BI/CH 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
- Introduction & overview; 2 phases
- Phase I
- Phase II
- Summary: logic, energetics, labeling studies

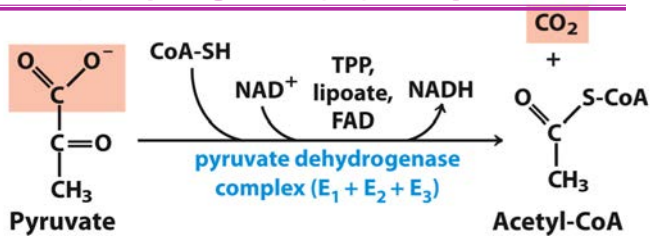
Other sugars
Pasteur: Anaerobic vs Aerobic
Fermentations
- Lactate-lactate dehydrogenase
- Acetoacetate decarboxylase
- Ethanol-pyruvate decarboxylase & alcohol dehydrogenase

Pyruvate
- pyruvate dehydrogenase

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

Pyruvate Oxidation

Conversion of Pyruvate to Acetyl-CoA

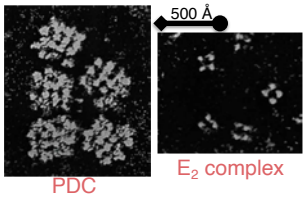


- Net reaction:
  - oxidative decarboxylation of pyruvate
    - Means pyruvate will get oxidized as the carboxylate leaves (as CO_2)
  - first carbons of glucose to be fully oxidized (C3 & C4)
- Fairly simple reaction done by a complicated process.
- Highly thermodynamically favorable/irreversible ($\Delta G^{\circ\prime} = -8$ kcal/mol); mostly due to the loss of 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 $E1_{96}$, $E2_{24}$, $E3_{24}$
  - requires 5 coenzymes
  - TPP, lipoic acid, and FAD are prosthetic groups.
  - NAD^+ and CoA-SH are co-substrates.

Pyruvate Oxidation

PDC is a large (up to **10 MDa**) multienzyme complex.

- pyruvate dehydrogenase (E_1)
- dihydrolipoyl transacetylase (E_2)
- dihydrolipoyl dehydrogenase (E_3)

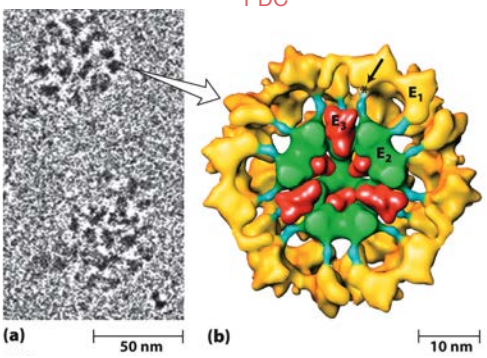


PDC E_2 complex

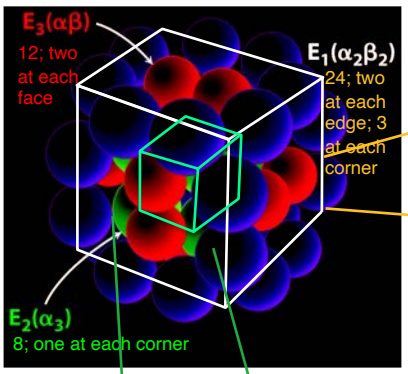
Cryo-Electron Microscopy

Nobel Prize for Chemistry in 2017

- Samples are in a near-native frozen hydrated state.
- Low temperature protects biological specimens against radiation damage.
- Electrons have a smaller wavelength and produce much higher-resolution images than light.
- No need for a crystal.

