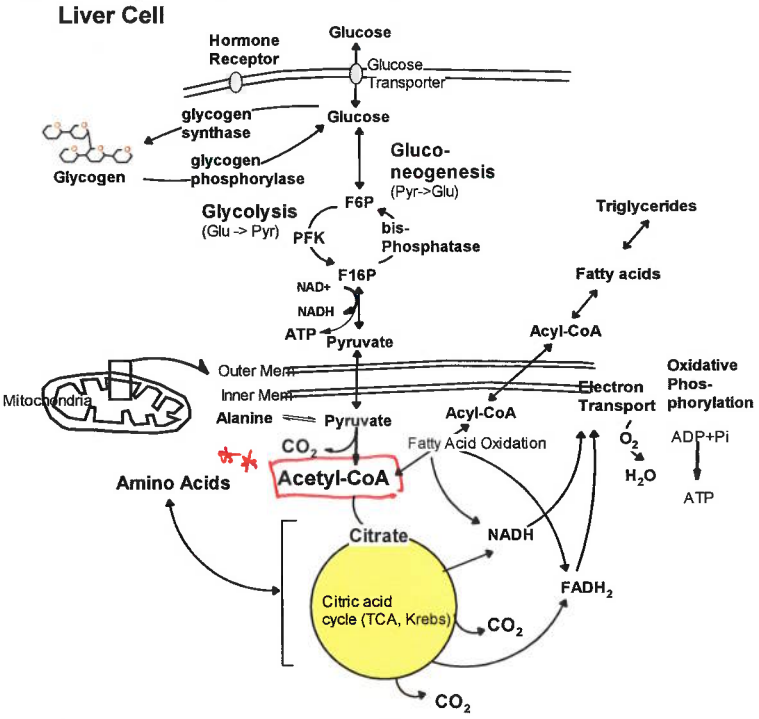
Lecture 32: Citric Acid Cycle

Expectations for Citric Acid Cycle:

i) Input - pyruvate
ii) Output CO_2, NADH, $FADH_2$, GTP
iii) Location-mitochondrial matrix
iv) Energy Generating Steps-oxidative decarboxylations (NADH produced)
v) Regulation – energy sensing (NADH, ATP).
vi) Thio-CoA – high energy compound.
vi) Biosynthesis of amino acids.

Features of Citric Acid Cycle:

- Also known as the TCA cycle (tricarboxylic acid) or the Krebs cycle.
- The enzymes that participate in the citric acid cycle are found in the **mitochondrial matrix,** except for one, which is in the inner membrane - Succinate dehydrogenase. This enzyme also participates in the electron transfer chain.
- **Catabolic role**: Amino acids, fats, and sugars enter the TCA cycle to produce energy. **Acetyl CoA** is a central intermediate
- **Anabolic role**: TCA cycle provides starting material for fats and amino acids. Note: carbohydrates cannot be synthesized from acetyl-CoA by humans. Pyruvate→Acetyl CoA is one way!
- In contrast to glycolysis, none of the intermediates are phosphorylated; but all are either di- or tricarboxylic acids.
- Regulation is largely by sensing energy levels.

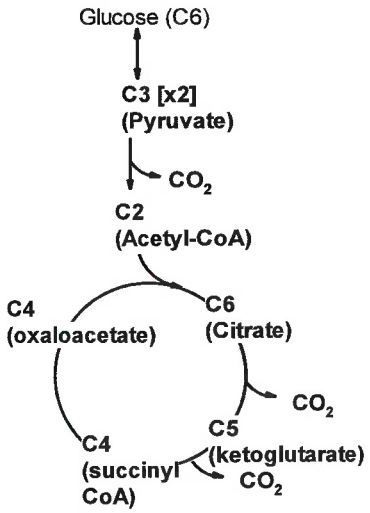


1: Overall Carbon Flow:

All of the carbon that is input as **pyruvate** are released as **CO_2.**

Three Locations of CO_2 release:

- Pyruvate Dehydrogenase: Pyruvate to acetyl-CoA
- Isocitrate dehydrogenase: Isocitrate to α-ketoglutarate
- α-ketoglutarate dehydrogenase:α-ketoglutarate to succinyl-CoA



The largest change in the carbon structure occurs at step 1, the citrate synthase reaction:

C2 (acetate) + C4 (oxaloacetate) → C6 (Citrate)

Subsequent reactions remove two carbons from citrate to generate the C4 compound, oxaloacetate at the end of the cycle. The oxaloacetate re-enters the cycle.

