Krebs cycle (tricarboxylic acid cycle; TCA)

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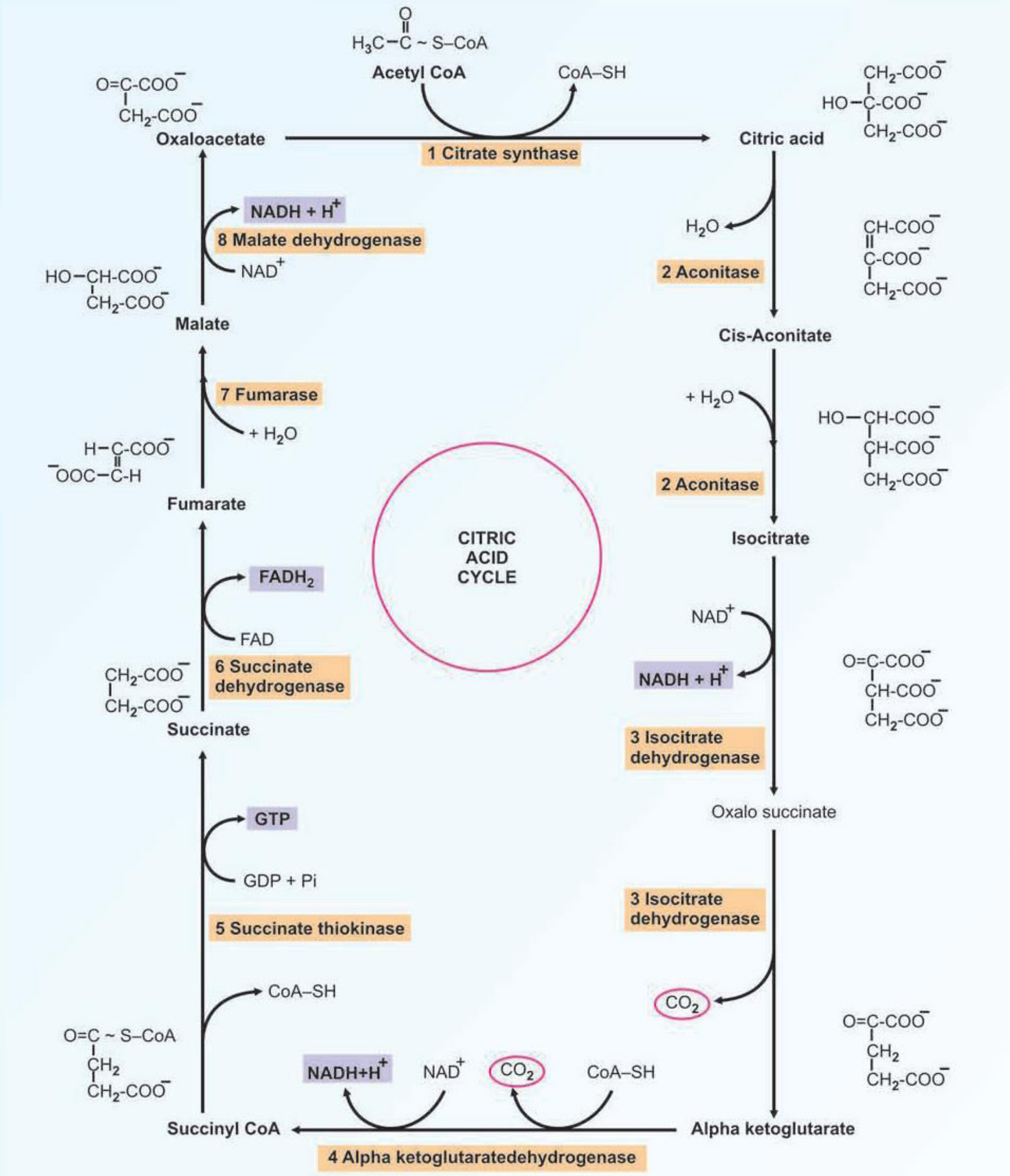
Introduction

- **Name:** tri-carboxylic acid cycle (TAC), Krebs cycle, or citric acid cycle
- It is the major common pathway of oxidation of CHO, lipids & protein (they all yield acetyl coA)
    - Generates 12 ATP (old) or 10 ATP (new) + 2 CO_2
- **Site:** totally in mitochondria & generates high amount of energy → ***mitochondria is power house of cell***
- ***To start, you need one molecule of acetyl coA & one molecule of oxaloacetate***



Acetyl CoA (2 carbon), enters the cycle. These are released as CO_2 in steps 3 and 4. So Acetyl CoA is completely oxidised by the time cycle reaches alpha ketoglutarate.