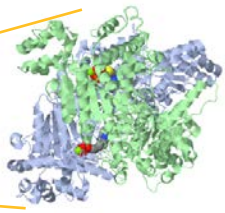


Pyruvate Oxidation

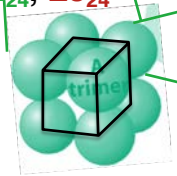


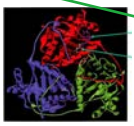
Box in a box

E1

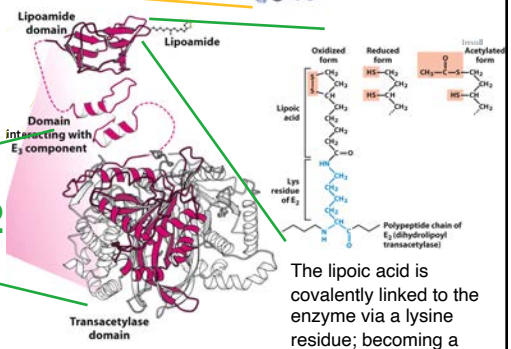


$E1_{96}$, $E2_{24}$, $E3_{24}$



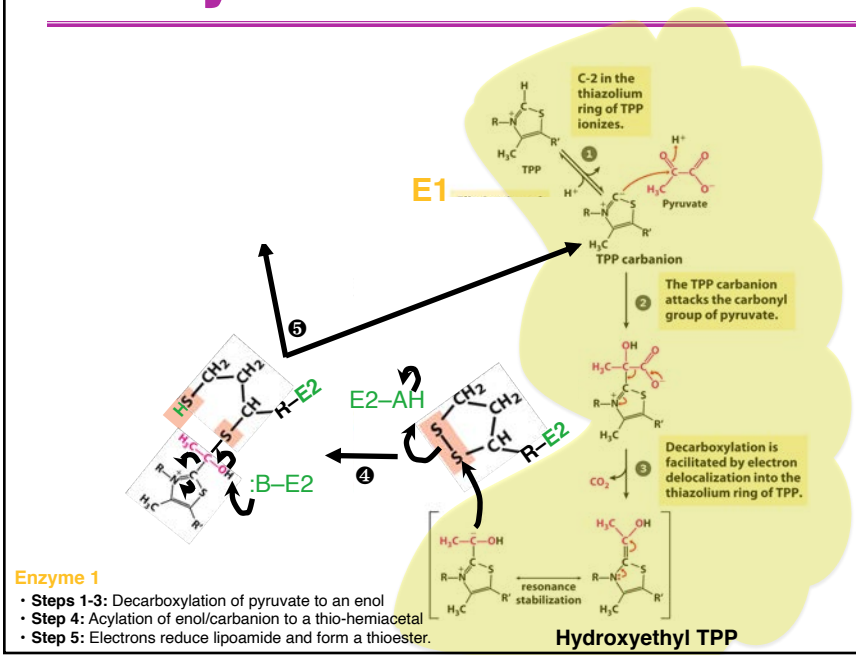


E2