Reflection: *Is there any oxygen required?* No

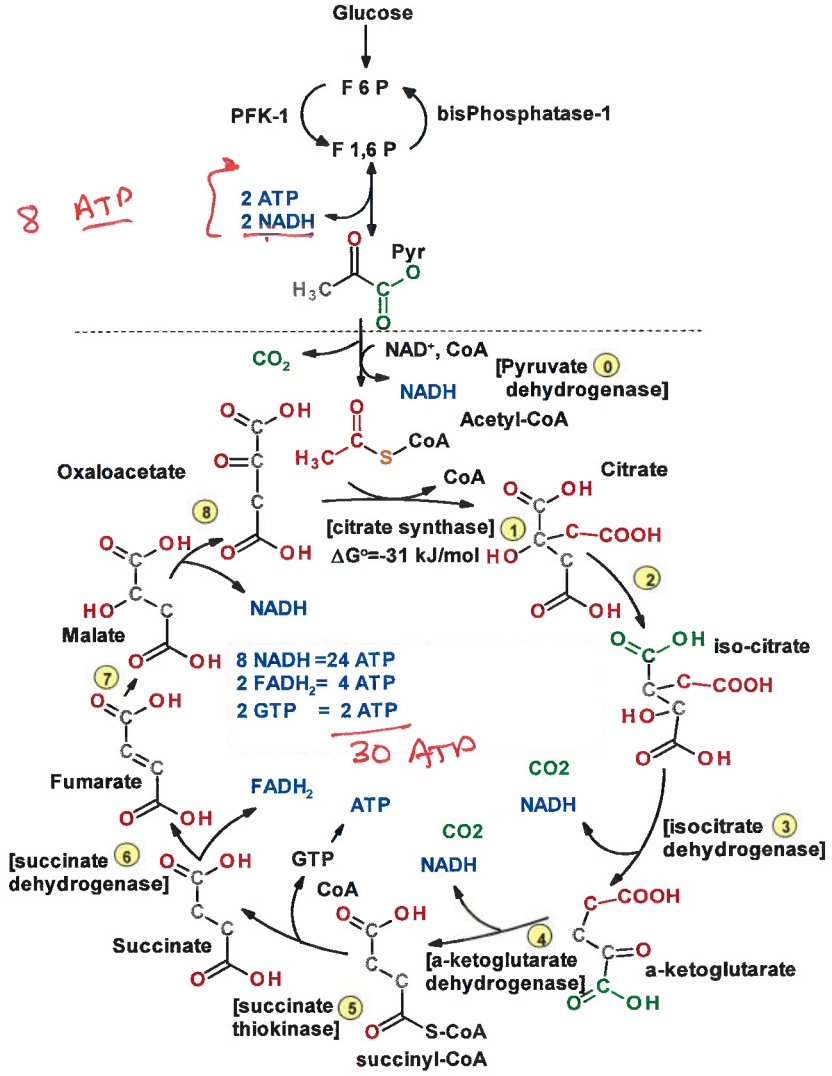
2. Energetics of the TCA Cycle:

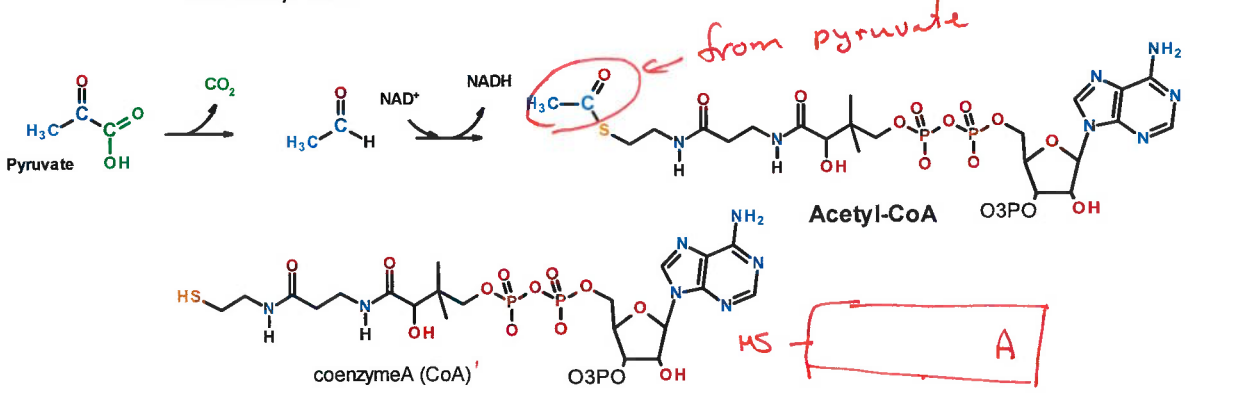
- Most of the energetic currency is in the form of redox reactions, only a single GTP (=ATP) is produced/pyruvate while four NADH and one $FADH_2$ are produced.
- Most of the energy from oxidation is of glucose is harvested in the TCA cycle. The TCA cycle is a slower but richer source of energy.

2a: Oxidative decarboxylations: These occur at three locations, leading to the loss of the three carbons from pyruvate. Each one captures the energy released by forming NADH.
1. Pyruvate dehydrogenase
2. Isocitrate dehydrogenase
3. α-ketoglutarate dehydrogenase

Pyruvate dehydrogenase (decarboxylase)(Step 0)
1. loss of the CO_2 group.
2. oxidation of the aldehyde and formation of the thio-ester.

The thio-ester is formed between the oxidized product and Coenzyme A, to form acetyl-CoA.

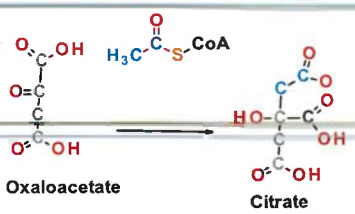




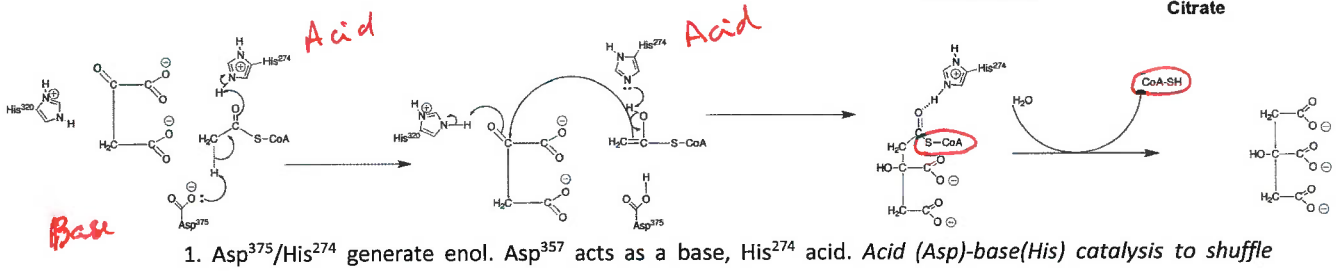
Reflection: *Which produces more ATP, glycolysis or the TCA cycle?*

TCA energy rich.

Thioesters in Biochemical Reactions: The relatively weak thioester bond facilitates the transfer of the attached group to other compounds.



