# BI/CH 422/622

Announcements:

Exam 1 is TONIGHT in Morse at 7:00 pm

**OUTLINE:**

Glycogenolysis

Glycolysis

- Introduction & overview; 2 phases
- Phase I
- Phase II
- Summary: logic, energetics, labeling studies

Other sugars

Pasteur: Anaerobic vs Aerobic

Fermentations

- Lactate-lactate dehydrogenase
- Ethanol-pyruvate decarboxylase & alcohol dehydrogenase
- Acetoacetate decarboxylase

Exam-1 material

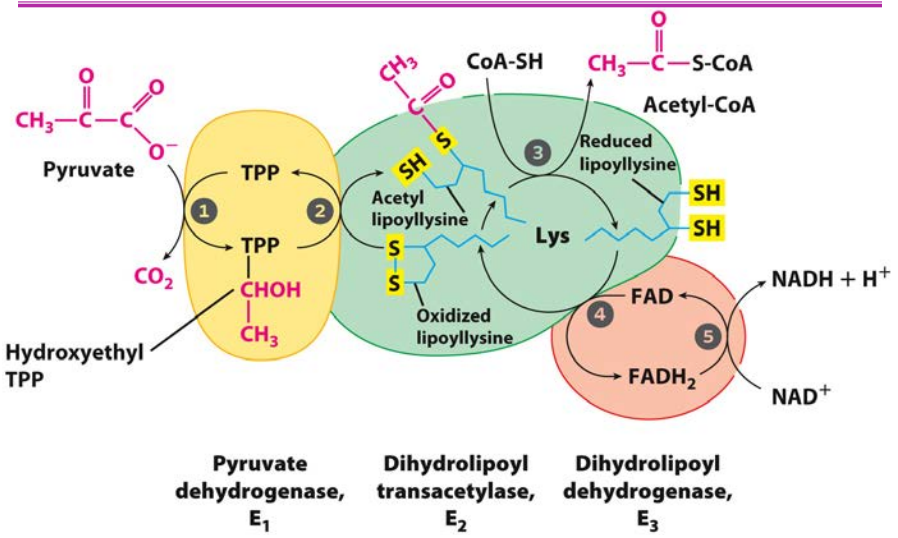
Pyruvate

- pyruvate dehydrogenase

Krebs' Cycle

- How did he figure it out?
- Overview
- 8 Steps
  - Citrate Synthase

# Pyruvate Oxidation

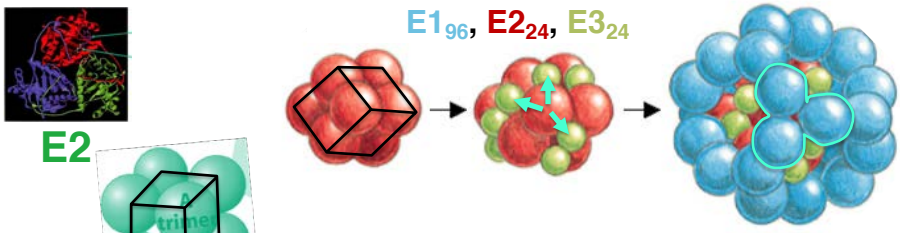



Enzyme 1
- **Step 1:** Decarboxylation of pyruvate to an enol (hydroxyethyl-TPP)
- **Step 2:** Acylation of enol to a thioester on lipoic acid.

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

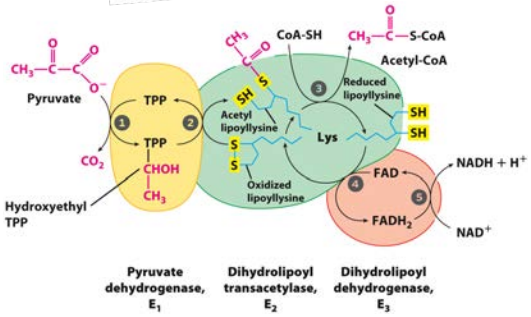
Enzyme 3
- **Step 4:** Reoxidation of the lipoamide cofactor; reduction of FAD/Cys/Cys
- **Step 5:** Regeneration of the oxidized FAD/Cys/Cys active site – forming NADH

