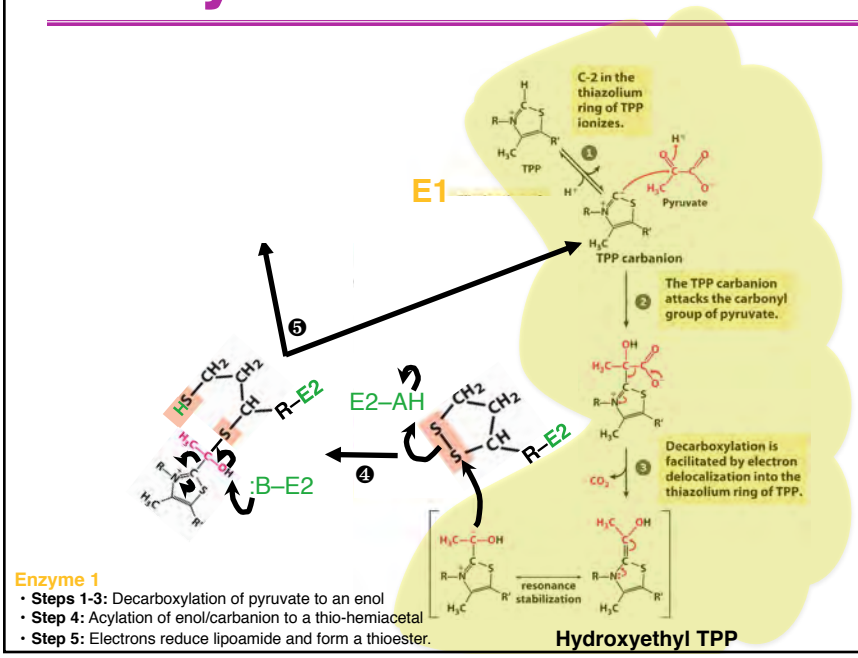
Pyruvate Oxidation



The lipoic acid is covalently linked to the enzyme via a lysine residue; becoming a Prosthetic group.

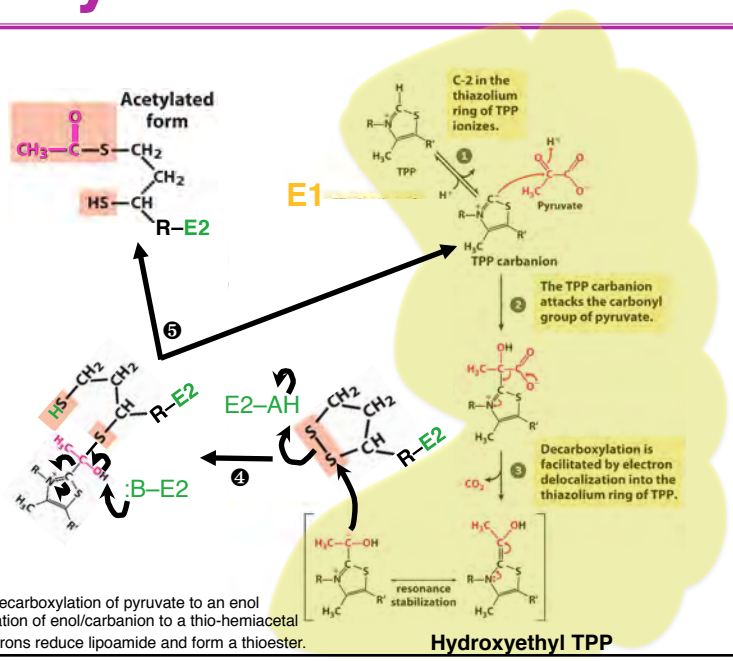
Pyruvate Oxidation



Enzyme 1

- **Steps 1-3:** Decarboxylation of pyruvate to an enol
- **Step 4:** Acylation of enol/carbanion to a thio-hemiacetal
- **Step 5:** Electrons reduce lipoamide and form a thioester.