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

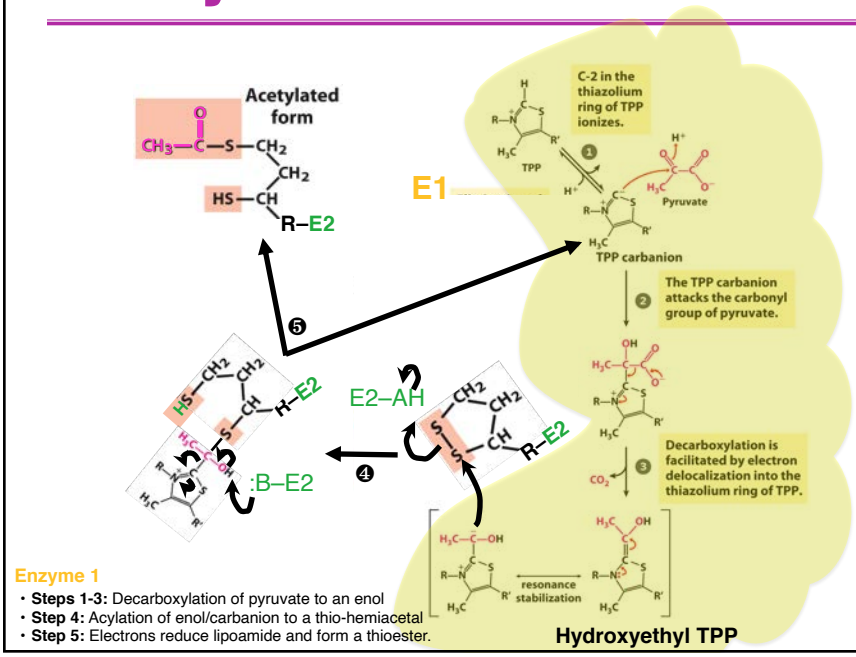
Pyruvate Oxidation



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



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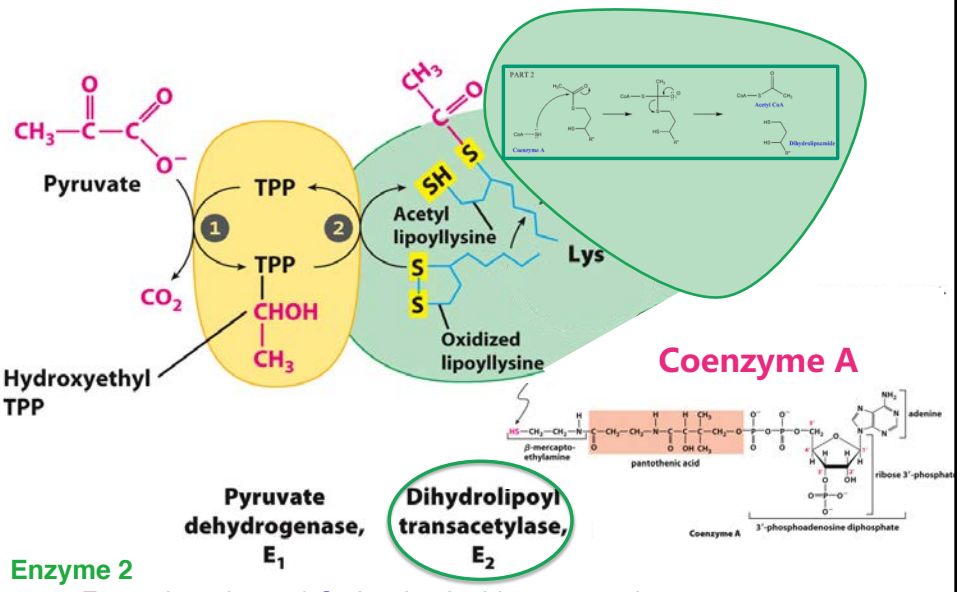
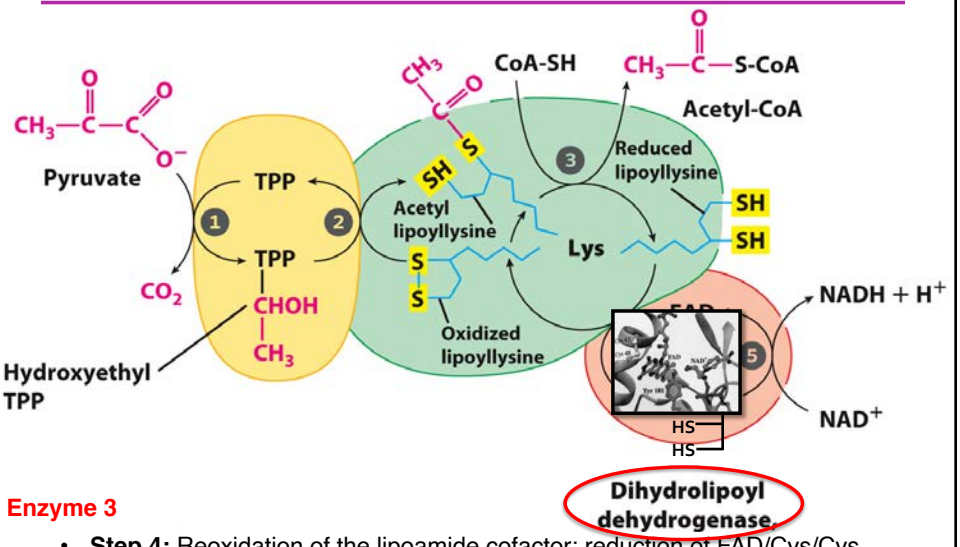