# Pyruvate Oxidation

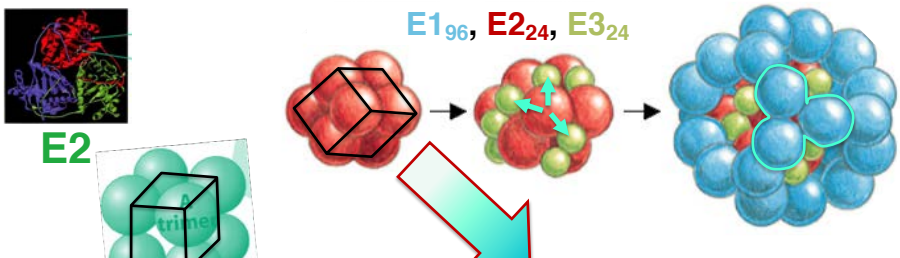


E2



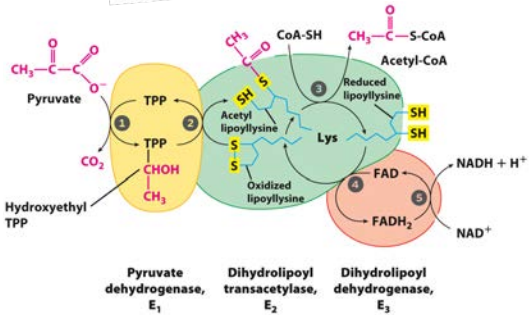

# Pyruvate Oxidation



E2







# Pyruvate Oxidation

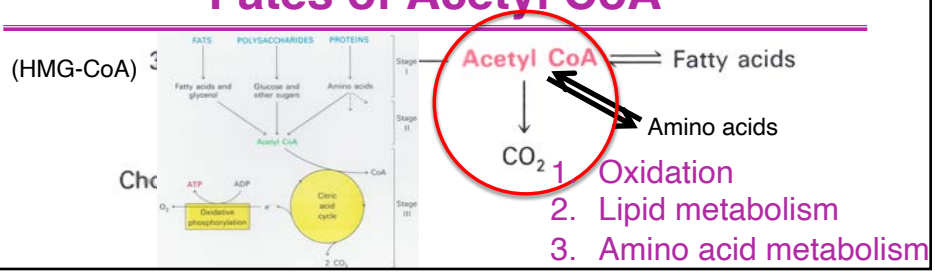
## Overall Reaction of PDC

Pyruvate + Coenzyme-A (CoASH) + $NAD^+$

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

$CO_2$ + Acetyl-Coenzyme-A (Ac-CoA) + NADH + $H^+$

## Fates of Acetyl CoA





# Pyruvate Oxidation

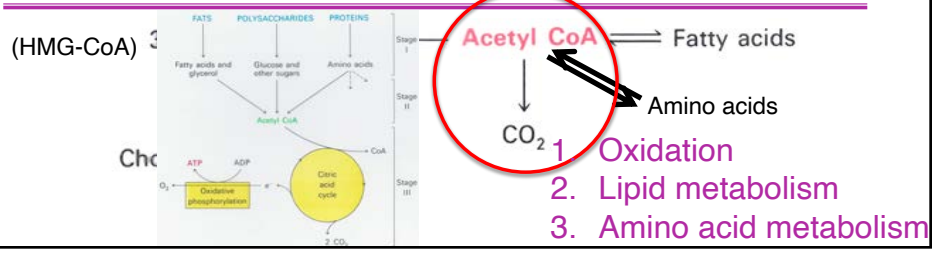
## Overall Reaction of PDC

Pyruvate + Coenzyme-A (CoASH) + $NAD^+$

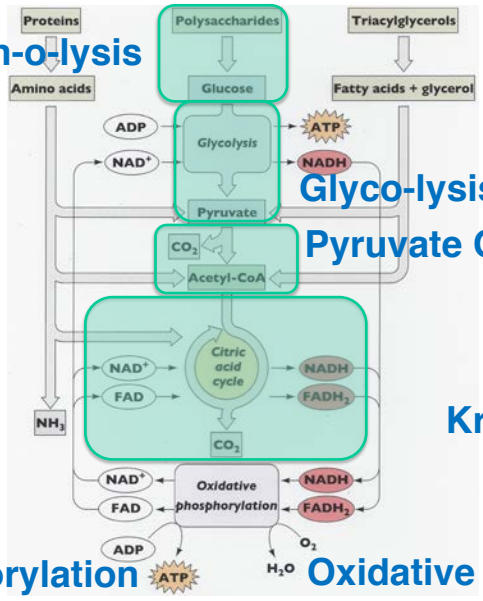
*PDC (TPP, lipoic acid, FAD)* ⇅ $\Delta G^{\circ\prime} = -8$ kcal/mol

