CREB'S CYCLE

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Polymer

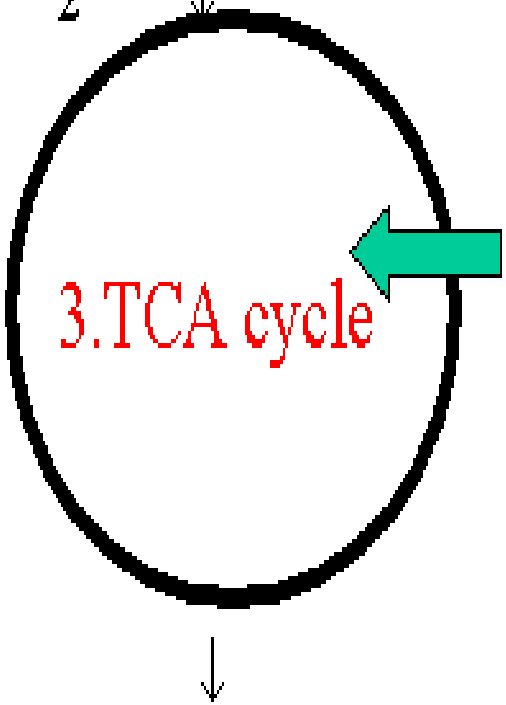
1 ↓

Monomer

2 ↓

Respiration

- 4 stages



