Tricarboxylic Acid Cycle

Purpose of the Citric Acid Cycle

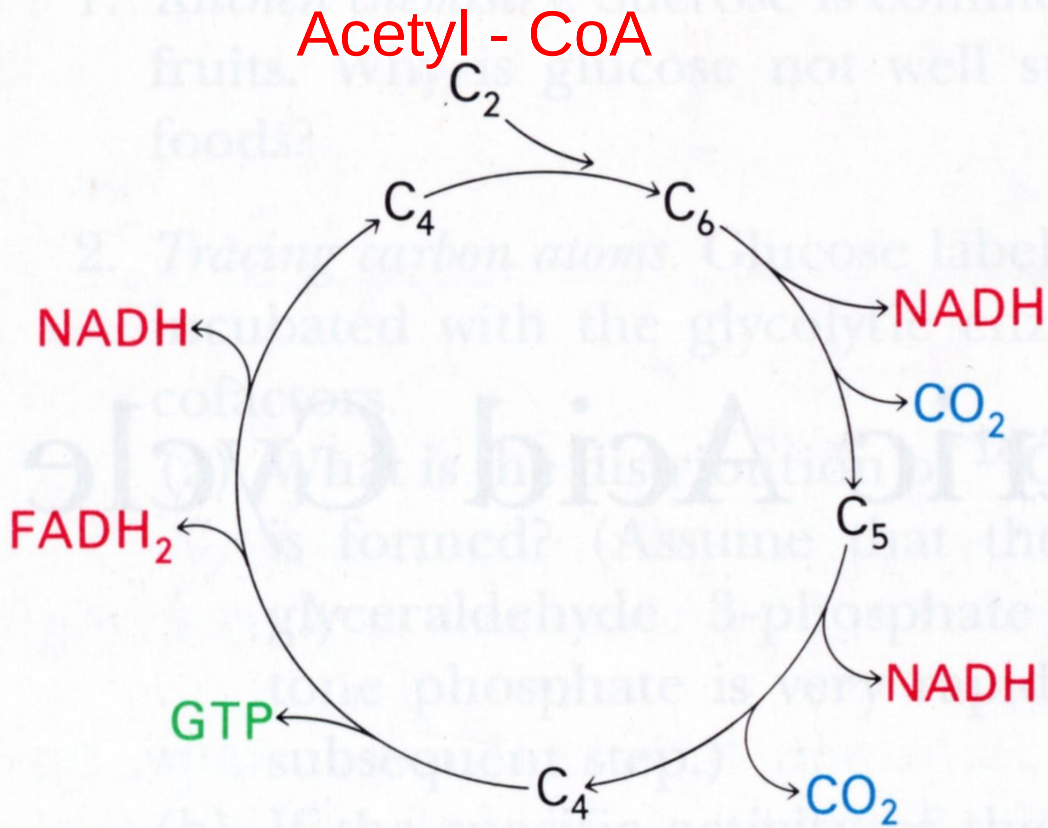
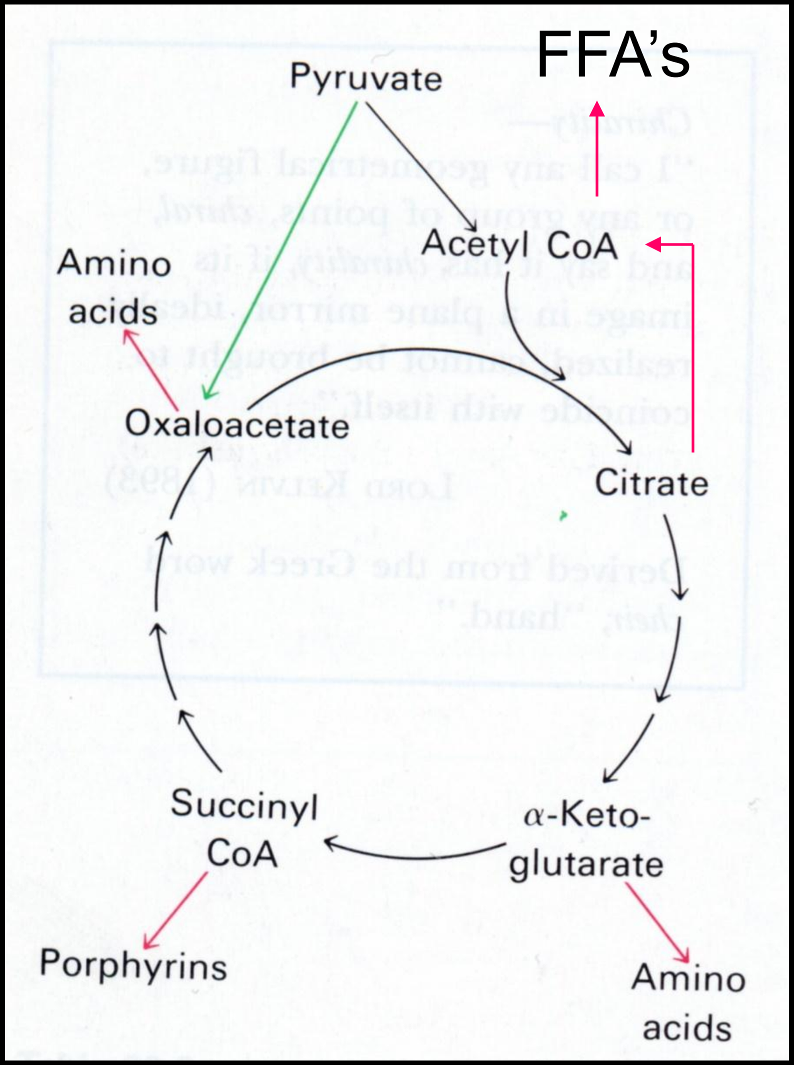


Figure 20-2
An overview of the citric acid cycle.

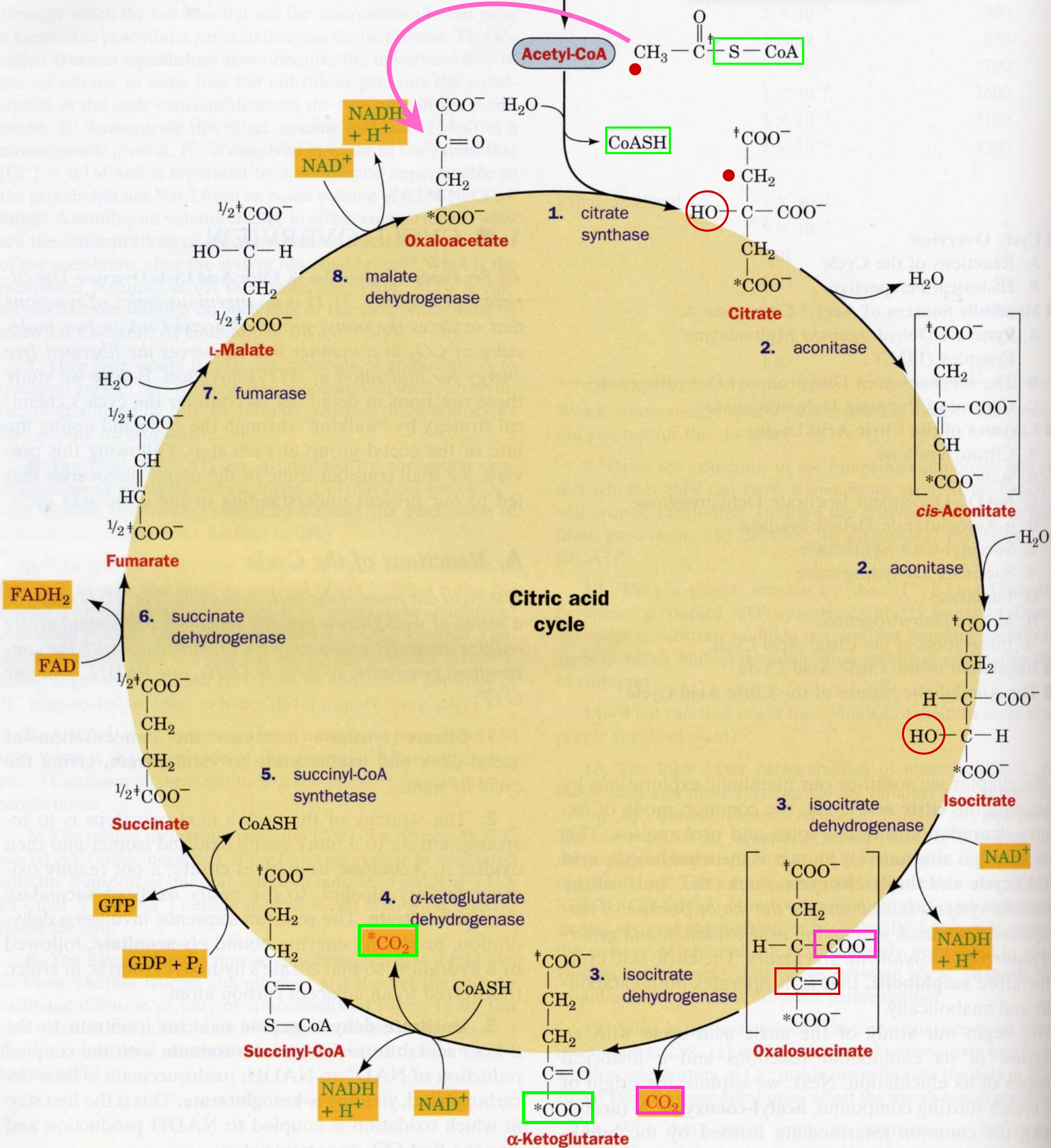
A) Complete Oxidation of Acetyl C's (& H's)