$CO_2$ + Acetyl-Coenzyme-A (Ac-CoA) + NADH + $H^+$

## Fates of Acetyl CoA

## The Citric Acid Cycle




Glycogen-o-lysis

Glyco-lysis

Pyruvate Oxidation

Krebs' Cycle

Phosphorylation

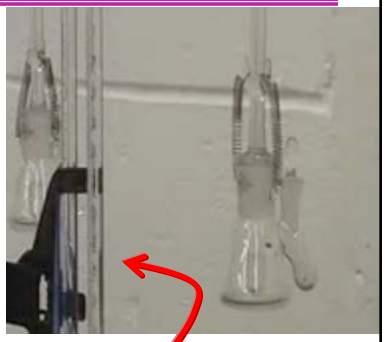
Oxidative

# The Citric Acid Cycle

a.k.a. Krebs Cycle,
a.k.a. Tricarboxylic 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
$CO_2 \rightarrow H_2CO_3 \rightarrow 2H^+ + CO_3^{-2}$
$\rightarrow K_2CO_3 + H_2O$

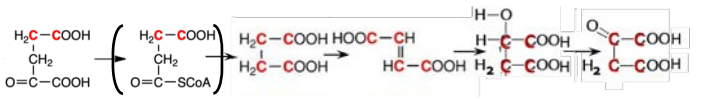
UTube instructions
(http://youtu.be/M-HYbZwN43o)

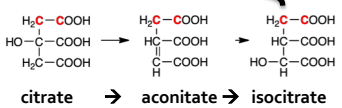
# Time B.C. (Before the Cycle)

O-leic
M-alonic
S-uccinic
G-lutaric
A-dipic
P-imelic

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: $2^{1/2}O_2 + C_3H_4O_3 \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow 3CO_2 + 2H_2O$
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.




Albert Szent-Györgyi
1893-1986

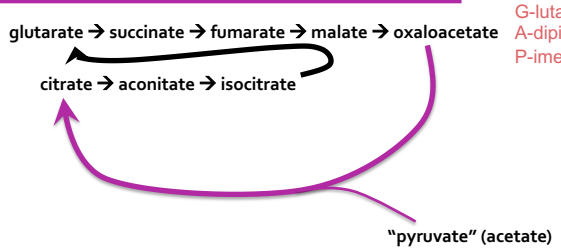
5) Others had already worked out several compounds and their interconversion. Specifically, Albert Szent-Györgi had worked out the interconversion of the dicarboxylic acids. Carl Martinus worked out the interconversion of the tricarboxylic acids

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)

O-leic
M-alonic
S-uccinic
G-lutaric
A-dipic
P-imelic

Hans Krebs
1900-1981




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.

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

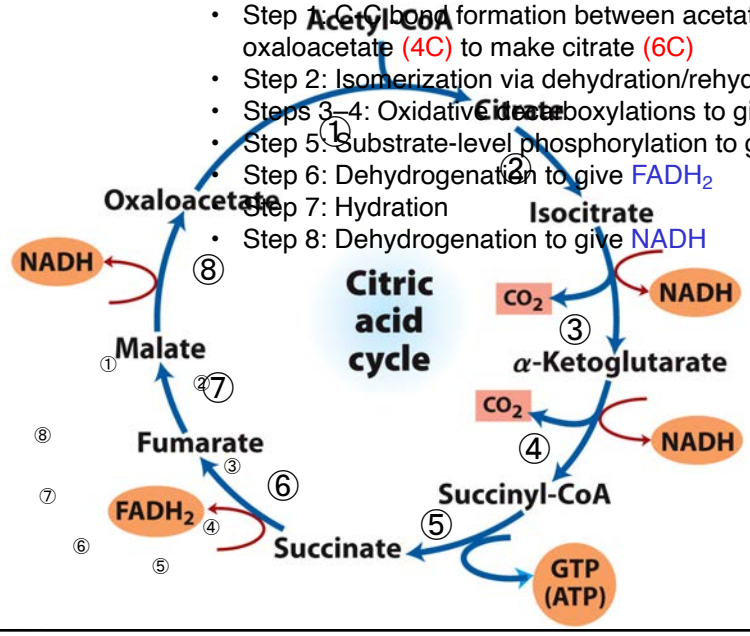
∴1 µmole fumarate would consume 3 µmole $O_2$

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.