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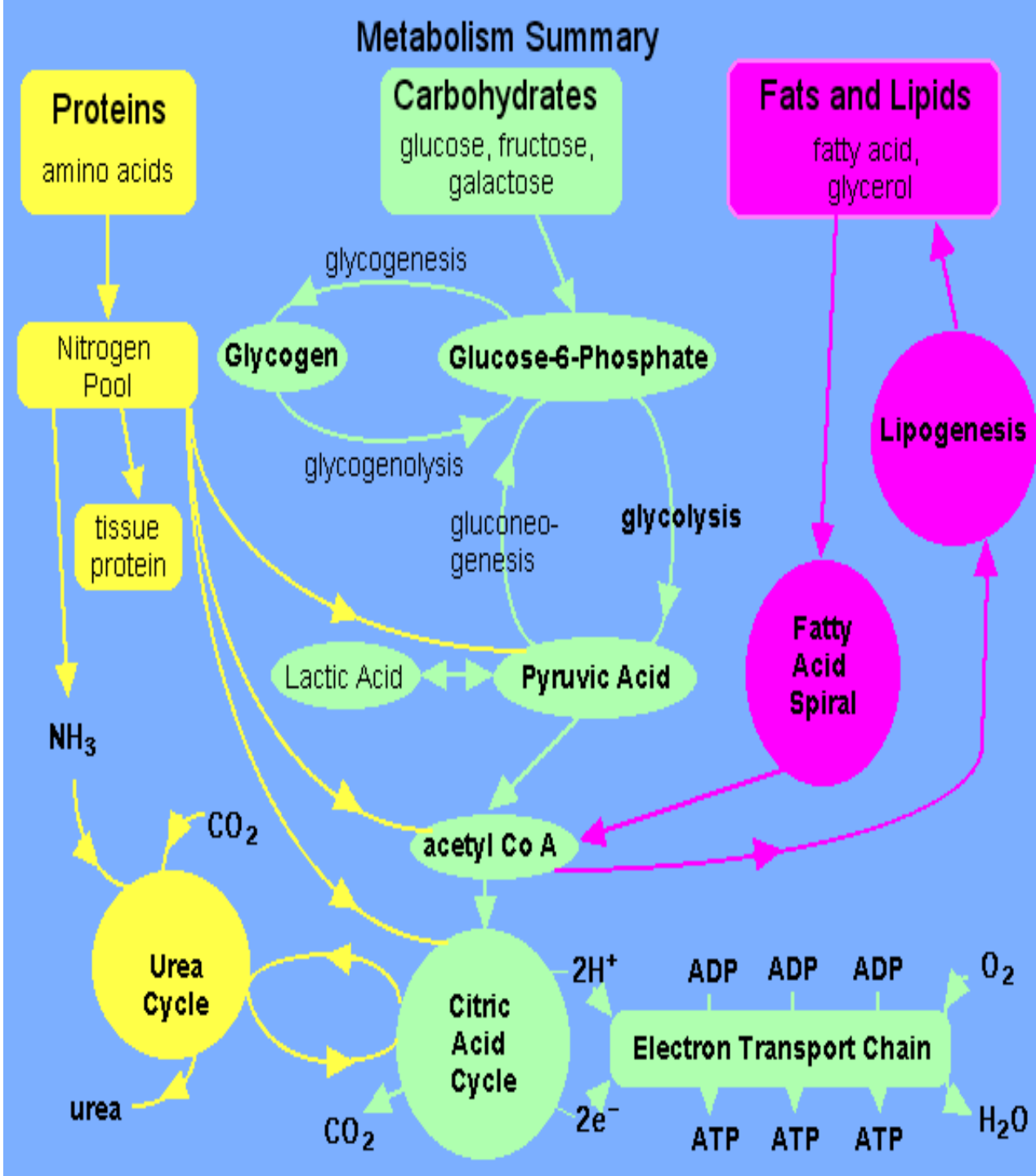
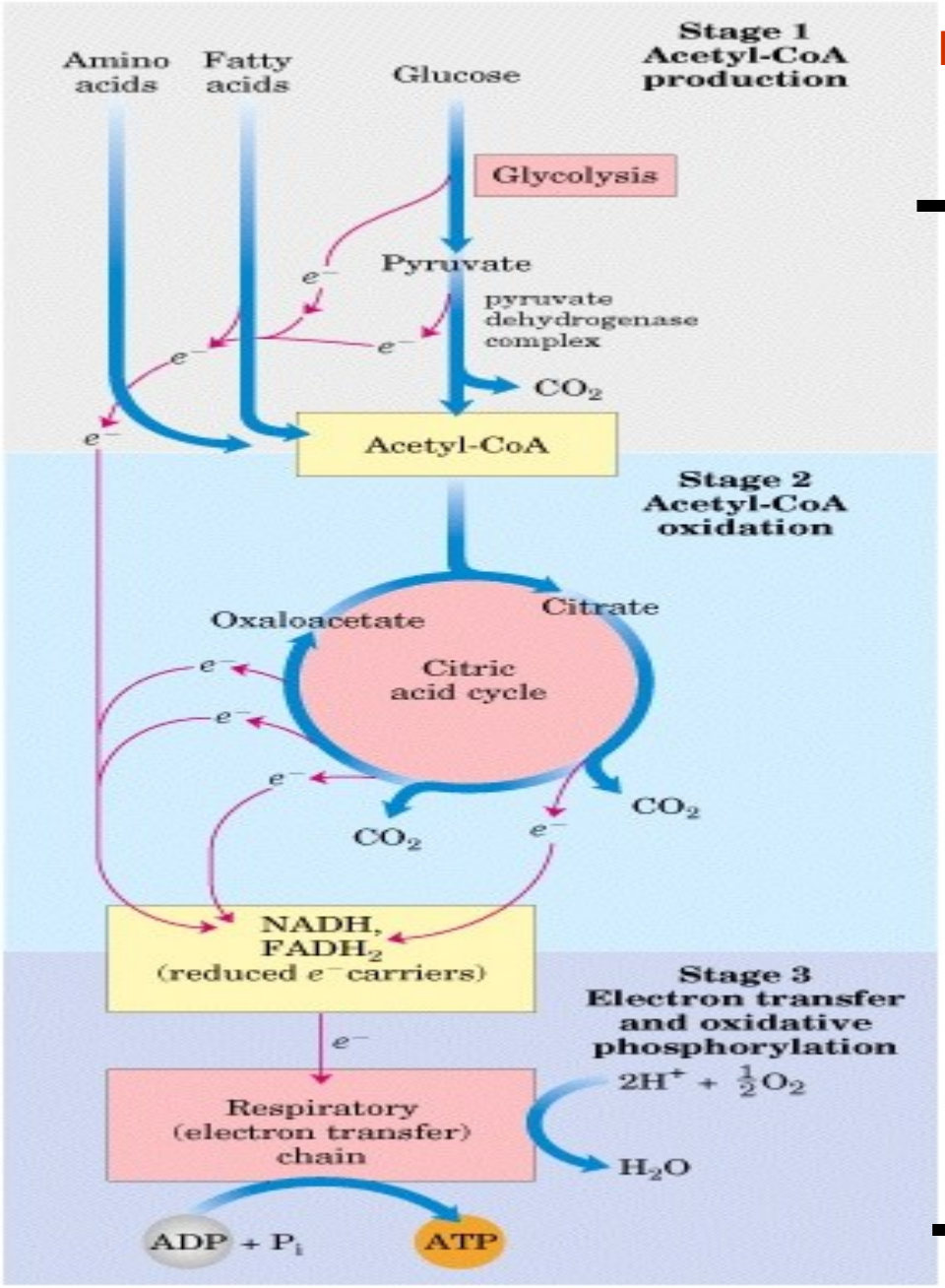
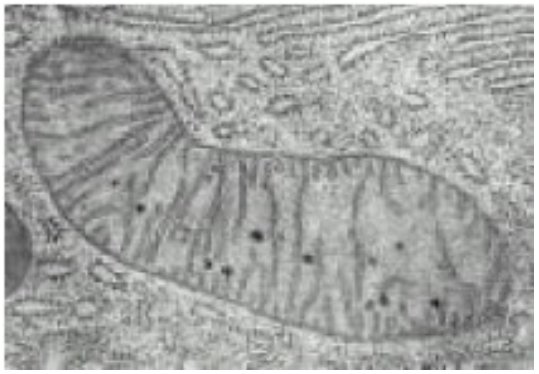
4.Electron Transport System