ATP

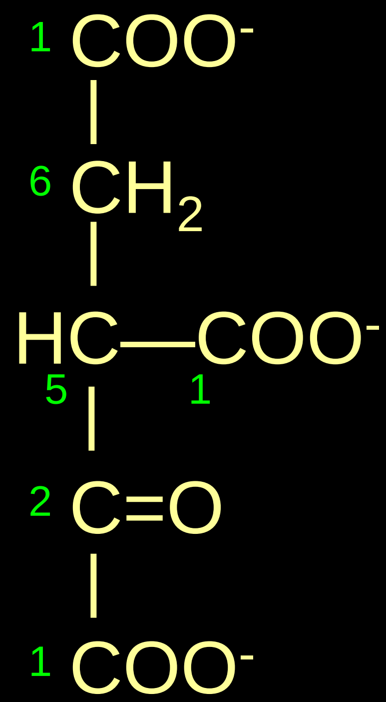


B) Generation of precursors for synthesis of macro-molecules

ATP



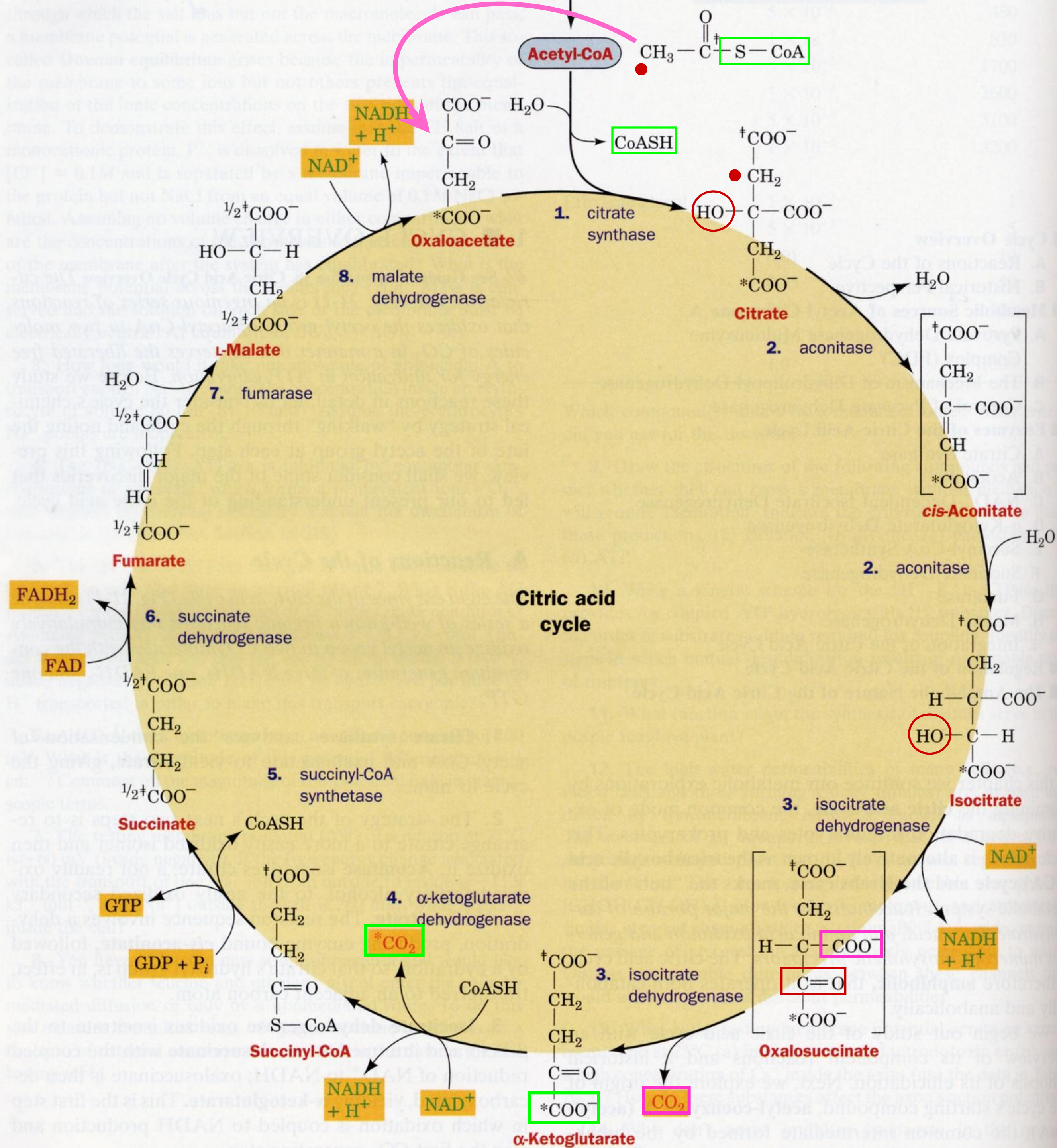
Acetyl-CoA
CoASH
NADH + H+
NAD+
Oxaloacetate
1. citrate synthase
Citrate
2. aconitase
cis-Aconitate
2. aconitase
Isocitrate
3. isocitrate dehydrogenase
NAD+
NADH + H+
Oxalosuccinate
3. isocitrate dehydrogenase
CO2
α-Ketoglutarate
NADH + H+
NAD+
CoASH
4. α-ketoglutarate dehydrogenase
*CO2
Succinyl-CoA
5. succinyl-CoA synthetase
GTP
GDP + Pi
CoASH
Succinate
6. succinate dehydrogenase
FAD
FADH2
Fumarate
7. fumarase
L-Malate
8. malate dehydrogenase
Citric acid cycle



oxalsuccinate

Major Steps:

1. Formation of oxalosuccinate from oxaloacetate and acetyl-CoA
2. Decarboxylation
3. Regeneration of oxaloacetate



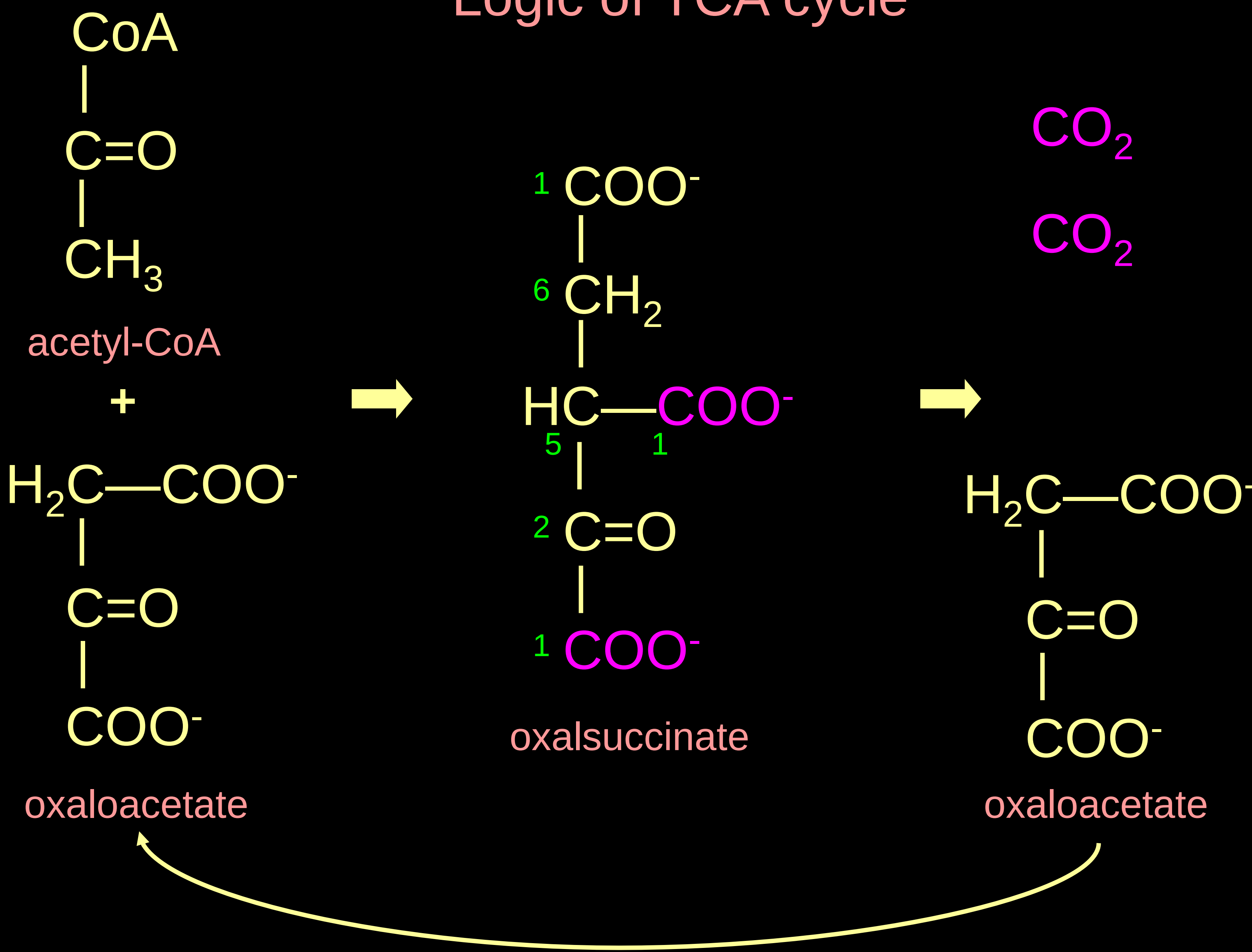
Acetyl-CoA
CoASH
1. citrate synthase
Citrate
2. aconitase
cis-Aconitate
2. aconitase
Isocitrate
3. isocitrate dehydrogenase
NAD+
NADH + H+
Oxalosuccinate
3. isocitrate dehydrogenase
CO2
α-Ketoglutarate
4. α-ketoglutarate dehydrogenase
CoASH
NAD+
NADH + H+
*CO2
Succinyl-CoA
5. succinyl-CoA synthetase
GDP + Pi
GTP
CoASH
Succinate
6. succinate dehydrogenase
FAD
FADH2
Fumarate
7. fumarase
H2O
L-Malate
8. malate dehydrogenase
NAD+
NADH + H+
Oxaloacetate
H2O
Citric acid cycle