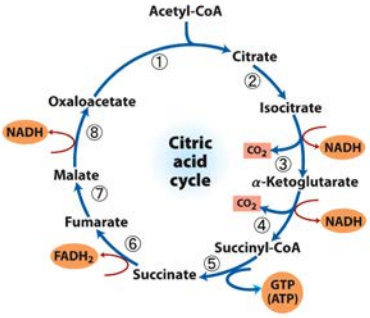
# The Citric Acid Cycle

- 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

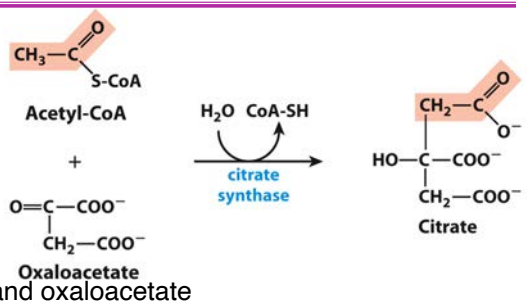


# The Citric Acid Cycle

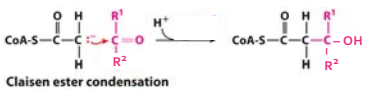
- Step 1: C-C bond formation between acetate (2C) and oxaloacetate (4C) to make citrate (6C)
- Step 2: Isomerization via dehydration/rehydration
- Steps 3–4: Oxidative decarboxylations to give 2 NADH
- Step 5: Substrate-level phosphorylation to give GTP
- Step 6: Dehydrogenation to give $FADH_2$
- Step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




# The Citric Acid Cycle: Citrate Synthase




- Condensation of acetyl-CoA and oxaloacetate
- The only reaction with C-C bond formation
- Highly thermodynamically favorable/irreversible ($\Delta G^{\circ\prime} = -7.7$ kcal/mol)
  - regulated by substrate availability and product inhibition
- Activity largely depends on [oxaloacetate].
- Rate-limiting step of CAC
- Uses acid/base catalysis
  - Carbonyl of oxaloacetate is a good electrophile.
  - Methyl of acetyl-CoA is not a good nucleophile…
  - …unless activated by deprotonation.



# The Citric Acid Cycle: Citrate Synthase

## Mechanism

- Conformational change occurs upon binding oxaloacetate.
- Avoids unnecessary hydrolysis of thioester in acetyl-CoA

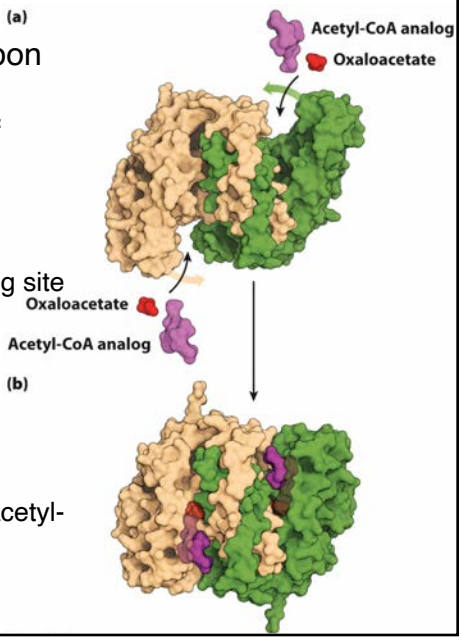
**a) Open conformation:**

Free enzyme does not have a binding site for acetyl-CoA. Ordered binding.

**b) Closed conformation:**

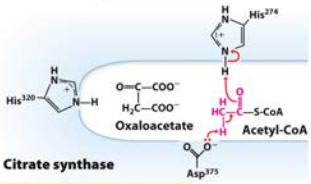
Binding of OAA creates binding for acetyl-CoA.

Reactive carbanion is protected.