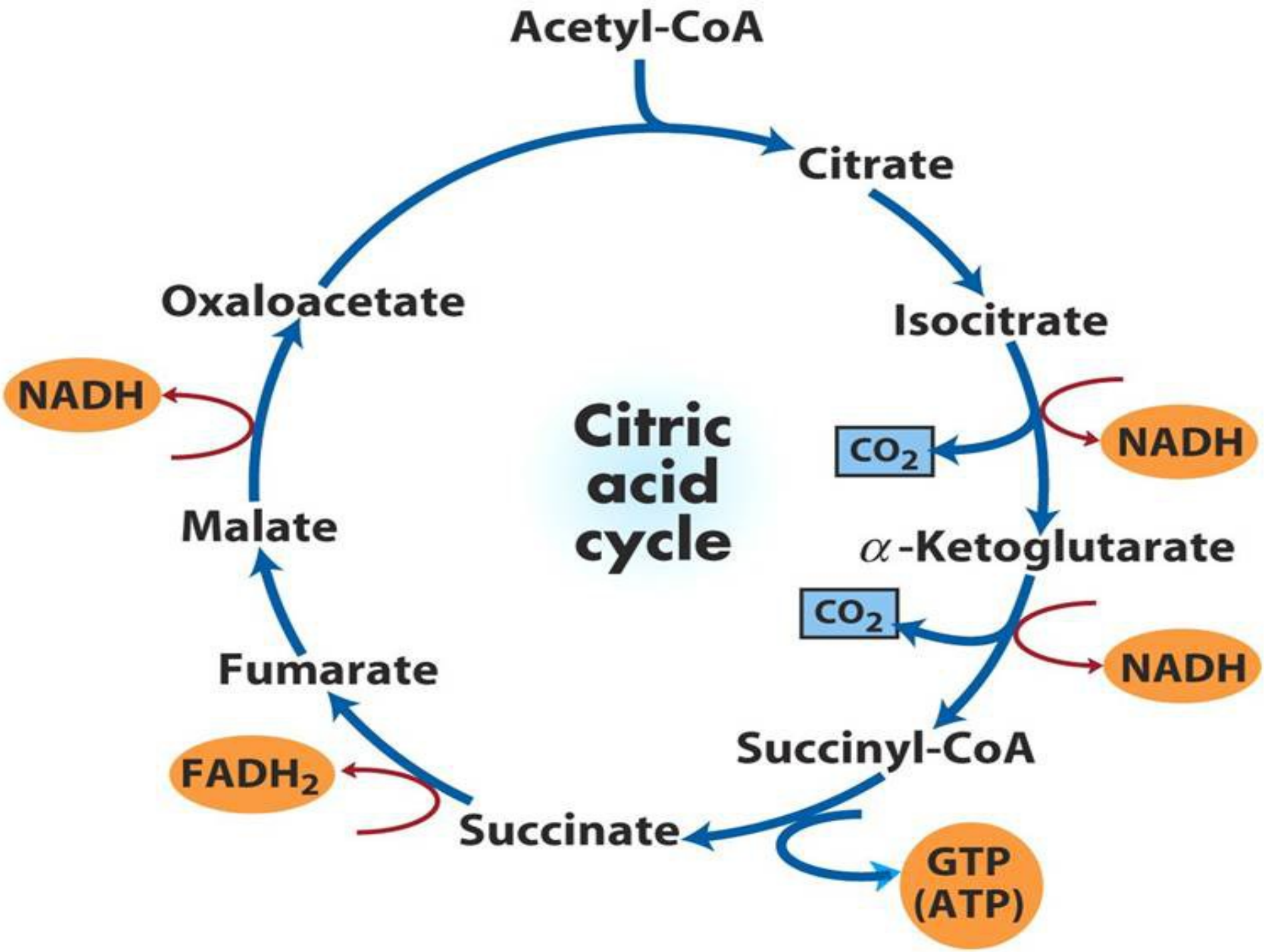
In Cytosol

In Mitochondria

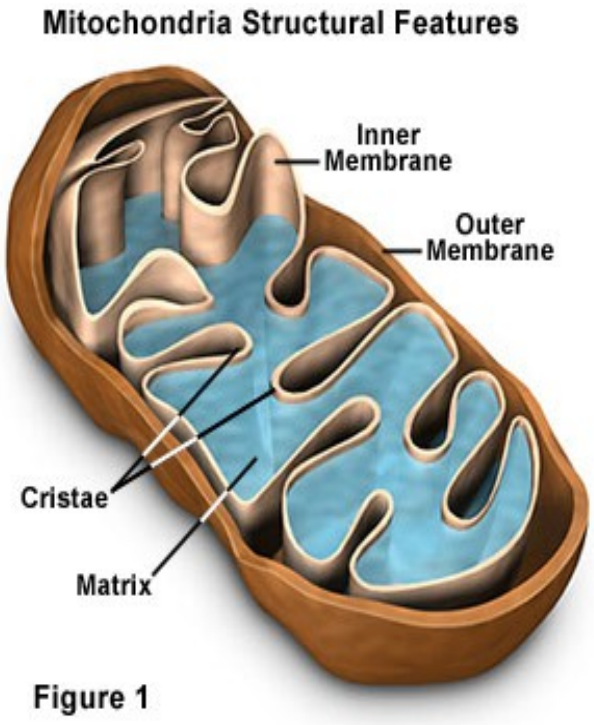


Overall goal

- Makes ATP
- Makes NADH
- Makes $FADH_2$
- Requires some carbohydrate to run


Acetyl-CoA
Citrate
Isocitrate
NADH
CO_2
α-Ketoglutarate
CO_2
NADH
Succinyl-CoA
GTP
(ATP)
Succinate
$FADH_2$