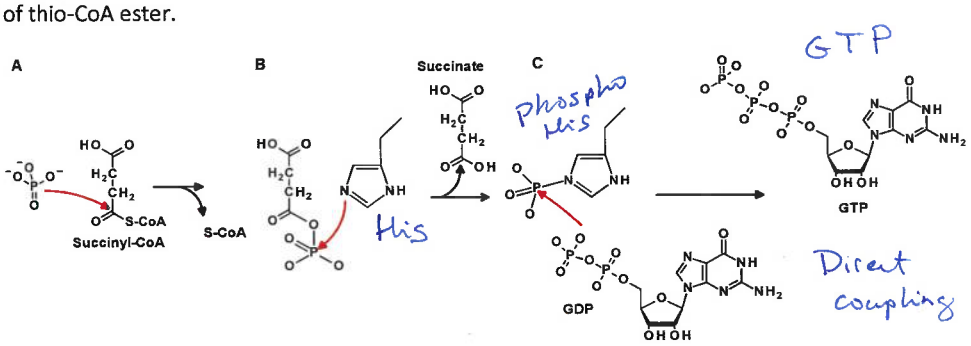
Citrate synthase (step 1). form C-C bond



1. Asp^{375}/His^{274} generate enol. Asp^{357} acts as a base, His^{274} acid. *Acid (Asp)-base(His) catalysis to shuffle proton.*
2. Double bond in enol attacks electropositive C=O, proton provided by His^{320} (acid) forms alcohol
3. Hydrolysis of CoA by H_2O.

Succinate thiokinase (Step 5): succinyl CoA provides enough energy to driving the synthesis of GTP.

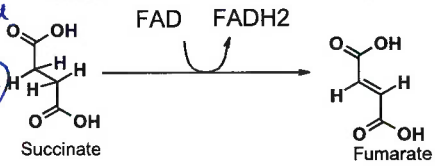
A) phosphorlysis of thio-CoA ester.
B) Transfer of phosphate to His
C) Transfer of phosphate from phosphoryl-His to GDP



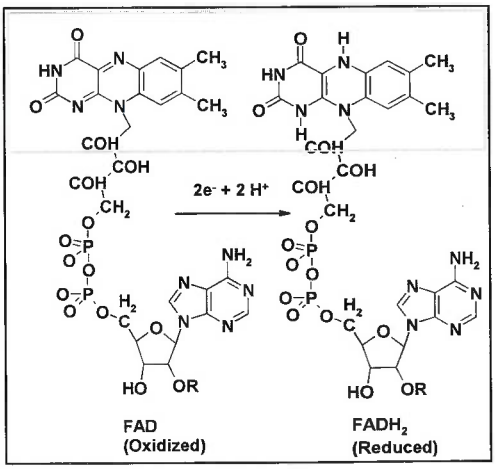
2b. The remaining section of the pathway, from **succinate** to **oxaloacetate** follows a classic three step oxidation scheme, which are also seen in fatty acid oxidation:

Alkane → Alkene → Alcohol → Ketone
REDOX REDOX

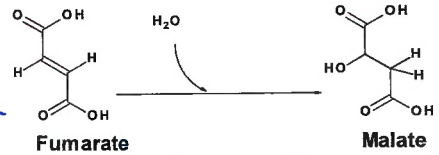
Step 6. Oxidation of succinate to fumarate reduces FAD to $FADH_2$. ***Alkane → Alkene***



Succinate Dehydrogenase (ETC chain) inner membrane

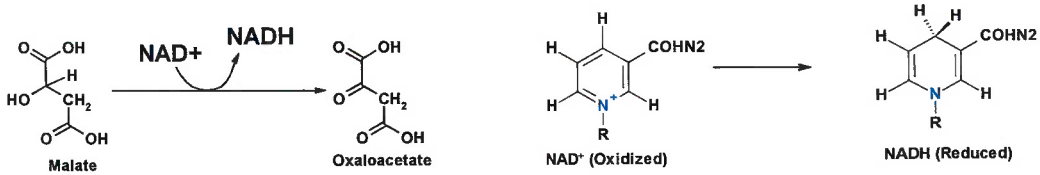


Step 7. Addition of water to the double bond, to make the alcohol. ***Alkene → Alcohol***