All reactions are readily reversible; except 1st and 4th steps

End result:

2 carbons from acetyl coA leave as 2 x CO2

Energy captured:

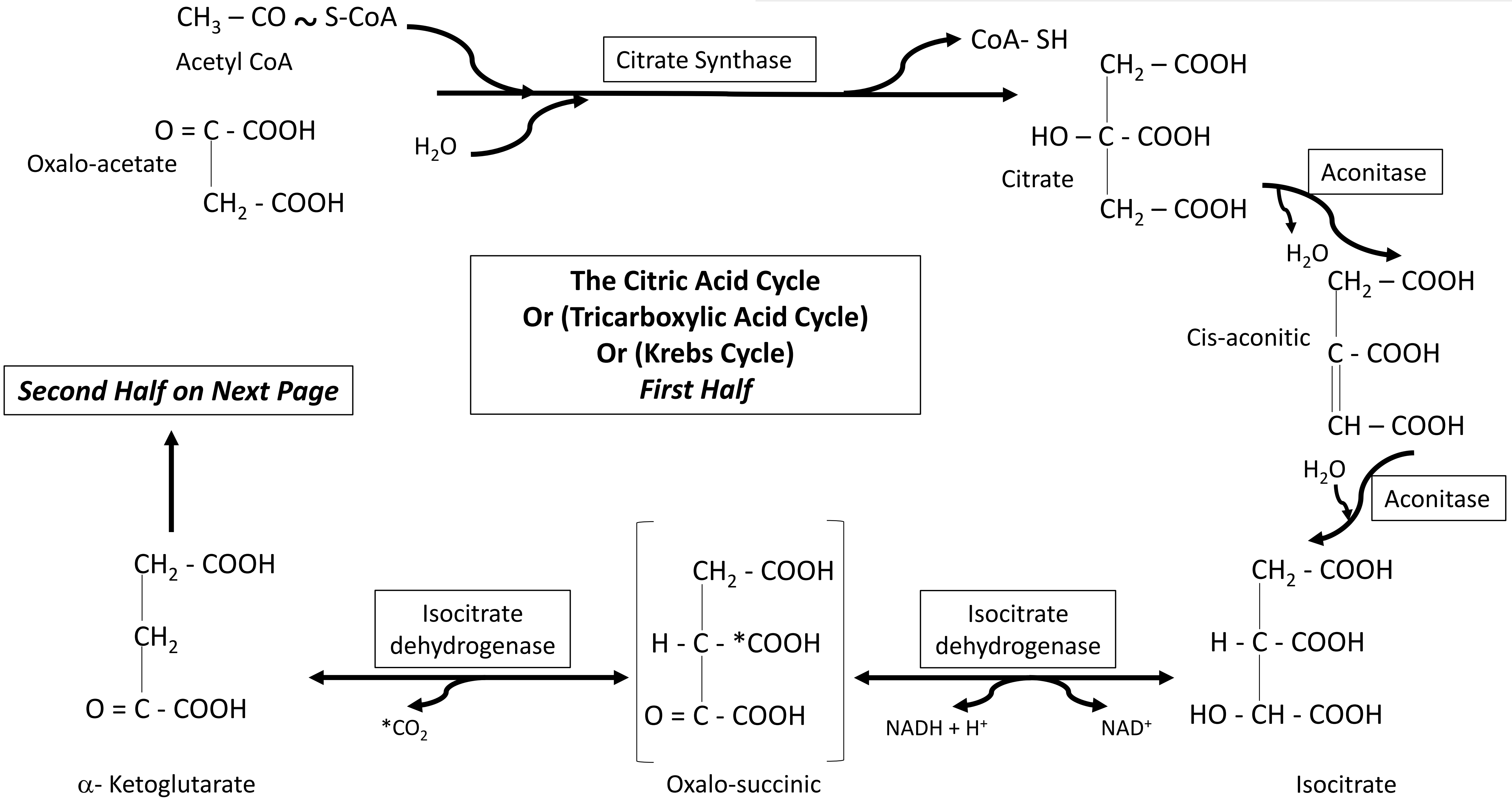
1 x GTP

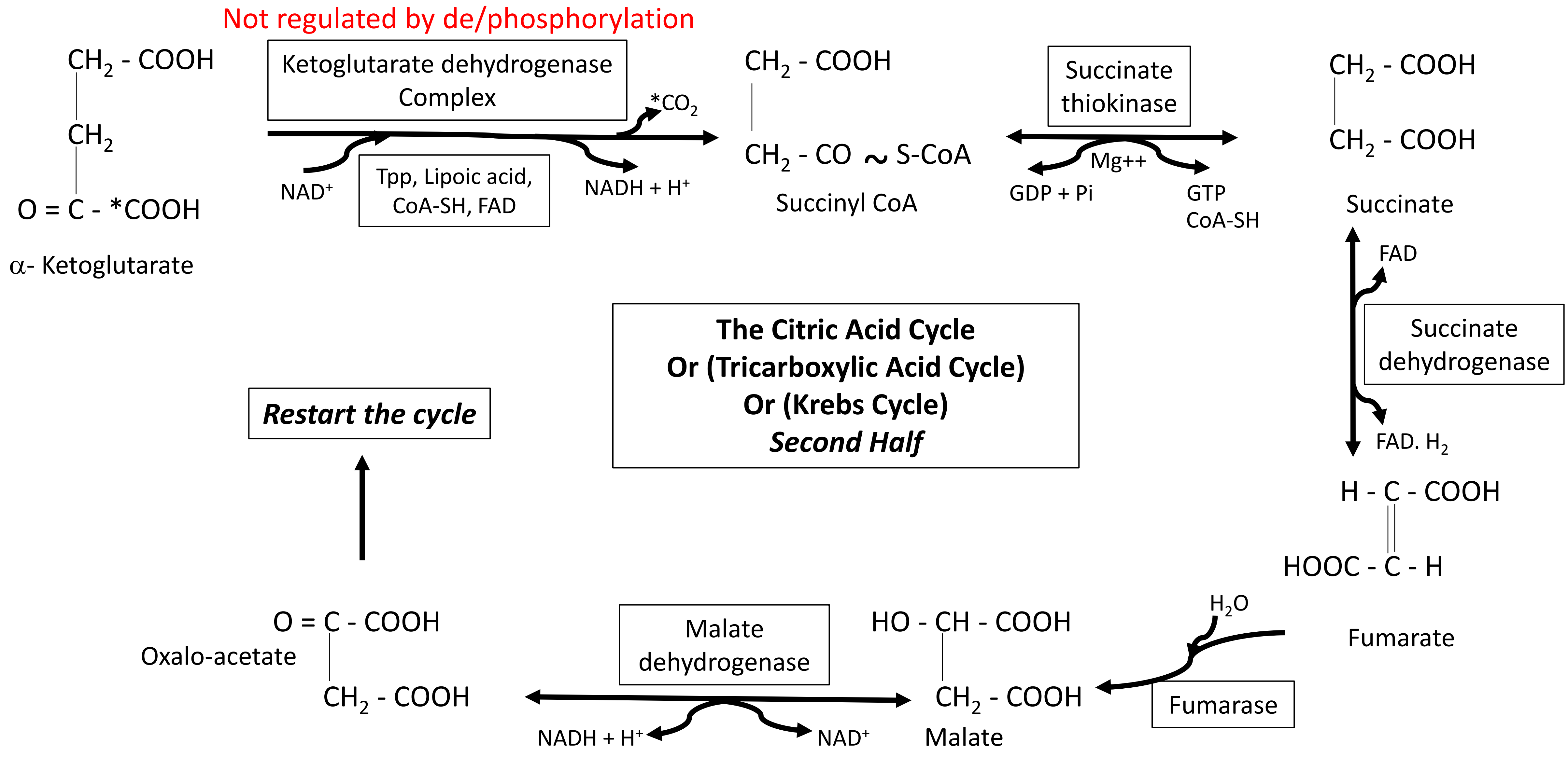
3 x NADH+H

1 x FADH2

Reversible vs irreversible reactions: look in upcoming slides

The Citric Acid Cycle
Or (Tricarboxylic Acid Cycle)
Or (Krebs Cycle)
First Half





Reversible vs irreversible reactions: look in upcoming slides

Pathway	Step	Enzyme	Source	Method of ATP formation	No of ATPs gained per glucose (new calculation)		No of ATPs as per old calculation
Glycolysis	1	Hexokinase	-		Minus	1	Minus 1
Do	3	Phospho-fructokinase	-		Minus	1	Minus 1
Do	5	Glyceralde-hyde-3-P DH	NADH	Respiratory chain	2.5 x 2 =	5	3 x 2 = 6
Do	6	1,3-BPG kinase	ATP	Substrate level	1 x 2 =	2	1 x 2 = 2
Do	9	Pyruvate kinase	ATP	Substrate level	1 x 2 =	2	1 x 2 = 2
Pyruvate to Acetyl CoA	-	Pyruvate dehydrogenase	NADH	Respiratory chain	2.5 x 2 =	5	3 x 2 = 6
TCA cycle	3	Isocitrate DH	NADH	Respiratory chain	2.5 x 2 =	5	3 x 2 = 6
Do	4	alpha keto glutarate DH	NADH	Respiratory chain	2.5 x 2 =	5	3 x 2 = 6
Do	5	Succinate thiokinase	GTP	Substrate level	I x 2 =	2	I x 2 = 2
Do	6	Succinate DH	$FADH_2$	Respiratory chain	1.5 x 2 =	3	2 x 2 = 4
Do	8	Malate DH	NADH	Respiratory chain	2.5 x 2 =	5	3 x 2 = 6
Net generation in glycolytic pathway					9 minus 2=	7	10 minus 2= 8
Generation in pyruvate dehydrogenase reaction					=	5	= 6
Generation in citric acid cycle					=	20	= 24
Net generation of ATP from one glucose mol					=	32	= 38