Fumarate
Malate
NADH
Oxaloacetate
Citric
acid
cycle

The **Krebs Cycle** is the **Third Stage** of Aerobic Respiration



Products of the **Krebs Cycle** enter the **Final Stage** of **Aerobic Respiration**

reused **released**

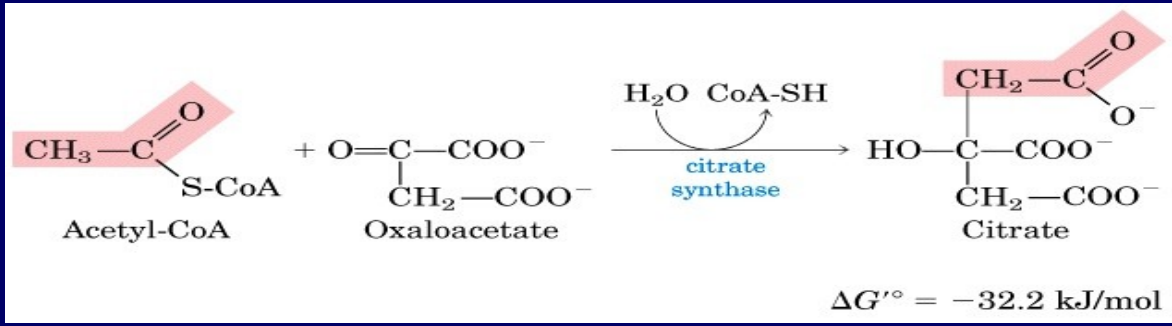
the final stage **oxidative phosphorylation**