No change in oxidation state

Step 8. Oxidation of Malate to Oxaloacetate reduces NAD^+ to NADH. ***Alcohol → Ketone***

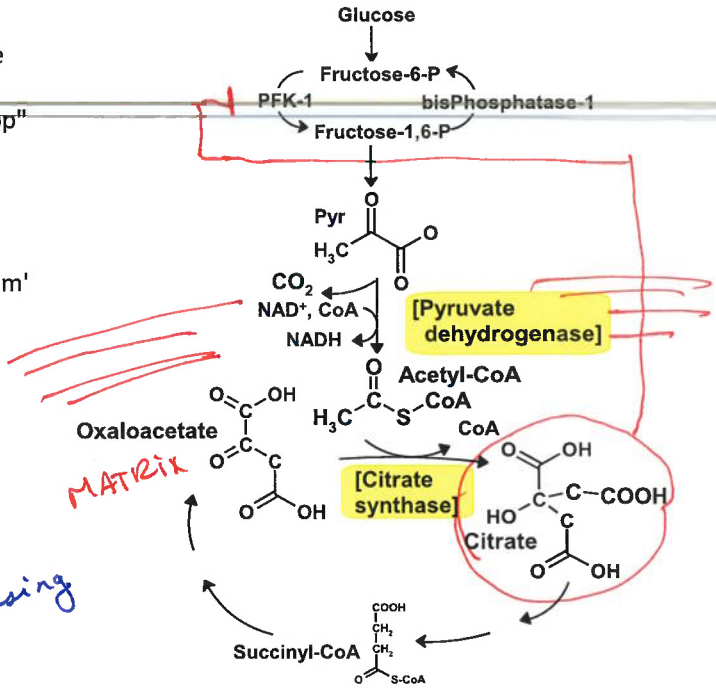


3. Regulation of the TCA Cycle:

1. High energy, irreversible steps are regulated.
2. Regulated reactions are at the "top" of the pathway.

Examples of:

1. Product Inhibition.
2. Allosteric inhibition by feedback inhibition by products 'downstream' in the pathway.



Energy sensing is the most important regulatory control (I=inhibited)

	High Energy		OTHER		
Step	**NADH**	**ATP**	**Compound**	**Product Inh**	**Feedback Inh**
Pyruvate Dehydrogenase	I	I	Inhibited by Acetyl Co-A	✓	
Citrate Synthase	I	I	Inhibited by succinyl-CoA		✓
			Inhibited by citrate	✓	

Regulation of glycolysis: Citrate stabilizes the tense-form of PFK, shutting down glycolysis.

Lecture 32B: Fatty Acid Oxidation.

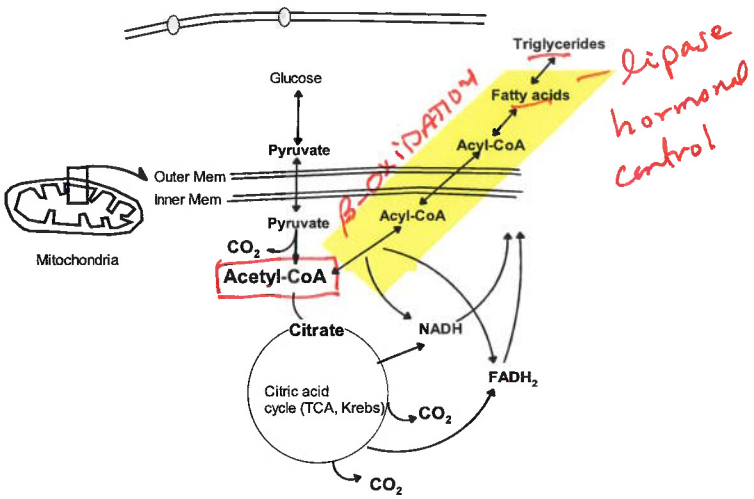
Expectations for Fatty Acid Oxidation:

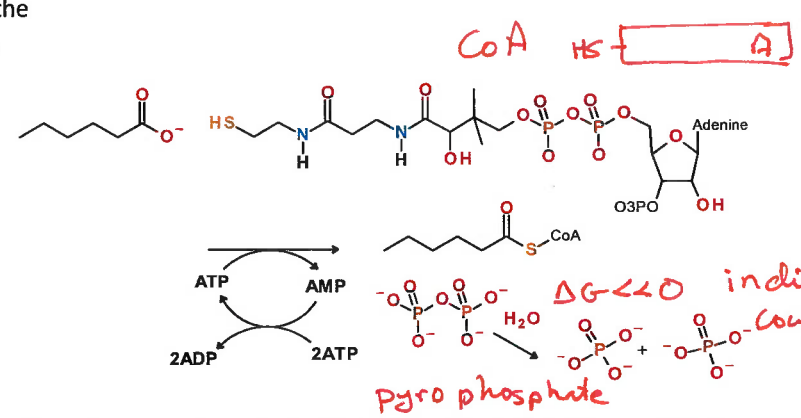
i) Input – triglycerides.
ii) Output Acetyl-CoA, NADH, $FADH_2$
iii) Location mitochondrial matrix
iv) Energy Generating Steps are oxidations.

Fatty Acid Oxidation (β-Oxidation):

A. Release of Fatty Acids: Triglycerides are hydrolyzed by lipases. This activity is also under hormonal control, in a similar fashion to glycogen/glucose metabolism.

B. Formation of Acyl-CoA (Cytosol): The fatty acids in the cytosol are coupled to coenzyme A to form acyl-CoA. The activation reaction is catalyzed by *acyl-CoA synthetase* and involves the hydrolysis of ATP to AMP, i.e. the equivalent of two high energy ATP molecules (60 kJ/mol). The released pyrophosphate is hydrolyzed to inorganic phosphate, making the overall ΔG negative for the reaction (indirect coupling). It is only necessary to utilize ATP once in the activation of the fatty acid, the activated fatty acid is transported to the mitochondria for oxidation.





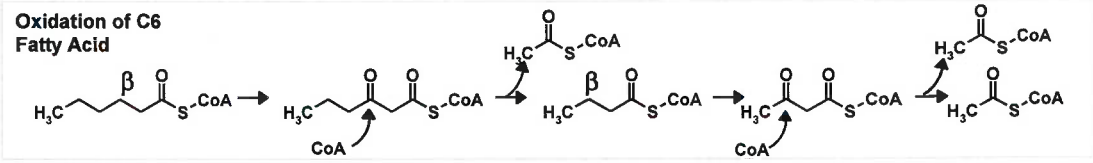
C. Transport into mitochondria matrix: The acyl-CoA is transported into the mitochondrial matrix for oxidation. This location is ideal for funneling the products of β-oxidation (NADH and $FADH_2$) to E. transport.