Enzyme 2

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

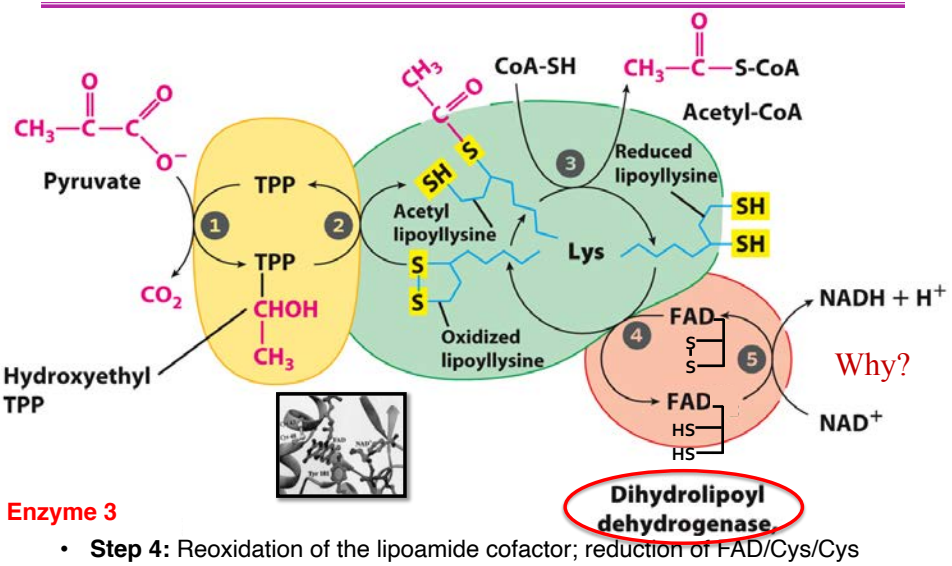
Pyruvate Oxidation



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



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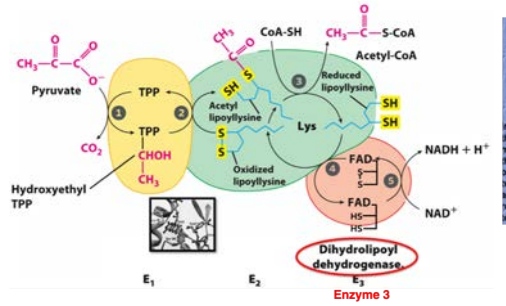
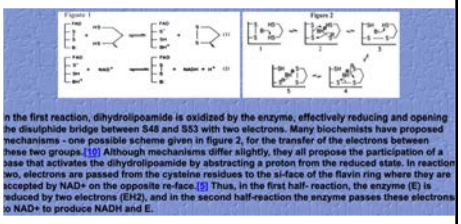
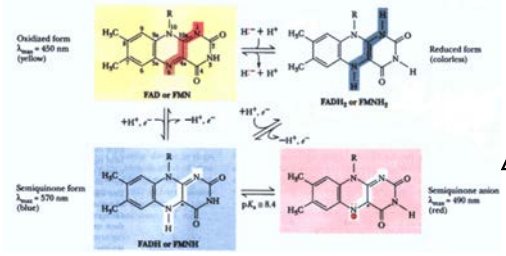
Pyruvate Oxidation



Enzyme 3



n the first reaction, dihydrolipoamide is oxidized by the enzyme, effectively reducing and opening he disulphide bridge between 548 and 553 with two electrons. Many biochemists have proposed mechanisms - one possible scheme given in figure 2, for the transfer of the electrons between hese two groups.[10] Although mechanisms differ slightly, they all propose the participation of a base that activates the dihydrolipoamide by abstracting a proton from the reduced state. In reaction wo, electrons are passed from the cysteine residues to the si-face of the flavin ring where they are accepted by NAD+ on the opposite re-face.[9] Thus, in the first half- reaction, the enzyme (E) is reduced by two electrons (EH_2), and in the second half-reaction the enzyme passes these electrons o NAD+ to produce NADH and E.