1. 2 C's are liberated from TCA cycle. Two enter via acetyl-CoA.
2. 8 e's are liberated. Eight enter via acetyl-CoA.

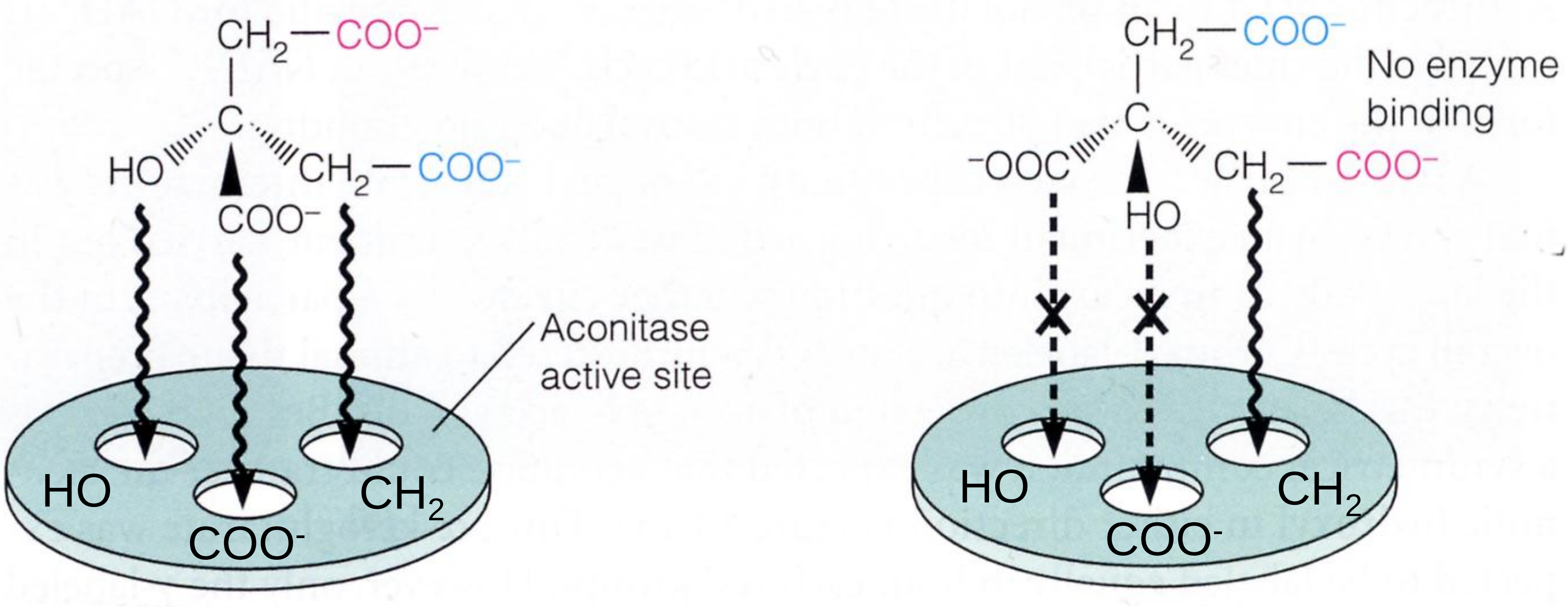
For Homework:

3. 4 O's are liberated. Where do they enter?
4. 8 H^+'s are liberated. Where do they enter?