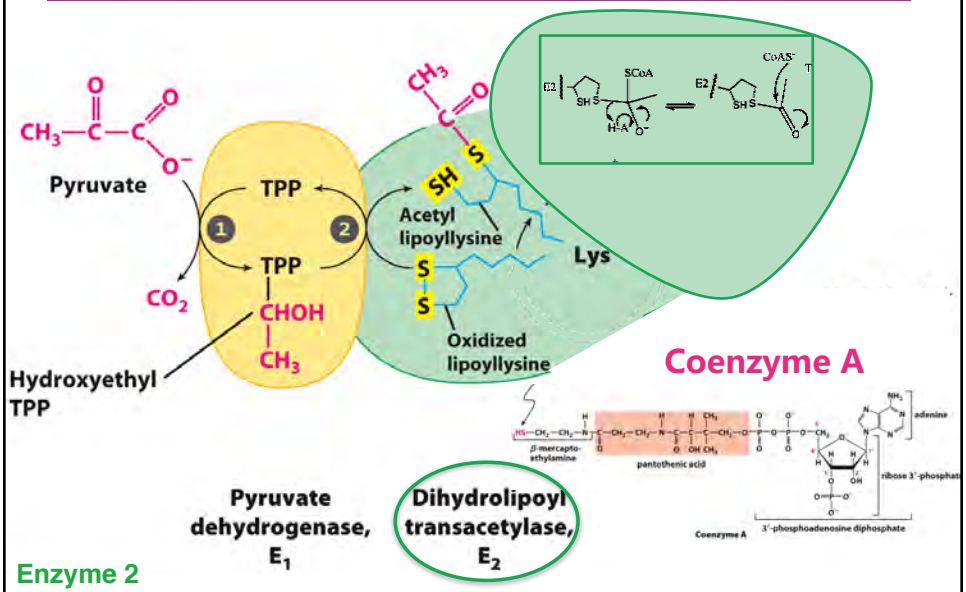
Pyruvate Oxidation



Enzyme 1

- **Steps 1-3:** Decarboxylation of pyruvate to an enol
- **Step 4:** Acylation of enol/carbanion to a thio-hemiacetal
- **Step 5:** Electrons reduce lipoamide and form a thioester.