$$\Delta E^{o'} = E^{o'}_{(reduction)} - E^{o'}_{(oxidation)}$$

$$\Delta E^{o'} = E^{o'}_{(NAD^+)} - E^{o'}_{(FAD)}$$

$$= -0.320\ V - (+0.031\ V^*)$$

$$= -0.351\ V$$

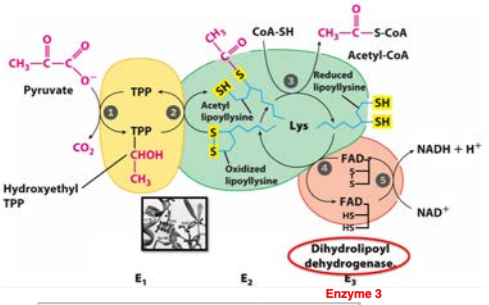
$$\Delta G^{o'} = -n\,\mathcal{F}\,\Delta E^{o'}$$

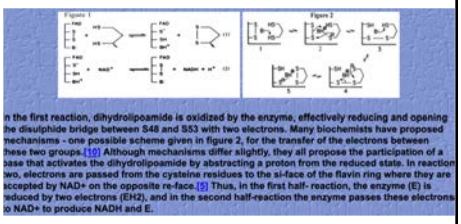
$$= -(2)(23.06 kcal V^{-1} mol^{-1})(-0.351\ V)$$

$$= +16\ kcal\ mol^{-1}$$

*from Maeda-Yorita et al., (1994) *Biochem.* **33**, 6213

Pyruvate Oxidation





n the first reaction, dihydrolipoamide is oxidized by the enzyme, effectively reducing and opening he disulphide bridge between S48 and S53 with two electrons. Many biochemists have proposed echanisms - one possible scheme given in figure 2, for the transfer of the electrons between hese two groups.[10] Although mechanisms differ slightly, they all propose the participation of a ase that activates the dihydrolipoamide by abstracting a proton from the reduced state. In reaction wo, electrons are passed from the cysteine residues to the si-face of the flavin ring where they are ccepted by NAD+ on the opposite re-face.[5] Thus, in the first half- reaction, the enzyme (E) is educed by two electrons (EH2), and in the second half-reaction the enzyme passes these electrons o NAD+ to produce NADH and E.

Standard Redox Potentials E_0			
Half reaction		Half reaction	E_0(V)
Succinate + CO_2 + $2H^+$ +2e⁻	↔	α-ketoglutarate + H_2O	-0.670
Acetate + $2H^+$ +2e⁻	↔	acetaldehyde	-0.581
$2H^+$ +2e⁻	↔	H_2	-0.421
α-ketoglutarate + CO_2 + $2H^+$ +2e⁻	↔	citrate	-0.380
Cystine + $2H^+$ +2e⁻	↔	2 cysteine	-0.340
NAD^+ + $2H^+$ +2e⁻	↔	NADH + H^+	-0.320
$NADP^+$ + $2H^+$ +2e⁻	↔	NADPH + H^+	-0.324
Acetaldehyde + $2H^+$ +2e⁻	↔	ethanol	-0.197
Pyruvate + $2H^+$ +2e⁻	↔	lactate	-0.185
Oxaloacetate + $2H^+$ +2e⁻	↔	malate	-0.166
FAD + $2H^+$ +2e⁻	↔	$FADH_2$	0.031
Fumarate + $2H^+$ +2e⁻	↔	succinate	0.031
Ubiquinone + $2H^+$ +2e⁻	↔	ubiquinol	0.045
2 cytochrome $b_{(ox)}$ +2e⁻	↔	2 cytochrome $b_{(red)}$	0.070
2 cytochrome $c_{(ox)}$ +2e⁻	↔	2 cytochrome $c_{(red)}$	0.254
2 cytochrome $a_{3(ox)}$ +2e⁻	↔	2 cytochrome $a_{3(red)}$	0.385
1/2 O_2 + $2H^+$ +2e⁻	↔	H_2O	0.816