Krebs Cycle (KC)

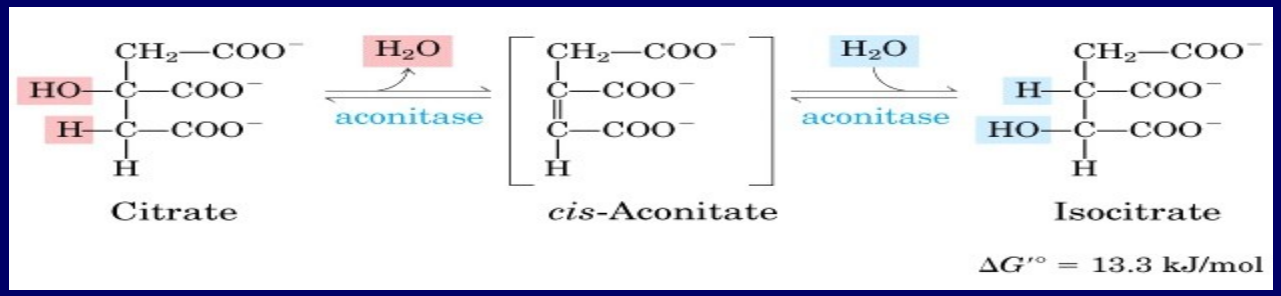
- Also known as tricarboxylic acid (TCA) cycle, or citric acid cycle (TCA)
- Common final degradative pathway for breakdown of monomers of CHO, fat and protein to CO_2 and H_20
  - Electrons removed from acetyl groups and attached to NAD^+ and FAD
  - Small amount of ATP produced from substrate level phosphorylation
- KC also provides intermediates for anabolic functions (eg gluconeogenesis)
- Under aerobic conditions, the pyruvate produced by glycolysis is oxidized to H_2O and CO_2 in a process called cellular respiration.
- The citric acid cycle is a hub in metabolism, with degradative pathways leading in and anabolic pathways leading out. It's also known as an amphibolic pathway because it operates both catabolically and anabolically.

Reactions of Citric Acid Cycle

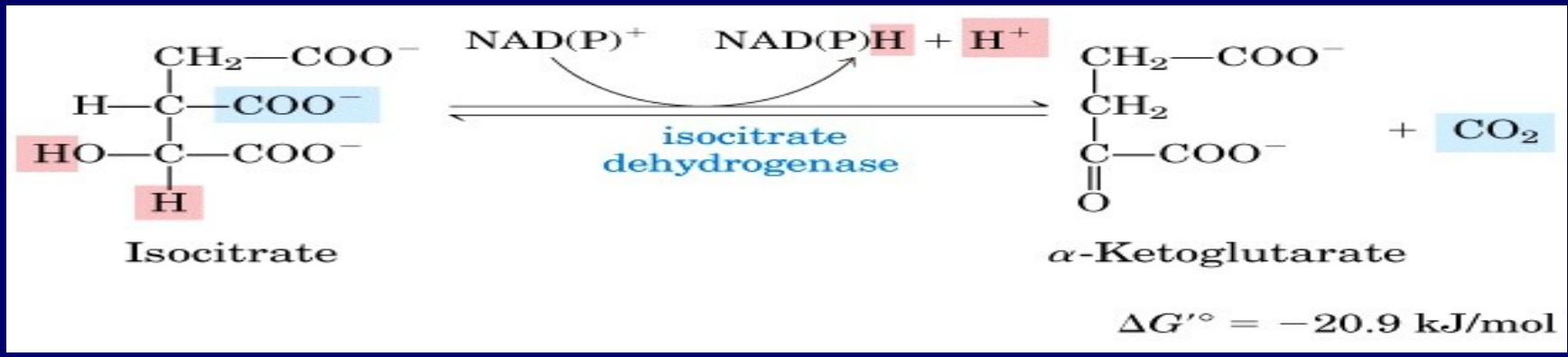
1. Citrate synthase: Formation of Citroyl CoA intermediate.
   Binding of Oxaloacetate to the enzyme results in conformational change which facilitates the binding of the next substrate, the acetyl Coenzyme A. There is a further conformational change which leads to formation of products. This mechanism of reaction is referred as induced fit model.