Logic of TCA cycle



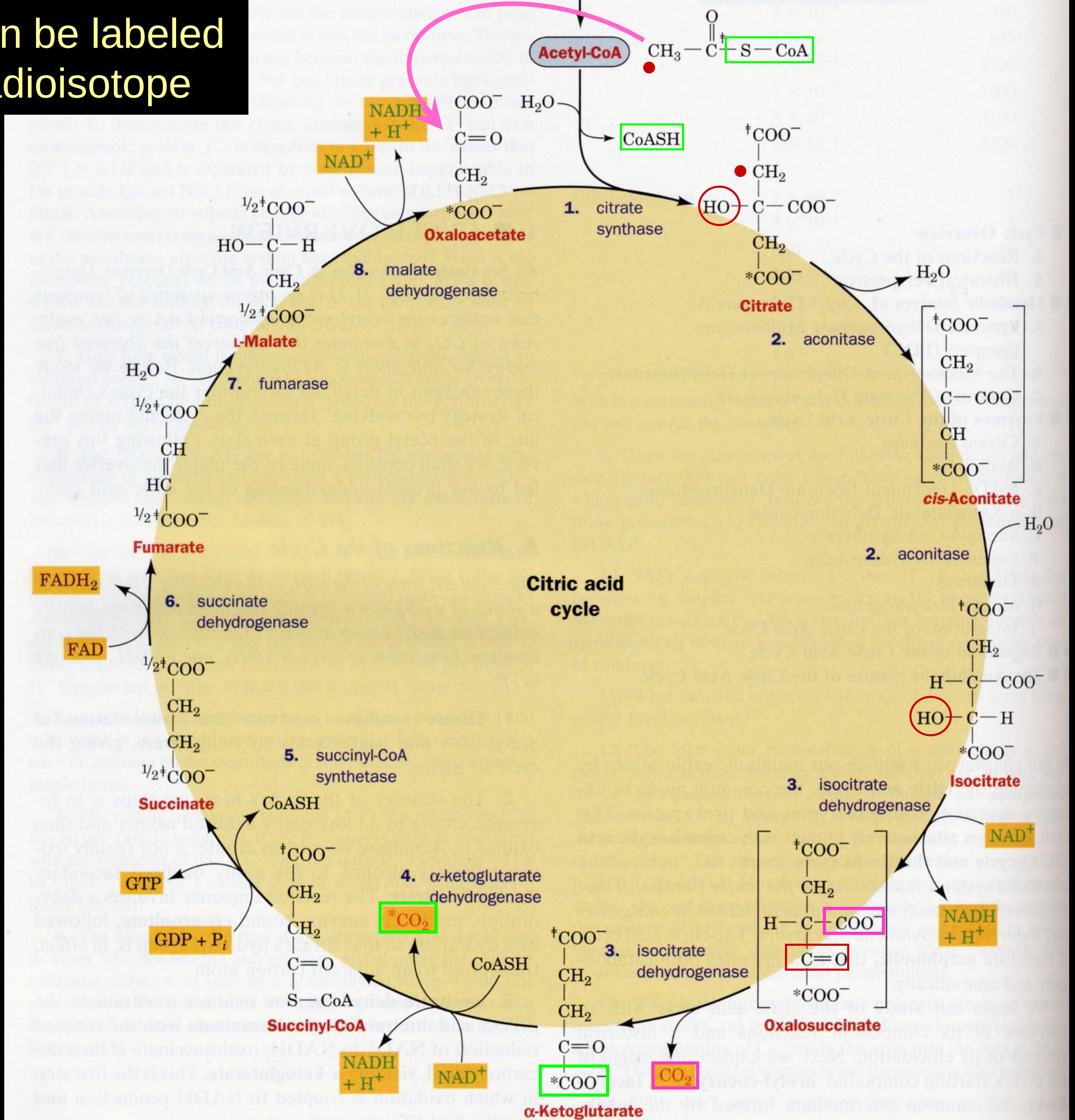
Citrate is *pro*chiral



Matthews 3rd ed. p500

Evidence for the TCA cycle

Carbon can be labeled with C^{14} radioisotope

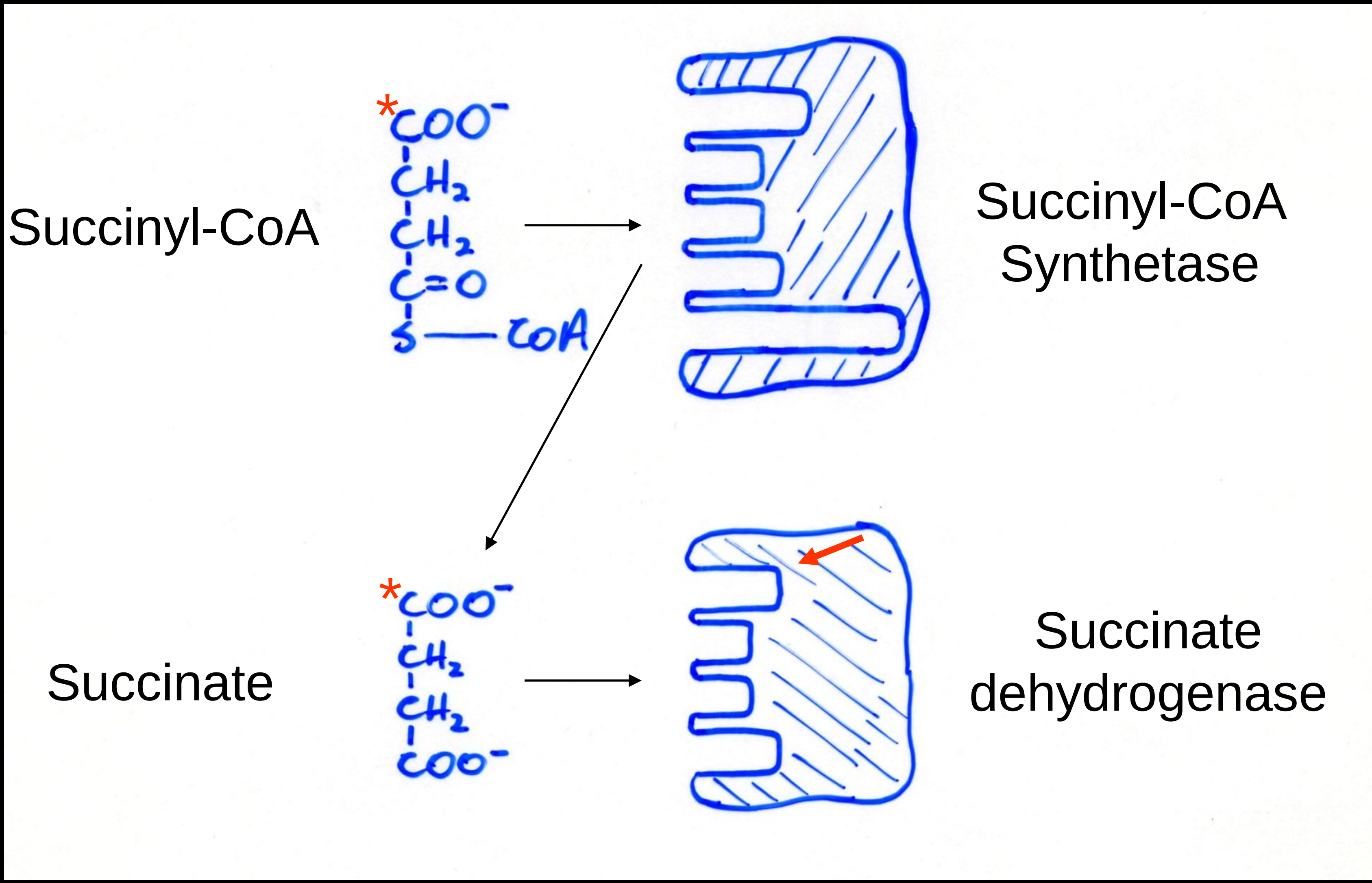


$$\text{Specific Radioactivity} = \frac{[\text{Hot}]}{[\text{Hot} + \text{Cold}]}$$