1.5 x 2 for FADH2 if glycerol phosphate shuttle

Important facts of Krebs cycle

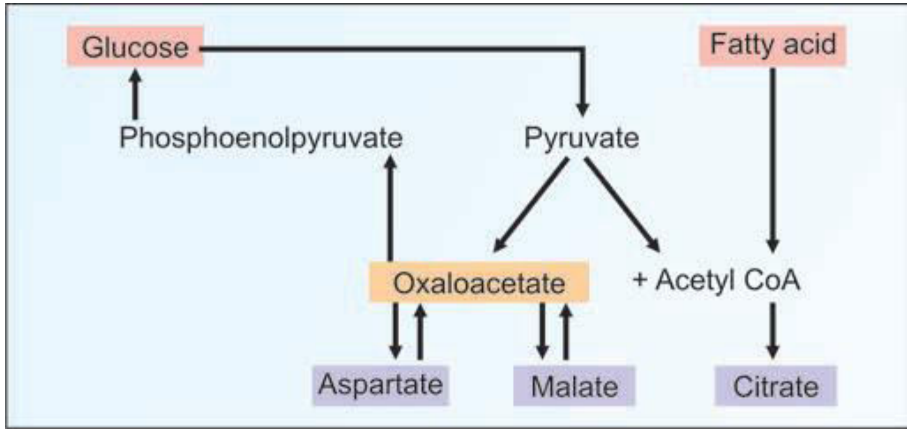
- Contrary to glycolysis, Krebs cycle can only happen under aerobic conditions

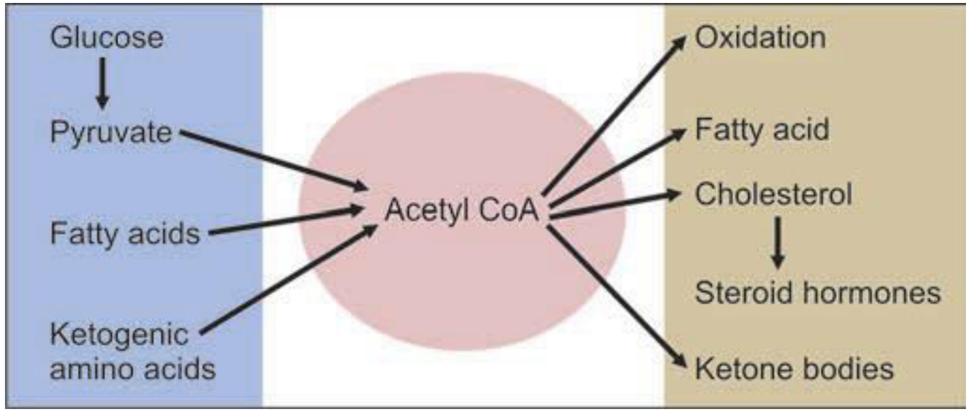
- Enzymes of TCA are found in the mitochondrial matrix, in close proximity to the enzymes of the respiratory chain

- Different isocitrate dehydrogenases are seen (isoenzymes)
  - NAD+ specific in mitochondria
  - NADP+ specific in cytoplasm

- **<u>Alpha ketoglutarate dehydrogenase is irreversible step</u>**
  - Citrate synthase is irreversible but body can reverse it via ATP-citrate lyase
  - IDH step of the citric acid cycle is often (**<u>but not always</u>**) an irreversible reaction due to its large negative change in free energy