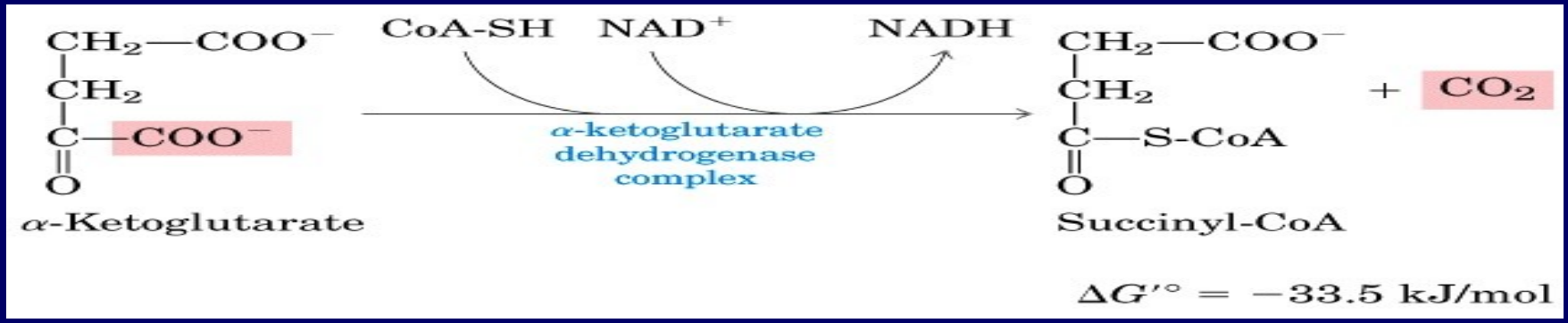
2. Aconitase: This enzyme catalyses the isomerization reaction by removing and then adding back the water (H and OH) to cis-aconitate in at different positions. Isocitrate is consumed rapidly by the next step thus deriving the reaction in forward direction.



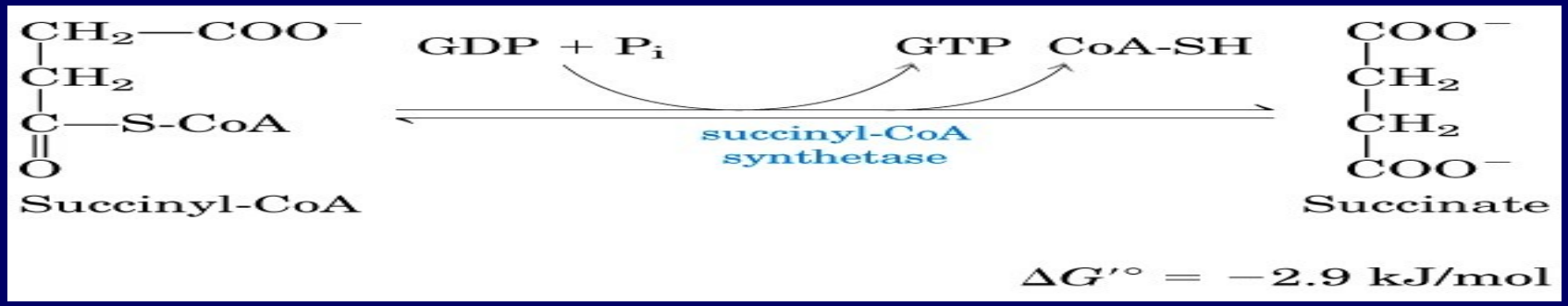
3. Isocitrate dehydrogenase: There are two isoforms of this enzyme, one uses NAD^+ and other uses $NADP^+$ as electron acceptor.