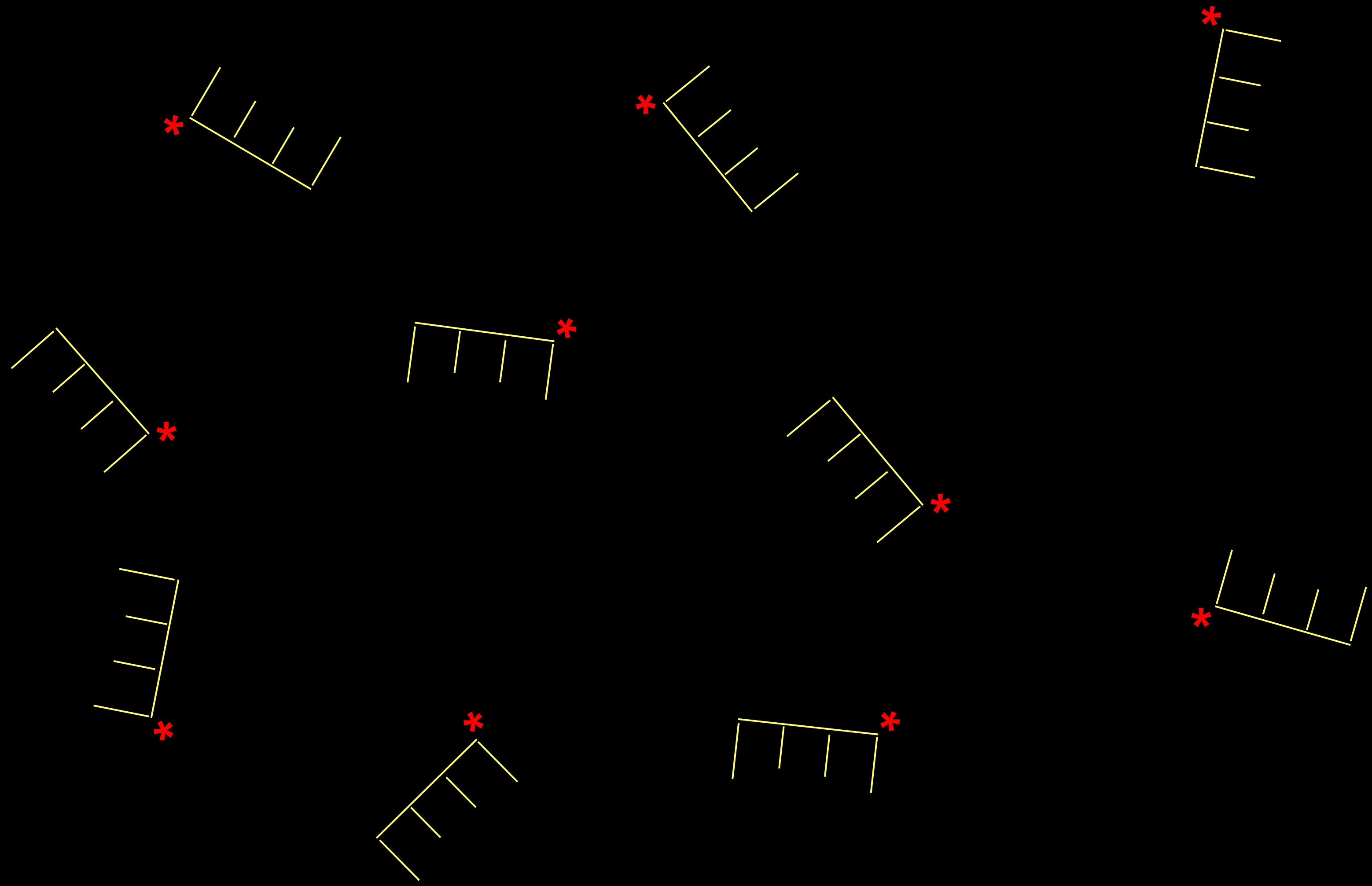
Hot = labeled

Cold = unlabeled

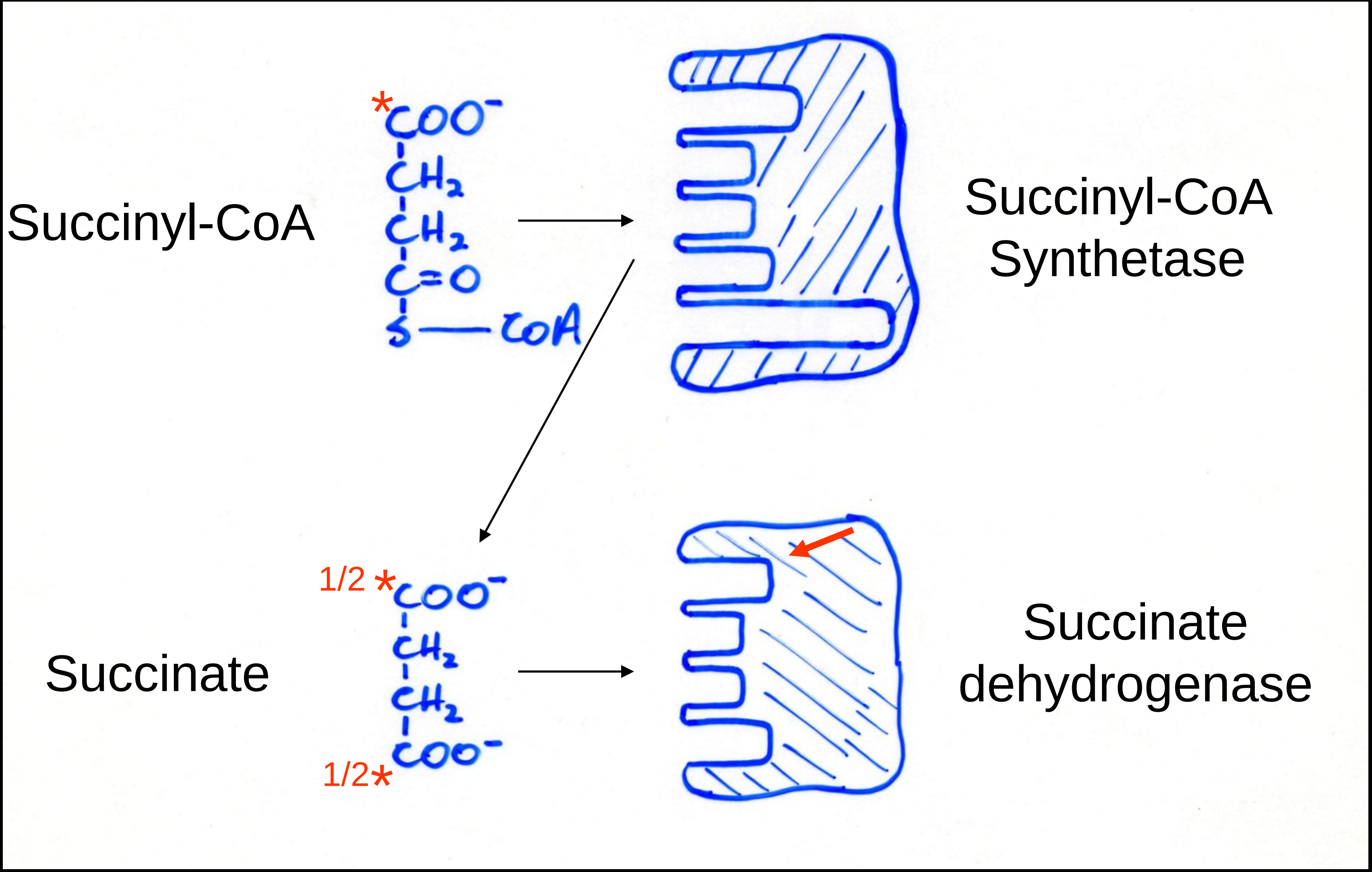
Radiolabel remains 100% on C1



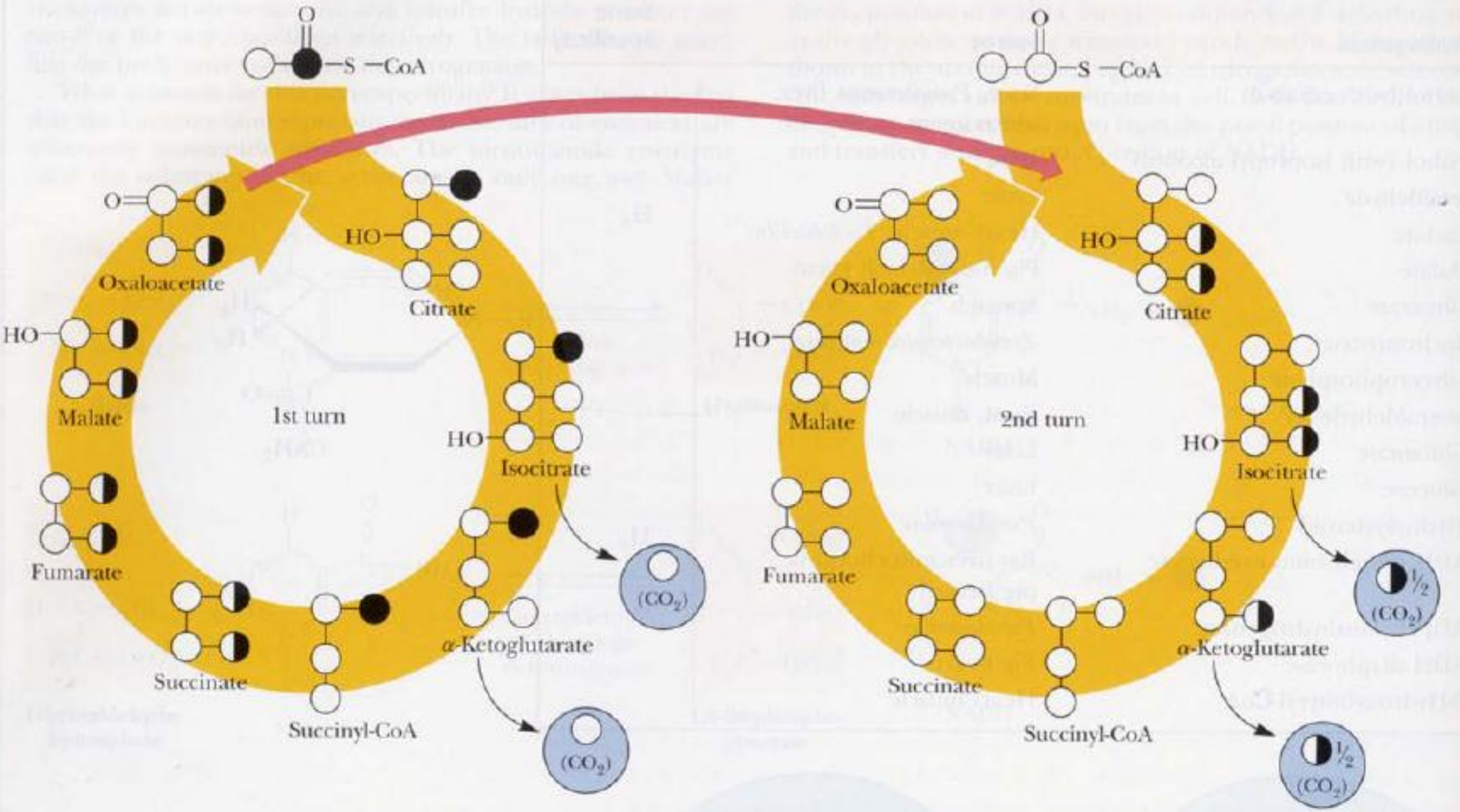
However, C1 cannot be distinguished from C4 in succinate.



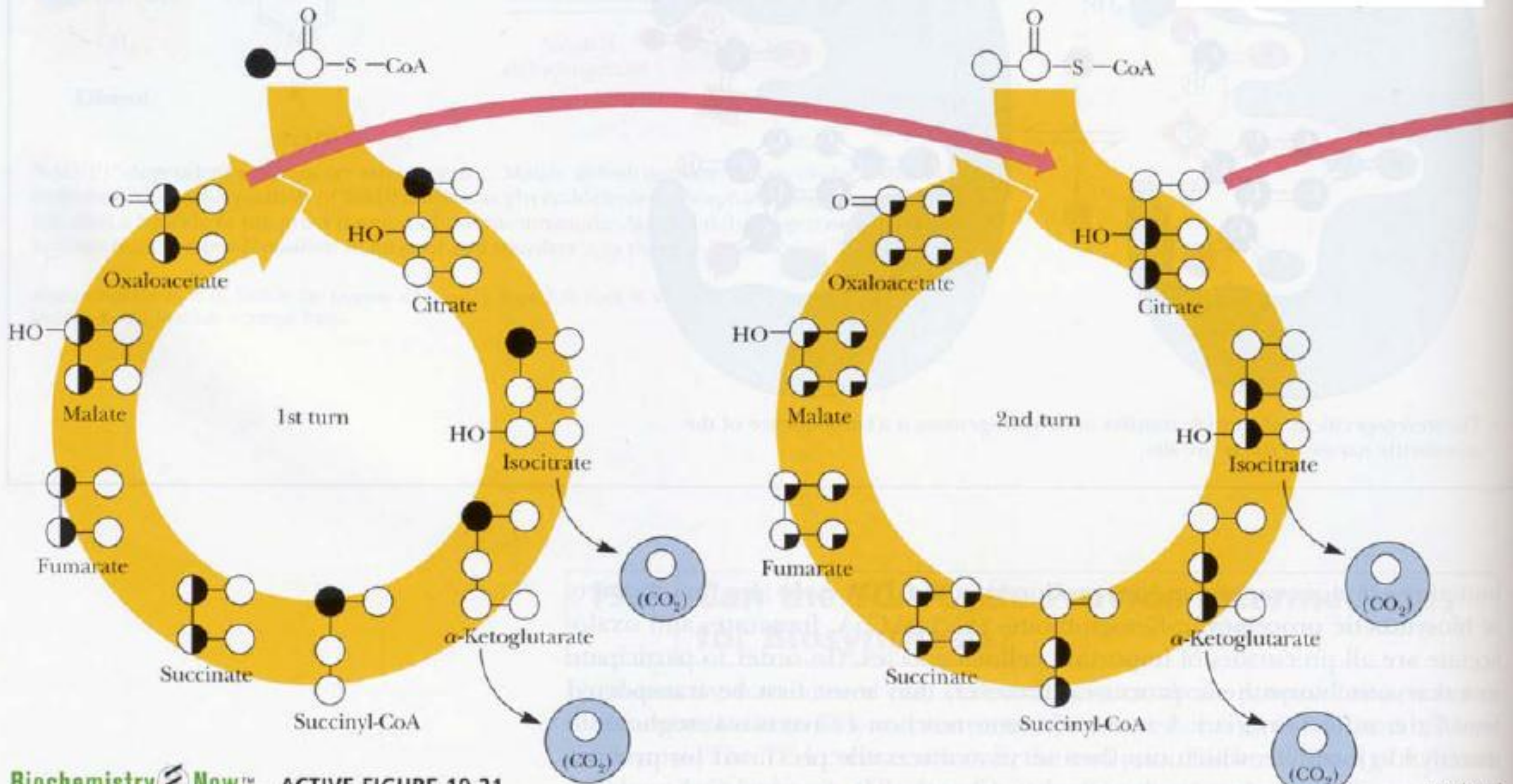
Thus, radioisotope is *scrambled* at succinate.
It appears to be 50% C1, 50% C4.