Oxaloacetate as a “catalyst” and junction point of metabolism

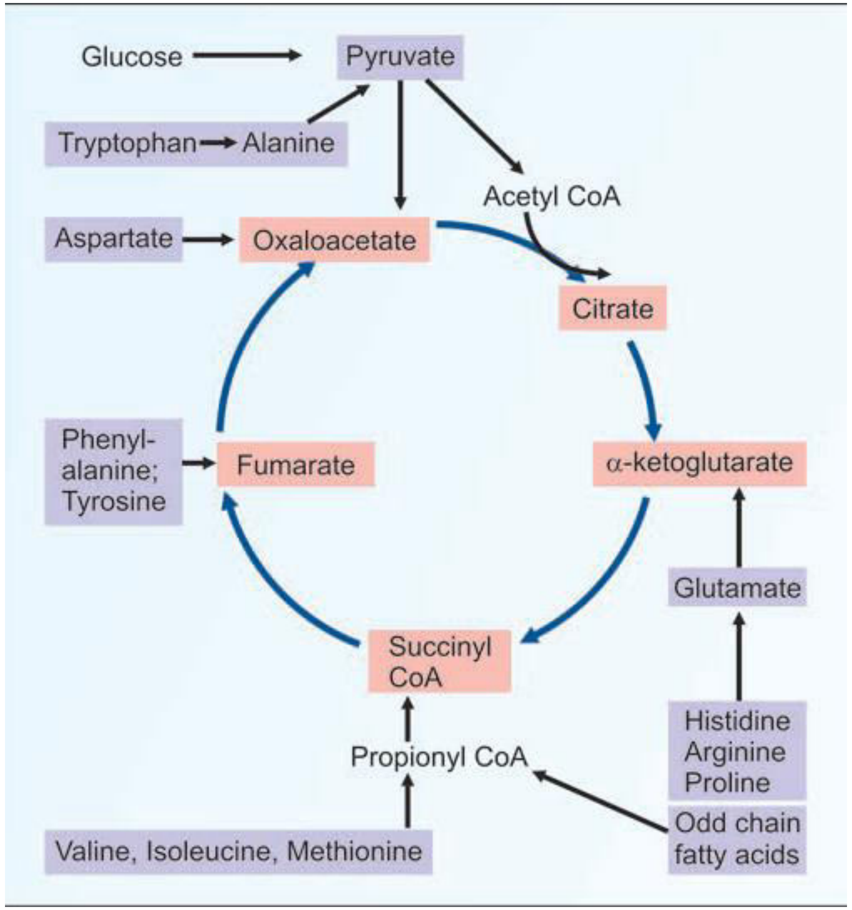




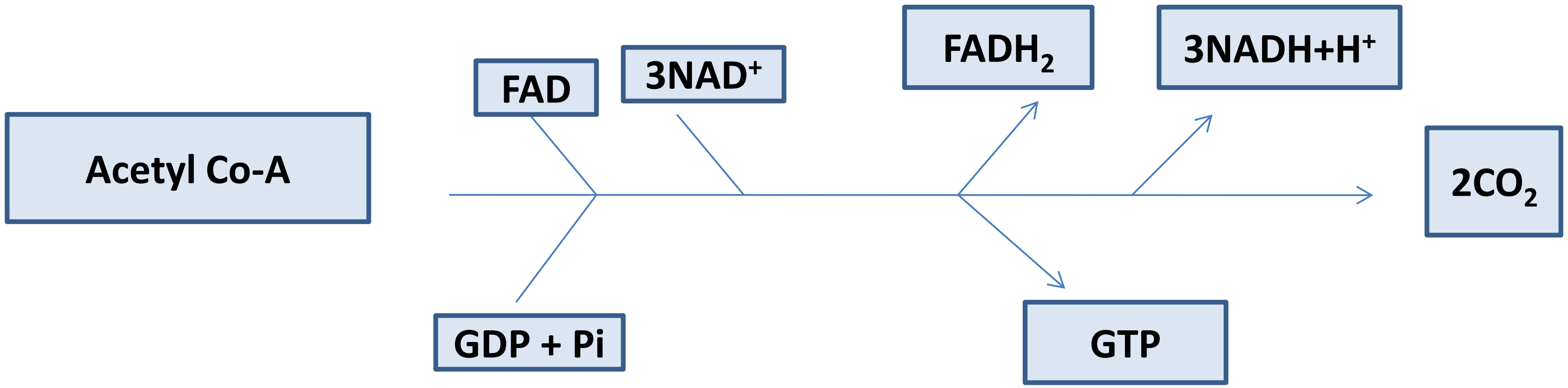
Sources and utilization of acetyl coA

Significance/ importance of Krebs cycle (1 of 2)