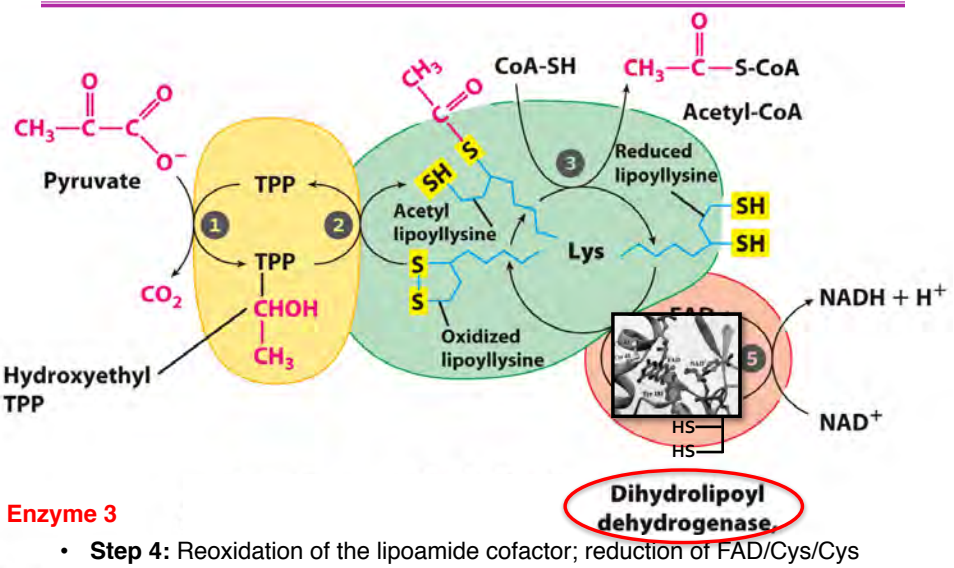
Pyruvate Oxidation



Enzyme 2

- Formation of acetyl-CoA: simple thio-ester exchange

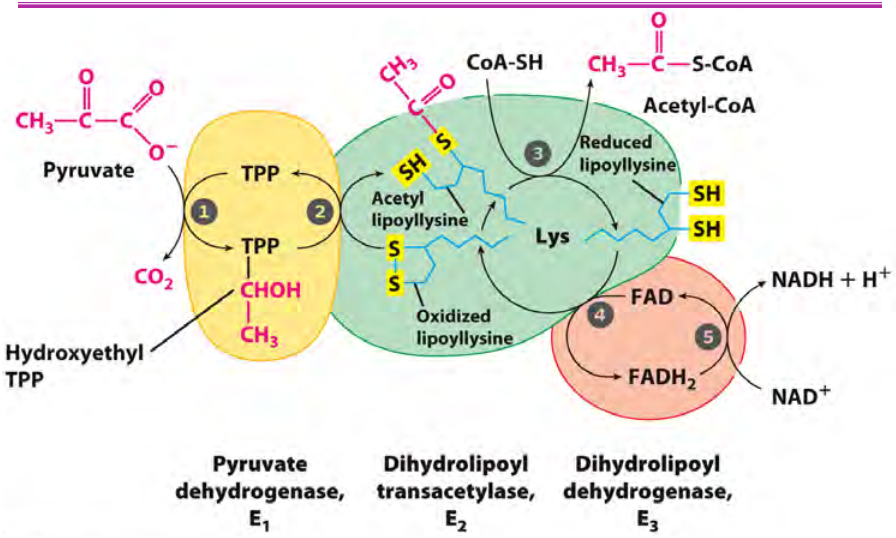
Pyruvate Oxidation



Enzyme 3

- **Step 4:** Reoxidation of the lipoamide cofactor; reduction of FAD/Cys/Cys
  - Disulfide interchange with dihydrolipoyllysine and 2 adjacent Cys in disulfide bond
  - The reduced Cys reform disulfide using FAD, which funnels electrons to NAD^+
- **Step 5:** Regeneration of the oxidized FAD/Cys/Cys active site – forming NADH

Pyruvate Oxidation



Enzyme 1
- **Step 1:** Decarboxylation of pyruvate to an enol (hydroxyethyl-TPPl
- **Step 2:** Acylation of enol to a thioester on lipoic acid.

Enzyme 2
- **Step 3:** Formation of acetyl-CoA: simple thio-ester exchange

Enzyme 3
- **Step 4:** Reoxidation of the lipoamide cofactor; reduction of FAD/Cys/Cys
- **Step 5:** Regeneration of the oxidized FAD/Cys/Cys active site – forming NADH