$$\Delta E^{o'} = E^{o'}(\text{reduction}) - E^{o'}(\text{oxidation})$$

$$\Delta E^{o'} = E^{o'}(NAD^+) - E^{o'}(FAD)$$

$$= -0.320\ \text{V} - (+0.031\ \text{V}^*)$$

$$= -0.351\ \text{V}$$

$$\Delta G^{o'} = -n\,\mathcal{F}\Delta E^{o'}$$

$$= -(2)(23.06\text{kcalV}^{-1}\text{mol}^{-1})(-0.351\ \text{V})$$

$$= +16\ \text{kcal mol}^{-1}$$

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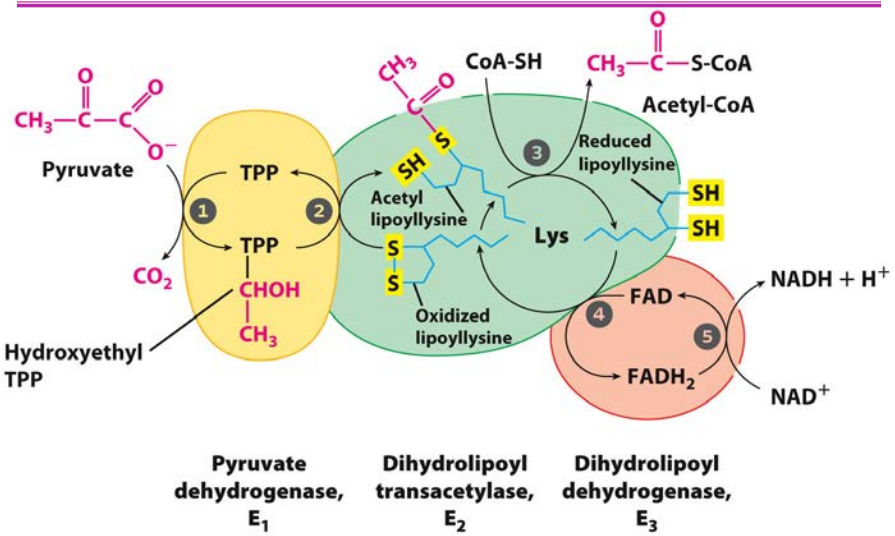
Pyruvate Oxidation



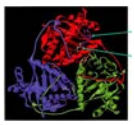
Pyruvate dehydrogenase, E_1 | **Dihydrolipoyl transacetylase, E_2** | **Dihydrolipoyl dehydrogenase, E_3**

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

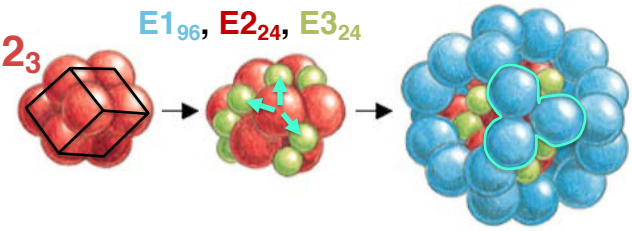
Enzyme 3
- **Step 4:** Reoxidation of the lipoamide cofactor; reduction of FAD/Cys/Cys
- **Step 5:** Regeneration of the oxidized FAD/Cys/Cys active site – forming NADH