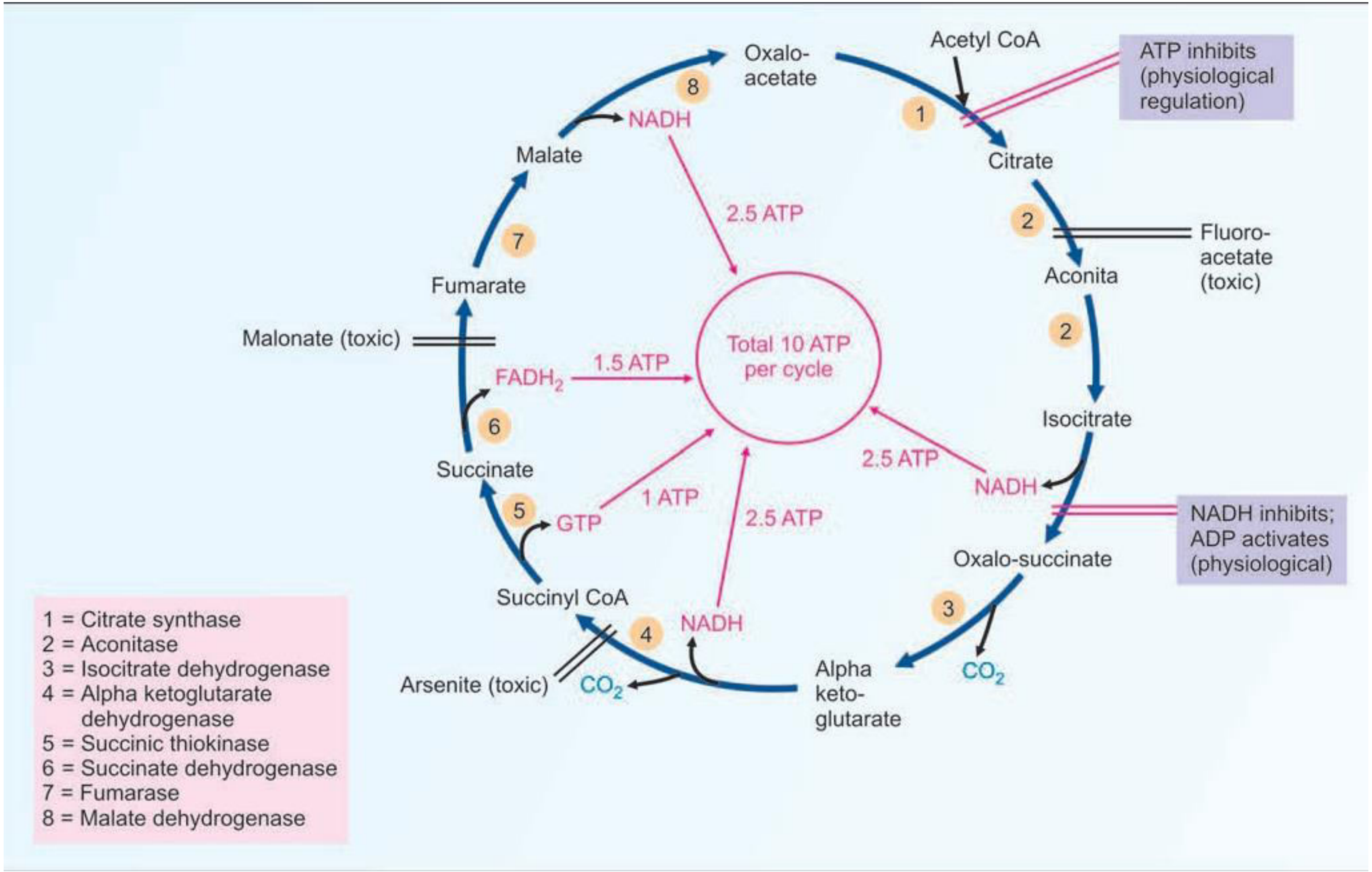
1. Complete oxidation of acetyl CoA
2. ATP generation
3. Final common oxidative pathway
4. Integration of major metabolic pathways
5. Fat is burned on the wick of carbohydrates



- The overall reaction for one turn of the TCA cycle is:



ENERGY YIELD OF KREBS CYCLE (NEW system):



- **ENERGY YIELD OF KREBS CYCLE (old system):**
- 3 ATP in step 4 from oxidation of NADH.
- 3 ATP in step 6 from oxidation of NADH.
- 1 GTP in step 7 substrate level.
- 2 ATP in step 8 from oxidation of FADH2.
- 3 ATP in step 10 from oxidation of NADH.
- So, total yield **12 ATPs**: 11 ATPs from respiratory chain & one ATP from substrate level.

Table 18.1. ATP generation steps

Step no.	Reactions	Co-enzyme	ATPs (old-calculation)	ATPs (new calculation)
3	Isocitrate → alpha ketoglutarate	NADH	3	2.5
4	Alpha ketoglutarate → succinyl CoA	NADH	3	2.5
5	Succinyl CoA → Succinate	GTP	1	1
6	Succinate → Fumarate	$FADH_2$	2	1.5
8	Malate → Oxaloacetate	NADH	3	2.5
		Total	12	10

Don’t worry about numbering of reactions as numbers vary from author to author; imp thing is to know the NAME of reaction where NADH+H… is produced

Significance/ importance of Krebs cycle (2 of 2)

6. Excess carbohydrates are converted as neutral fat
7. No net synthesis of carbohydrates from fat
8. Carbon skeletons of amino acids finally enter the citric acid cycle
9. Amphibolic pathway
10. Anaplerotic role