Pyruvate Oxidation

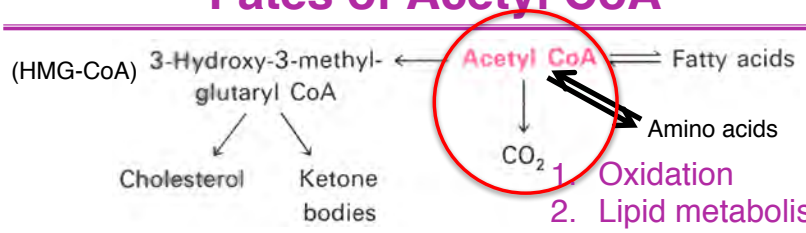
Overall Reaction of PDC

Pyruvate + Coenzyme-A (CoA) + NAD^+

PDC (TPP, lipoic acid, FAD) ⇅ $\Delta G^{o'} = -8$ kcal/mol

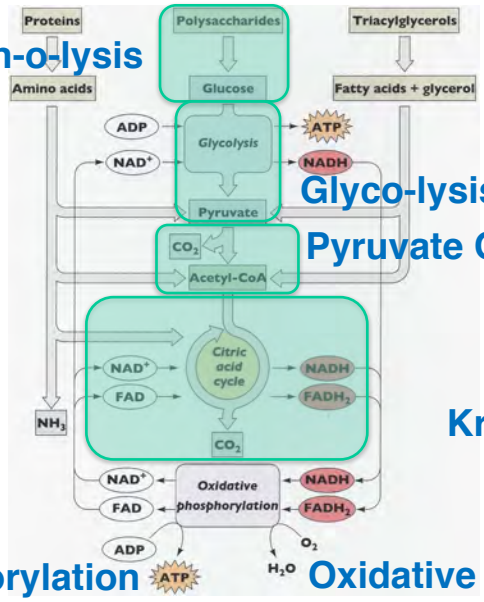
CO_2 + Acetyl-Coenzyme-A + NADH + H^+

Fates of Acetyl CoA



1. Oxidation
2. Lipid metabolism
3. Amino acid metabolism

The Citric Acid Cycle



Glycogen-o-lysis

Glyco-lysis

Pyruvate Oxidation

Krebs' Cycle

Phosphorylation

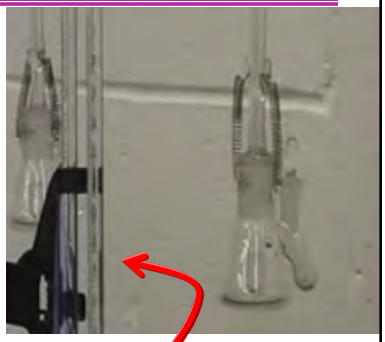
Oxidative

The Citric Acid Cycle

a.k.a. Krebs Cycle,
a.k.a. Tricarboxylatic Acid Cycle (TCA)

Time B.C. (Before the Cycle)

Otto Warburg
1883-1970



Manometer

Warburg Apparatus
-respiration
-Measure rates of O_2 consumption

Substrates
(e.g., glucose)

Tissues

CO_2 trap

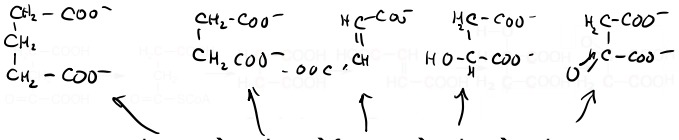
$CO_2 \rightarrow H_2CO_3 \rightarrow 2H^+ + CO_3^{-2} \rightarrow K_2CO_3 + H_2O$

UTube instructions
(http://youtu.be/M-HYbZwN43o)

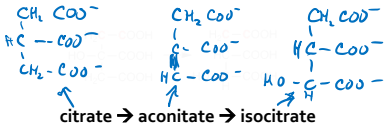
Time B.C. (Before the Cycle)

In 1920 BC, what was known about respiration?

1) Glycolysis gives rise to pyruvate
2) Adding pyruvate to respiring tissues in a Warburg apparatus, there are 2.5 O_2 consumed: $2^{1/2}O_2 + C_3H_4O_3 \rightarrow 3CO_2 + 2H_2O$
3) Any intermediate in the process will be oxidized at a rate ≥pyruvate
4) Many intermediates were tried, but few met this criteria, they were: succinate, fumarate, malate, alpha-ketoglutarate, etc.



glutarate → succinate → fumarate → malate → oxaloacetate



citrate → aconitate → isocitrate

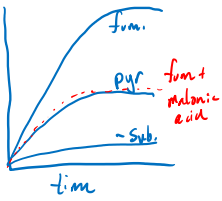
Albert Szent-Györgyi
1893-1986

5) Others had already worked out several compounds and their interconversion. Specifically, Albert Szent-Györgi had worked out the interconversion of the dicarboxylic acids. Carl Martinus worked out the interconversion of the tricarboxylic acids

6) In 1937, with help of German biochemist Franz Koop, Carl Martinus, demonstrated a series of reactions using citrate that produced α–ketoglutarate. Thus tricarboxylic acid and dicarboxylic acids would be interconverted with loss of CO_2, but also support respiration.