Pyruvate Oxidation

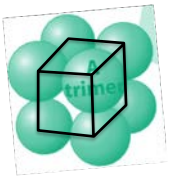


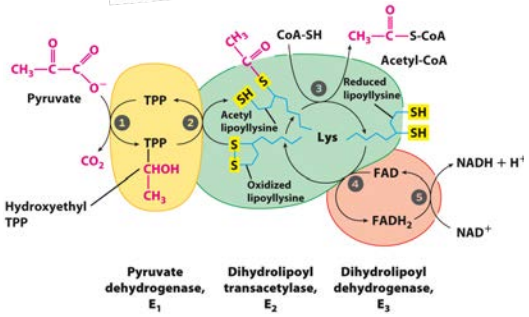
8-E2$_3$

E1$_{96}$, E2$_{24}$, E3$_{24}$

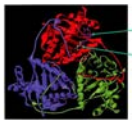


E2

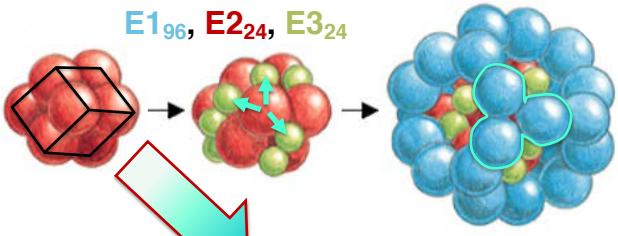




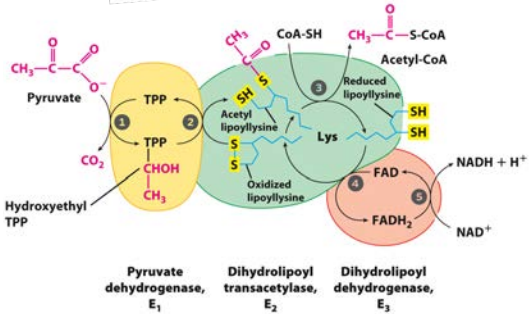
Pyruvate Oxidation



E1$_{96}$, E2$_{24}$, E3$_{24}$



E2



Pyruvate Oxidation

Overall Reaction of PDC

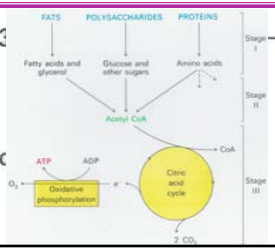
Pyruvate + Coenzyme-A (CoASH) + NAD^+

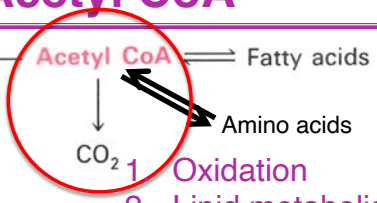
PDC (TPP, lipoic acid, FAD) ⇅ $\Delta G^{o'} = -8$ kcal/mol

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

Fates of Acetyl CoA

(HMG-CoA)

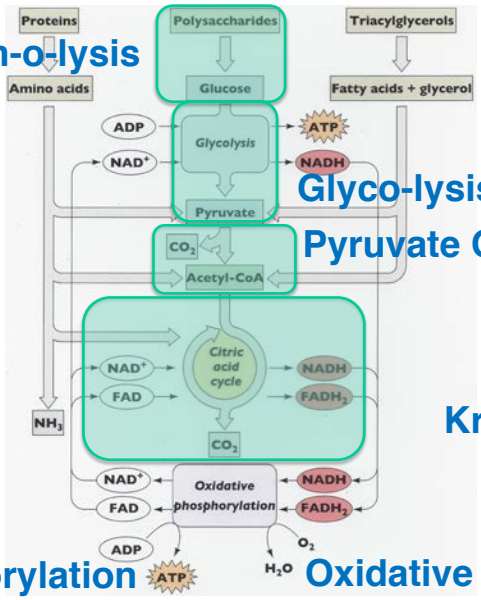




1. Oxidation
2. Lipid metabolism
3. Amino acid metabolism

The Citric Acid Cycle

Glycogen-o-lysis



Glyco-lysis

Pyruvate Oxidation

Krebs' Cycle

Phosphorylation

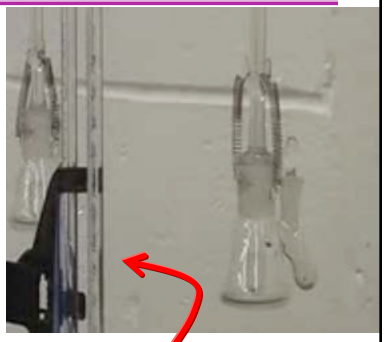
Oxidative

The Citric Acid Cycle

a.k.a. Krebs Cycle,
a.k.a. 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

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

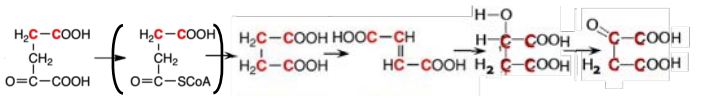
UTube instructions
(http://youtu.be/M-HYbZwN43o)

Time B.C. (Before the Cycle)

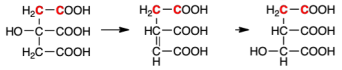
O-leic
M-alonic
S-uccinic
G-lutaric
A-dipic
P-imelic

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.



α-ketoglutarate → 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)

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

Hans Krebs
1900-1981

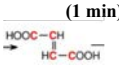
glutarate → succinate → fumarate → malate → oxaloacetate

citrate → aconitate → isocitrate

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

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.

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



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

∴1 µmole fumarate would consume 3 µmole O_2