(a) Fate of the carboxyl carbon of acetate unit

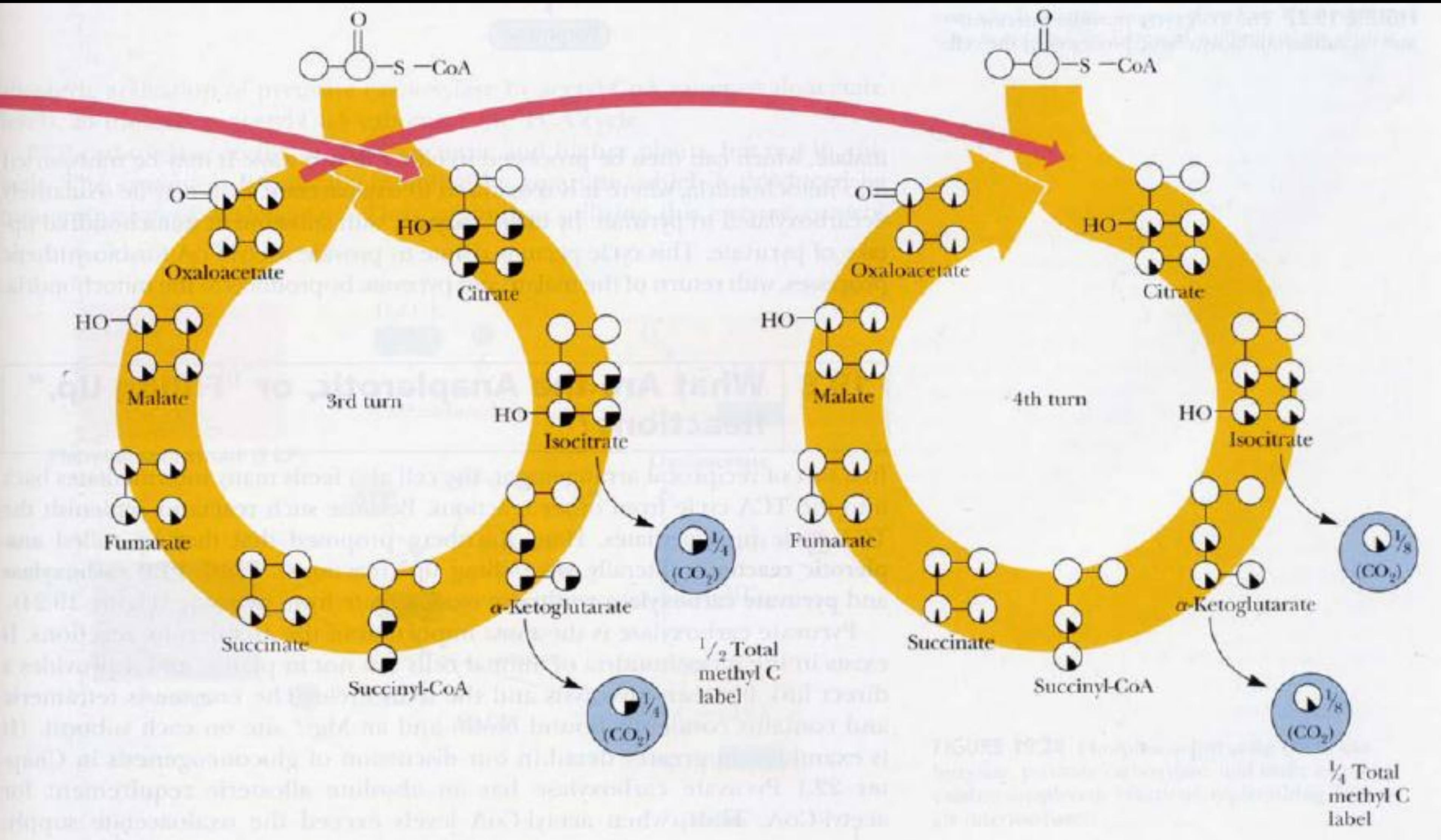


(b) Fate of methyl carbon of acetate unit



Biochemistry Now™ ACTIVE FIGURE 19.21

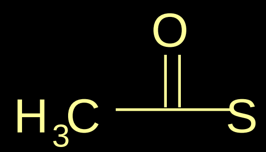
continued



S —CoA
Oxaloacetate
Citrate
Malate
3rd turn
Isocitrate
Fumarate
α-Ketoglutarate
Succinate
Succinyl-CoA
1/4 (CO2)
1/4 (CO2)
1/2 Total methyl C label
S —CoA
Oxaloacetate
Citrate
Malate
4th turn
Isocitrate
Fumarate
α-Ketoglutarate
Succinate
Succinyl-CoA
1/8 (CO2)
1/8 (CO2)
1/4 Total methyl C label

Stoichiometry of the TCA Cycle

- For every one turn of the cycle:

1.  $\longrightarrow$ $2\ CO_2$ 4 e- pair process

2. $3\ NAD^+ \xrightarrow{+6H} 3NADH + 3H^+$

   $1\ FAD \xrightarrow{+2H} 1FADH_2$

3. $2O_2 \xrightarrow{+8H} 4H_2O$

4. 1 GTP produced.

   $GTP + ADP \rightleftharpoons GDP + ATP$ $\Delta G° = 0$

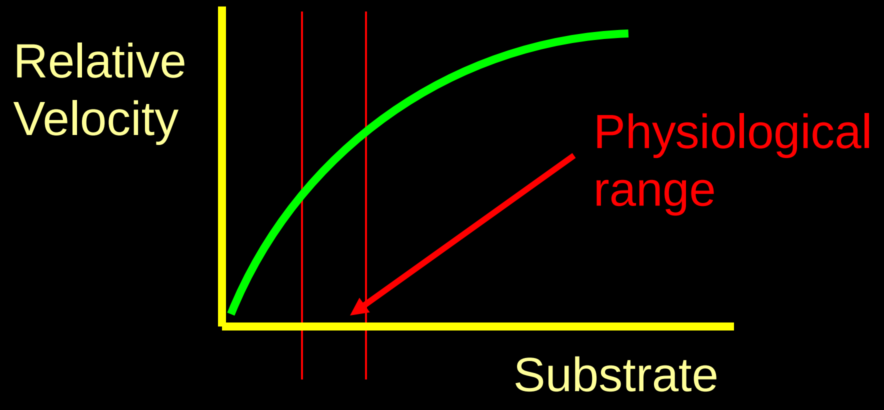
4.2 kJ = 1 kcal

Enzyme	ΔG (kJ/mol)
Citrate synthase	-53.9
Aconitase	$+0.8$
Isocitrate dehydrogenase	-17.5
α-Ketoglutarate dehydrogenase complex	-43.9
Succinyl-CoA synthetase	≈ 0
Succinate dehydrogenase	$\neq 0$
Fumarase	≈ 0
Malate dehydrogenase	≈ 0
	$\approx (-115)$

Control of Citrate Synthase, Isocitrate DH, α-Ketoglutarate DH

- Large degree of control is achieved by substrate availability and product inhibition.
- Acetyl CoA & Oxaloacetate and NADH are critical.
- Both Acetyl CoA & Oxaloacetate are subsaturating

Therefore : ↑ [substrate] → ↑ activity of citrate synthase.