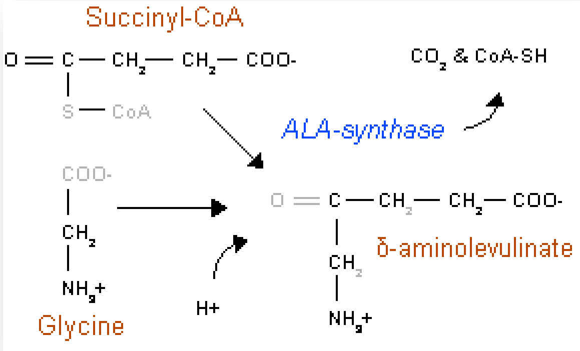
Amphibolic aspects of Krebs cycle (serve as catabolic and anabolic pathway)

Catabolic role

Anabolic role:

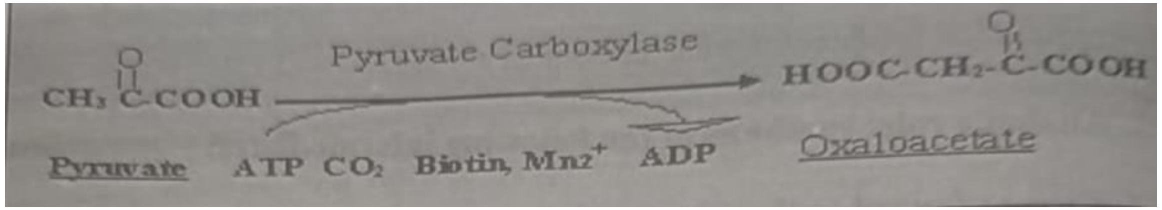
- Citrate → ***acetyl coA*** + oxaloacetate (**citrate lyase** in cytoplasm) → FA synthesis
- Non essential amino acids