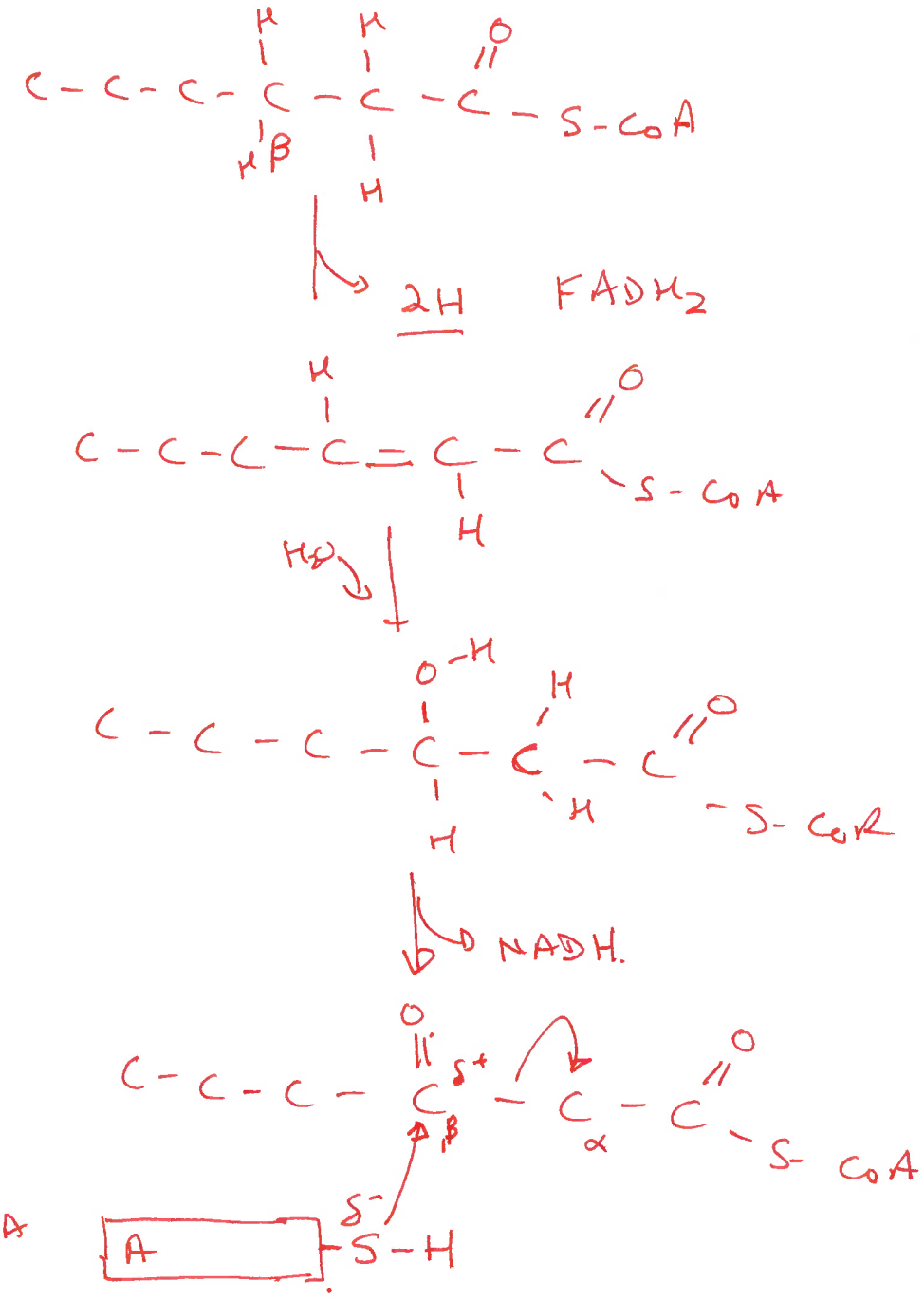
D. β-Oxidation (Mito. matrix): Acyl-CoA is shortened **2 carbons at a time** from the carboxyl end of the fatty acid using the following steps:

1. Formation of trans α-β double bond by **acyl-CoA dehydrogenase**, an FAD enzyme.
2. Addition of water to the newly formed double bond to generate the alcohol by **enoyl-CoA hydratase**
3. Oxidation of the alcohol by NAD^+ to give the ketone, catalyzed by **3-L-hydroxyacyl-CoA dehydrogenase.**
4. Cleavage reaction by **β-ketoacy-CoA thiolase** (thiolysis), generates acetyl-CoA and an acyl-CoA that is two carbons shorter. The acetyl-CoA enters the TCA cycle.
5. Steps 1-4 are repeated until only acetyl-CoA remains. The last cycle produces two acetyl-CoA.

Example: 6 carbon fatty acid:

2 cycles: 2 NADH, 2 $FADH_2$, and 3 Acetyl-CoA produced




2H FADH2
NADH.
CoA
A
SH
3 Acetyl CoA
2 FADH2
2 NADH.
FADH2
NAD
Acetyl CoA