- [Acetyl-CoA] is regulated by PDH activity

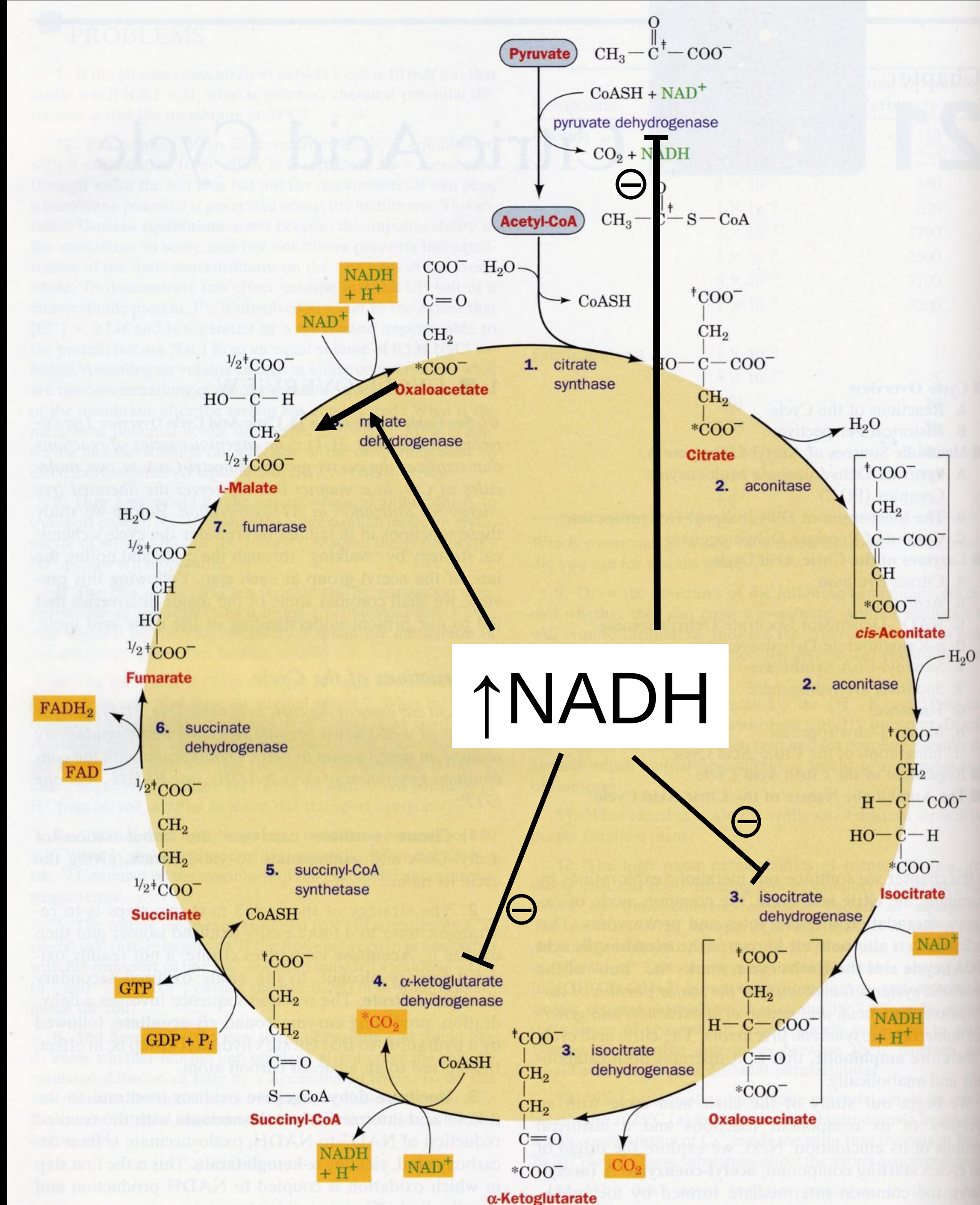
- [Oxaloacetate] is regulated by intracellular redox potential: [NADH]/[NAD^+]

- Malate + NAD^+ $\rightleftharpoons$ Oxaloacetate + NADH + H^+ $\Delta G \sim 0$

For example:

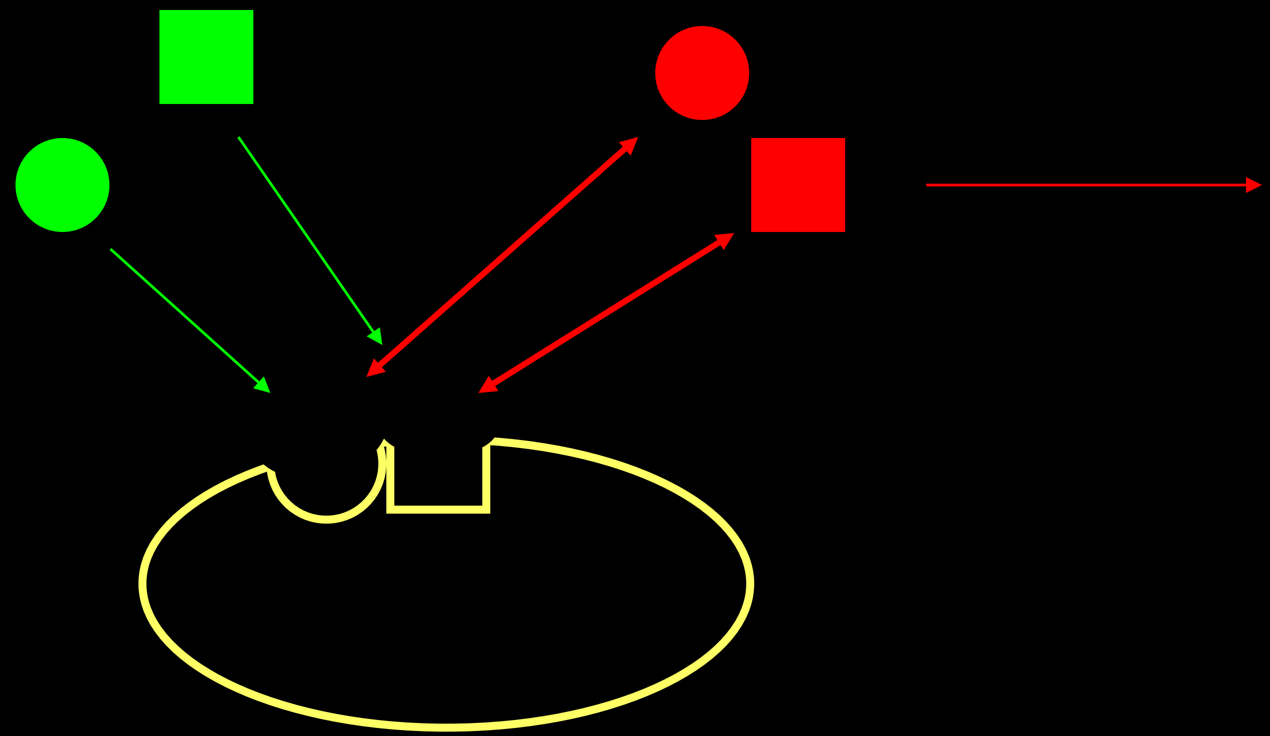
↑ Exercise → ↓ $NADH/NAD^+$ ratio

↓ NADH, ↑ NAD^+ → ↑ Oxaloacetate → ↑ Citrate Synthesis



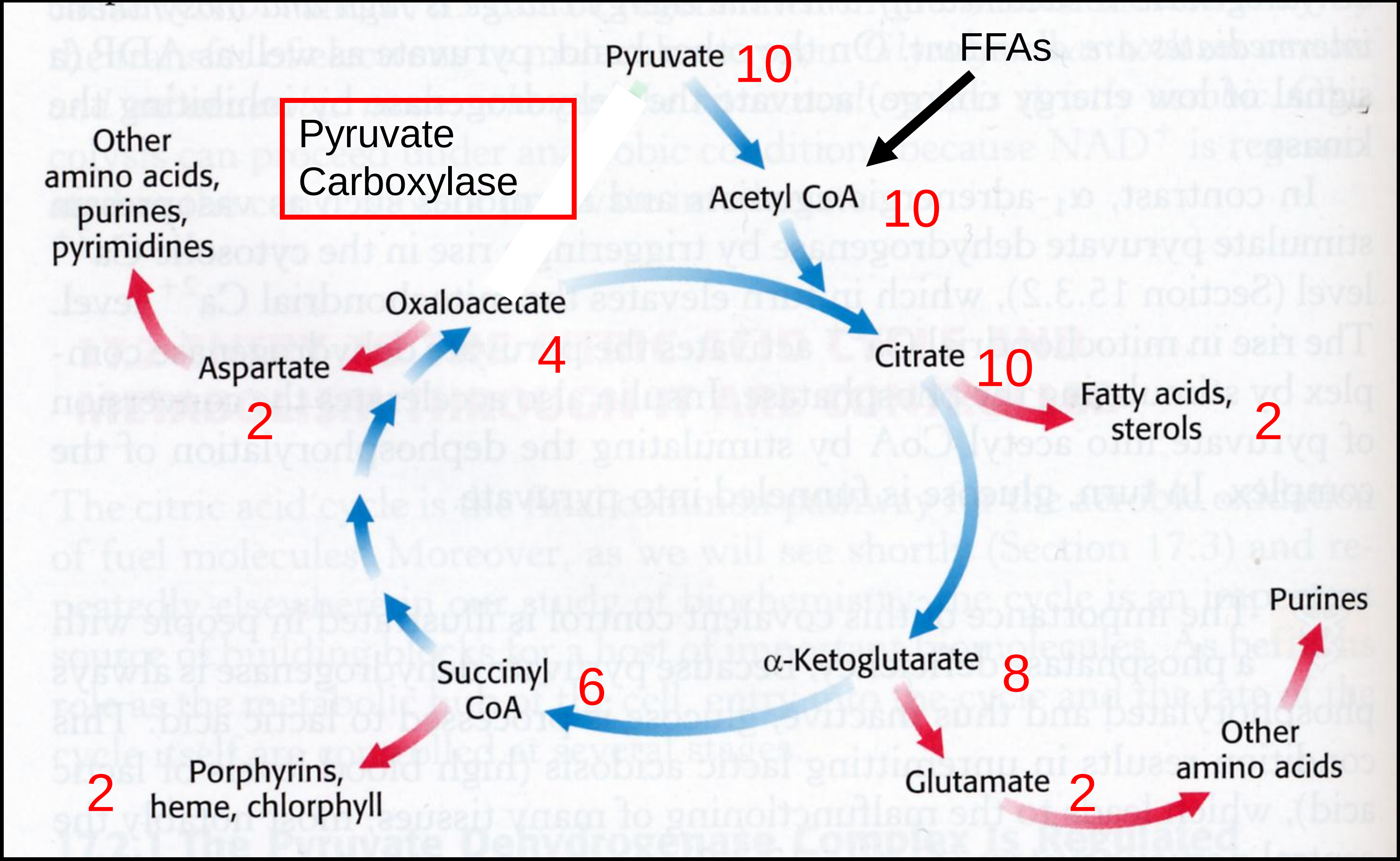
Pyruvate
CoASH + NAD+
pyruvate dehydrogenase
CO2 + NADH
Acetyl-CoA
CoASH
1. citrate synthase
Citrate
2. aconitase
cis-Aconitate
2. aconitase
Isocitrate
3. isocitrate dehydrogenase
NAD+
NADH + H+
Oxalosuccinate
CO2
3. isocitrate dehydrogenase
α-Ketoglutarate
NAD+
NADH + H+
CoASH
4. α-ketoglutarate dehydrogenase
*CO2
Succinyl-CoA
GDP + Pi
GTP
CoASH
5. succinyl-CoA synthetase
Succinate
FAD
FADH2
6. succinate dehydrogenase
Fumarate
7. fumarase
L-Malate
8. malate dehydrogenase
NAD+
NADH + H+
Oxaloacetate
↑NADH