- Aminolevulinate required in heme synthesis
- Gluconeogenesis

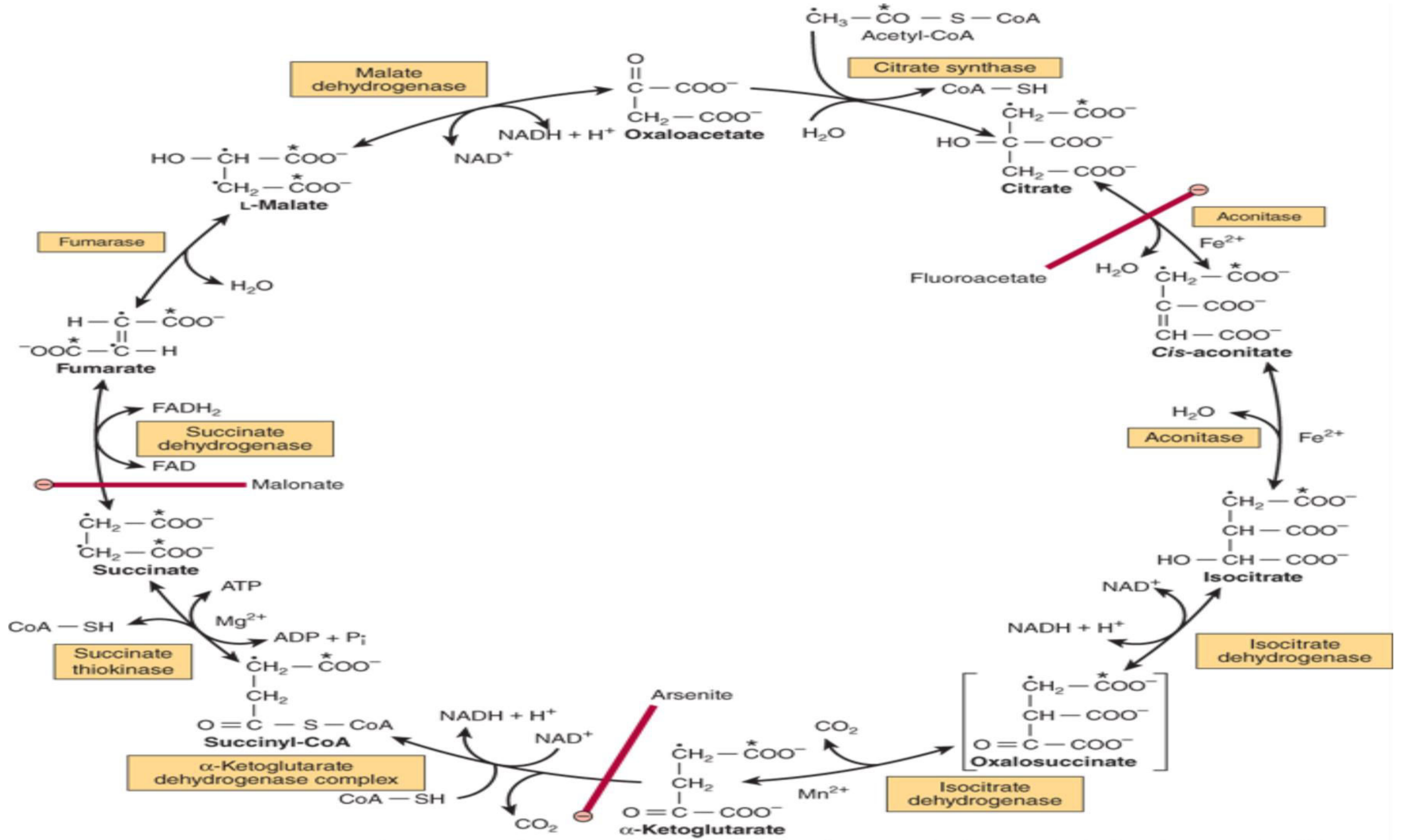


Anaploretic reactions to replenish Krebs cycle intermediates:

- Carboxylation of pyruvate to oxaloacetate



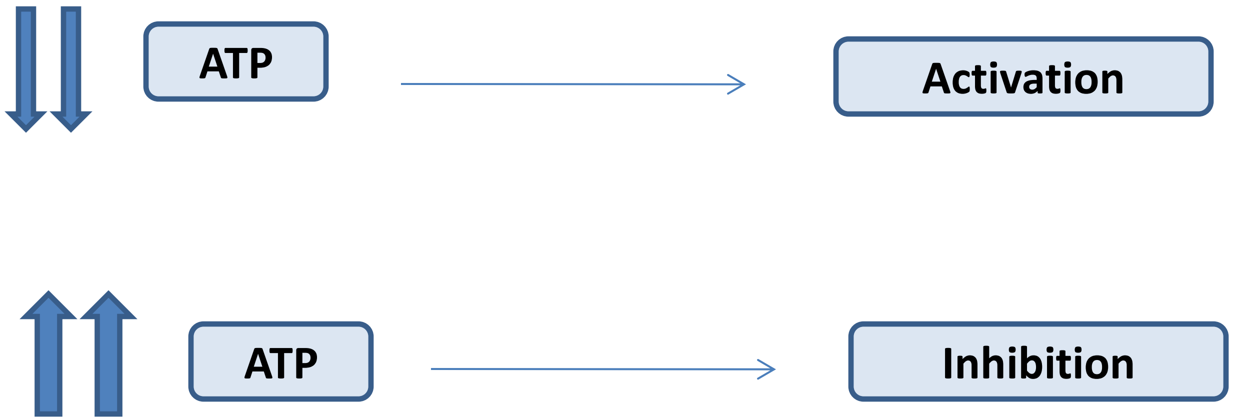
- Transamination reactions



Source: V. W. Rodwell, D. A. Bender, K. M. Botham, P. J. Kennelly, P. A. Weil: Harper's Illustrated Biochemistry, 13th Edition
www.accessmedicine.com

Regulation of Krebs cycle :

- The main function of Krebs cycle is ATP production so:



↑ ATP

↑ NADH

-

-

Allosteric regulation

Citrate synthase

Isocitrate DH

α-ketoglutarate DH

+

+

↑ ADP
↑ AMP
↑ NAD

Rate of glycolysis and Krebs cycle are matched via:

- Both inhibited by high levels of ATP & NADH
- Citrate is an important allosteric inhibitor of ***phosphofructokinase 1***

Substrate availability is required for Krebs cycle:

- **NAD and FAD:** NADH+H and FADH2 must be re-oxidised via active respiratory chain
    - Active respiratory chain needs high oxygen and high ADP concentrations

- **Acetyl coA** (via glucose oxidation, FA & KB oxidation and catabolism of ketogenic AA)

- **Oxaloacetate** (from malate, aspartate and pyruvate)

Table 18.2. Metabolic defects of oxidative metabolism

Enzymes	Reactions catalyzed	Abnormalities
Pyruvate dehydro-genase	Pyruvate → acetyl CoA	Lactic acidosis Neurological disorders
Acyl CoA-dehydro-genase	Fatty acyl CoA → alpha, beta-unsaturated fatty acyl CoA	Organic aciduria, glutaric aciduria, acidosis, hypoglycemia Electron flow from FAD → CoQ affected
Pyruvate carboxylase	Pyruvate → Oxaloacetate	Oxaloacetate needed for sparking TCA cycle is deficient. Lactic acidosis, hyperammonemia and hyperalaninemia