# The Citric Acid Cycle: Citrate Synthase

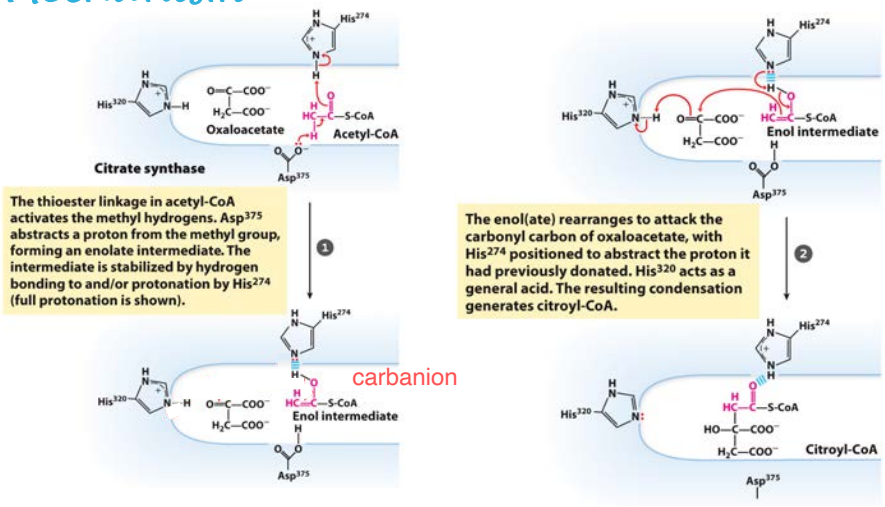
## Mechanism




The thioester linkage in acetyl-CoA activates the methyl hydrogens. $Asp^{375}$ abstracts a proton from the methyl group, forming an enolate intermediate. The intermediate is stabilized by hydrogen bonding to and/or protonation by $His^{274}$ (full protonation is shown).

# The Citric Acid Cycle: Citrate Synthase

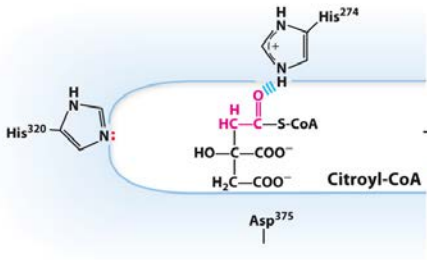
## Mechanism




# The Citric Acid Cycle: Citrate Synthase

## Mechanism

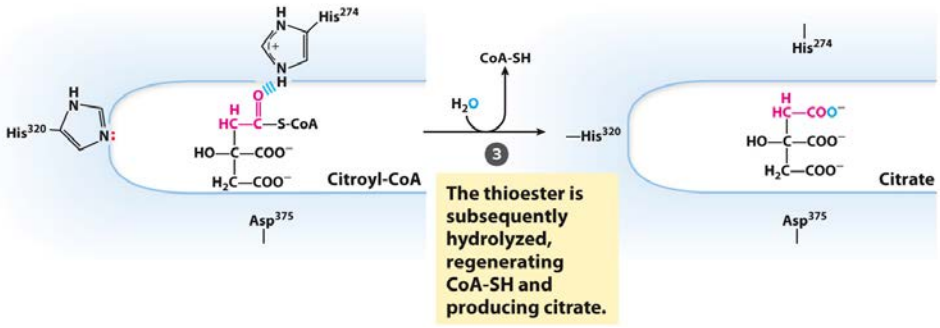
Hydrolysis of Thioester; citroyl-CoA



# The Citric Acid Cycle: Citrate Synthase

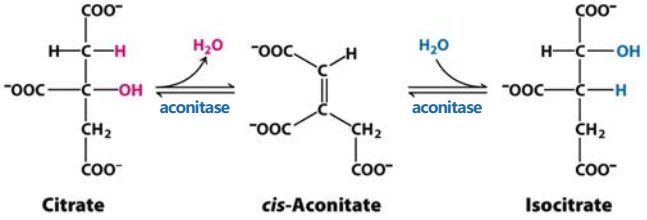
## *Mechanism*

### Hydrolysis of Thioester; citroyl-CoA




# The Citric Acid Cycle: Aconitase

### Isomerization by Dehydration/Rehydration




- Elimination of $H_2O$ from the symmetrical molecule, citrate, gives a cis C=C bond.
  - lyase

- Rationale:
  - Citrate, a tertiary alcohol, is a poor substrate for oxidation.
  - Isocitrate, a secondary alcohol, is a good substrate for oxidation.
- Thermodynamically unfavorable/reversible ($\Delta G^{\circ\prime}$ = +3.2 kcal/mol)
  - product concentration kept low to pull forward; citrate tends to "pool" with higher conc.
- Addition of $H_2O$ to *cis*-aconitate is stereospecific.
  - This was initially very confusing to bio/organic chemists
  - Only R-isocitrate is produced by aconitase.
  - A biochemist names A.G. Ogston clarified the situation by realizing that the enzyme spatially templates this symmetrical molecule by binding in only one way (e.g., clockwise or counter clockwise, not both)
  - Distinguished by three-point attachment to the active site