Product Inhibition vs. Reaction Reversal

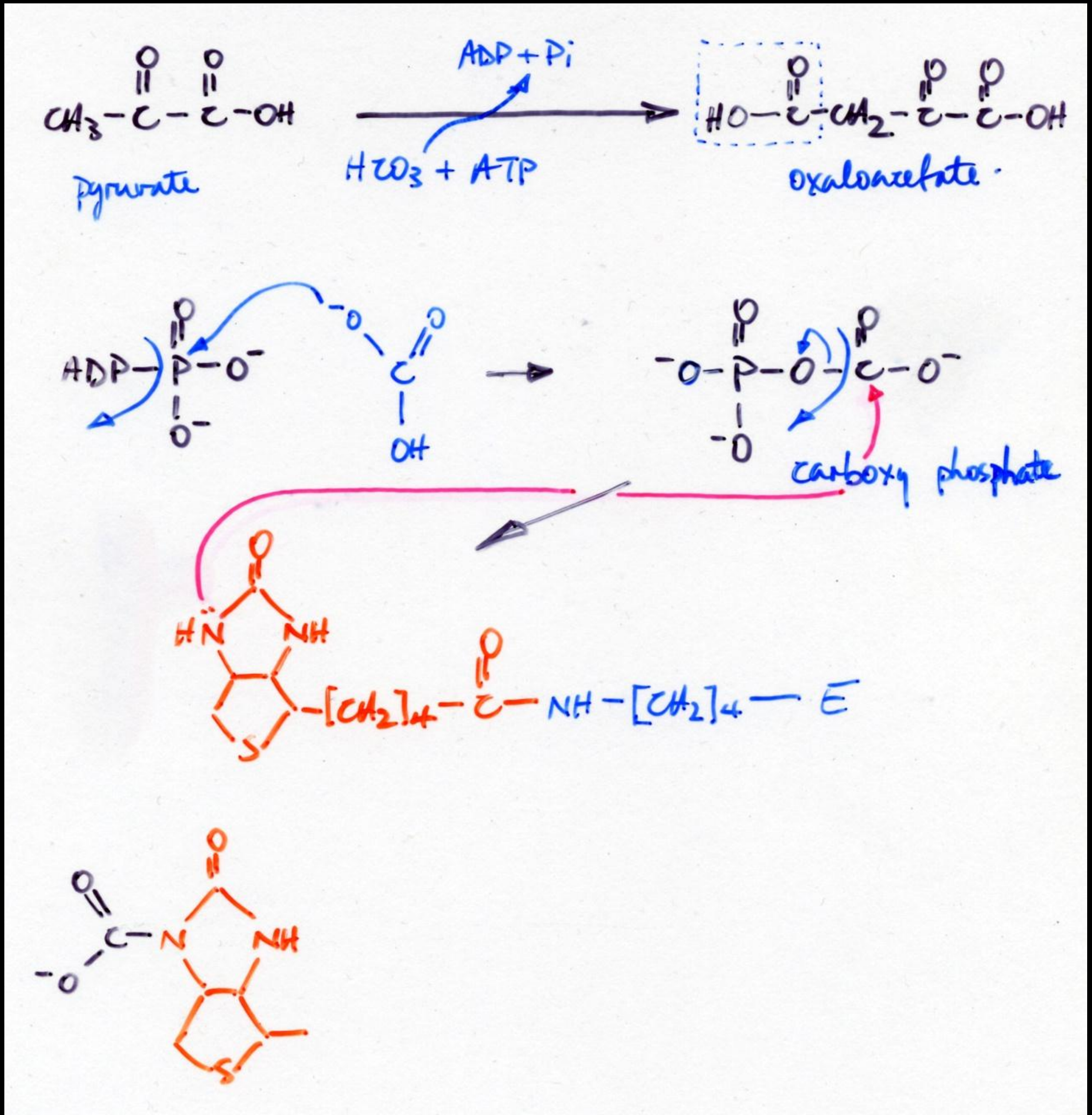


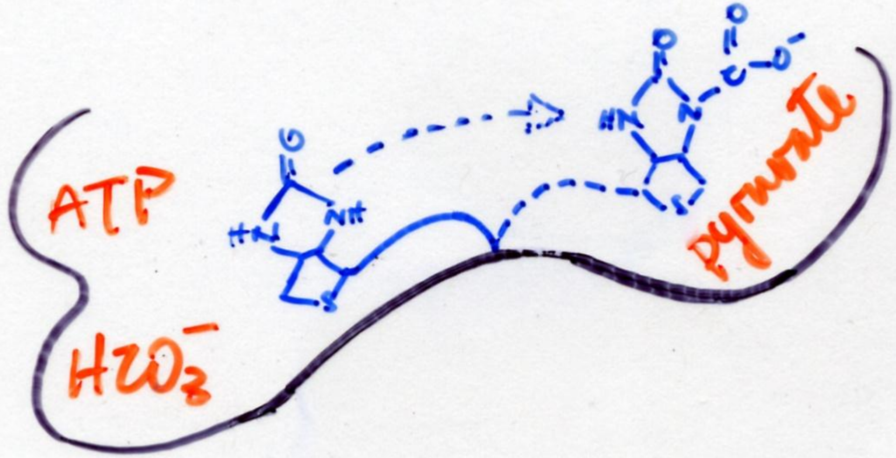
Two conditions for reaction reversal:
1. Both products must be present
2. Must be thermodynamically possible

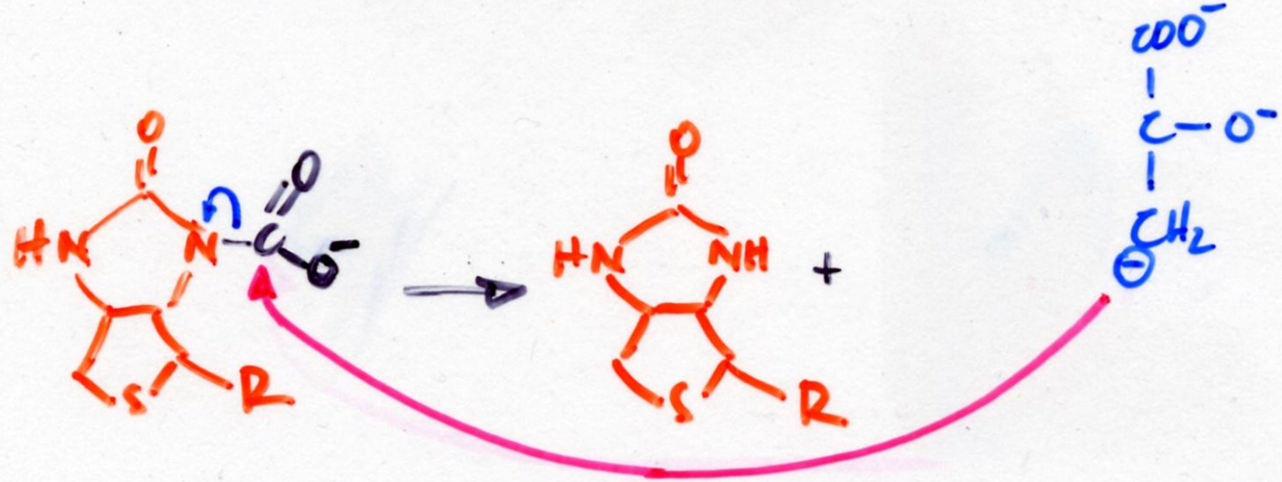
Anaplerotic Reactions

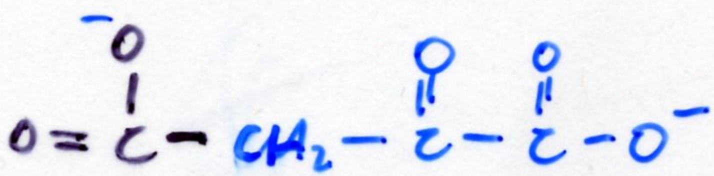


Mechanism of Pyruvate Carboxylase









oxaloacetate.

Coordinate Regulation of Pyruvate Metabolism

FFAs