Time B.C. (Before the Cycle)

Hans Krebs
1900-1981



glutarate → succinate → fumarate → malate → oxaloacetate

citrate → aconitate → isocitrate

"pyruvate" (acetate)

Krebs confirmed that the pathway was consistent with succinate, fumarate, and malate proved to be useful because all these molecules increased oxygen consumption in the pigeon breast muscle.

Dr. Kornberg: **Lecture 02.08.17** (19:54-20:39) **(1 min)**

The first clue came from an experiment with fumarate. Krebs did careful measurements using the Warburg manometer. Fumarate gave greater than expected oxygen consumption in the pigeon breast muscle.

$3O_2 + C_4H_4O_4 \rightarrow 4CO_2 + 2H_2O$

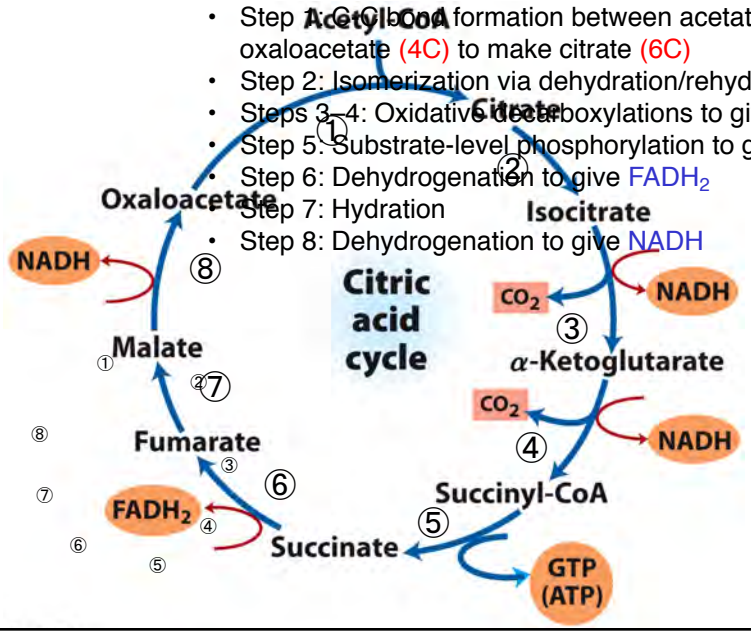
∴ 1 µmole fumarate would consume 3 µmole O_2

1) Malonic acid inhibition of the succinate → fumarate step prevented this increase & succinate accumulated
2) How can fumarate give rise to succinate? There must be a cycle
3) Tested by showing that using succinate or fumarate you could detect the formation of citrate.

Later in 1937, he proposed that pyruvate would combine with oxaloacetate to make citrate in a cycle he called the Citric Acid Cycle. Later, Fritz Lipmann showed that it was acetyl-CoA and not pyruvate.

The Citric Acid Cycle

- Step 1: C-C bond formation between acetate (2C) and oxaloacetate (4C) to make citrate (6C)
- Step 2: Isomerization via dehydration/rehydration
- Steps 3–4: Oxidative decarboxylations to give 2 NADH
- Step 5: Substrate-level phosphorylation to give GTP
- Step 6: Dehydrogenation to give $FADH_2$
- Step 7: Hydration
- Step 8: Dehydrogenation to give NADH



The Citric Acid Cycle

- Step 1: C-C bond formation between acetate (2C) and oxaloacetate (4C) to make citrate (6C)
- Step 2: Isomerization via dehydration/rehydration
- Steps 3–4: Oxidative decarboxylations to give 2 NADH
- Step 5: Substrate-level phosphorylation to give GTP
- Step 6: Dehydrogenation to give $FADH_2$
- Step 7: Hydration
- Step 8: Dehydrogenation to give NADH