4. a-Ketoglutarate dehydrogenase: This is a complex of different enzymatic activities similar to the pyruvate dyhdogenase complex. It has the same mechanism of reaction with E1, E2 and E3 enzyme units. NAD+ is an electron acceptor.

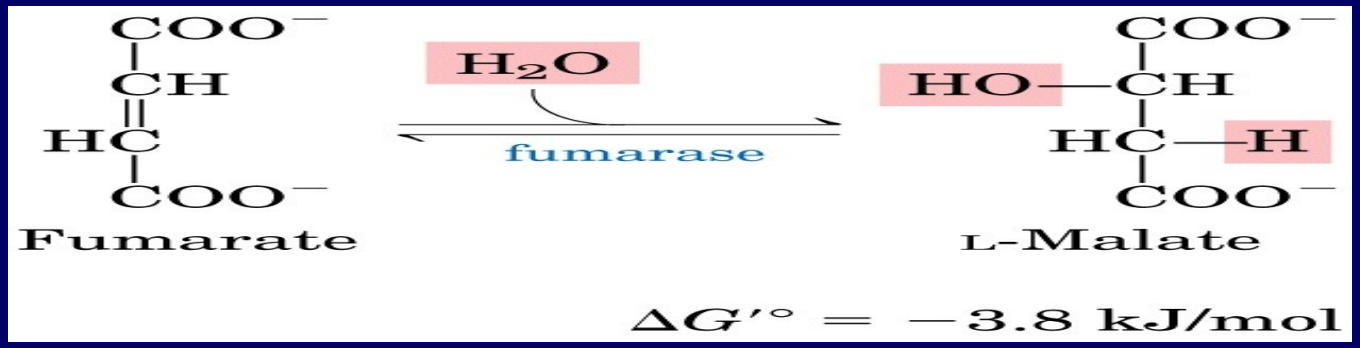


5. Succinyl CoA synthatse: Sccinyl CoA, like Acetyl CoA has a thioester bond with very negative free energy of hydrolysis. In this reaction, the hydrolysis of the thioester bond leads to the formation of phosphoester bond with inorganic phosphate. This phosphate is transferred to Histidine residue of the enzyme and this high energy, unstable phosphate is finally transferred to GDP resulting in the generation of GTP.

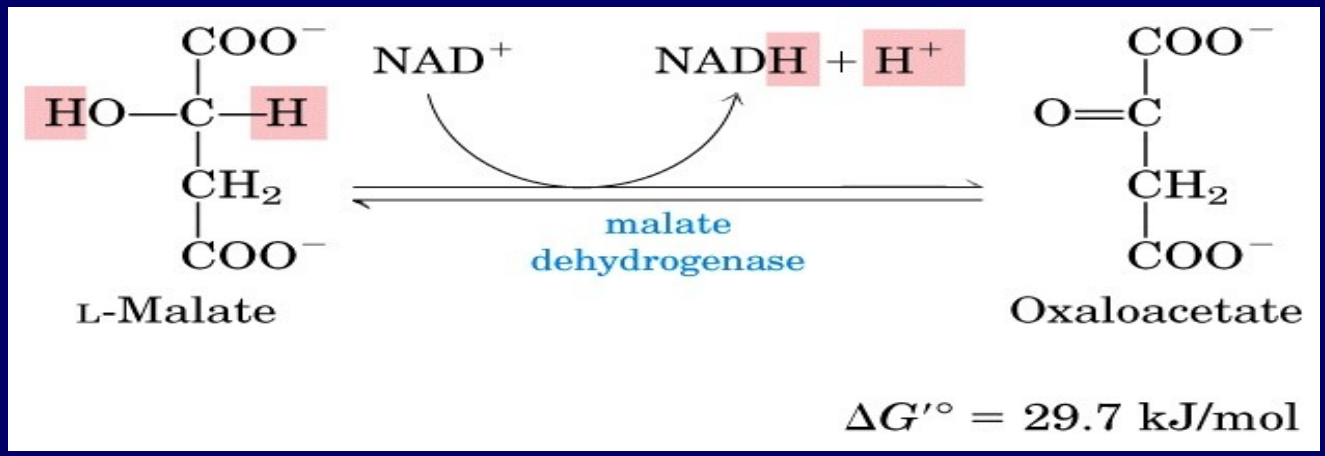


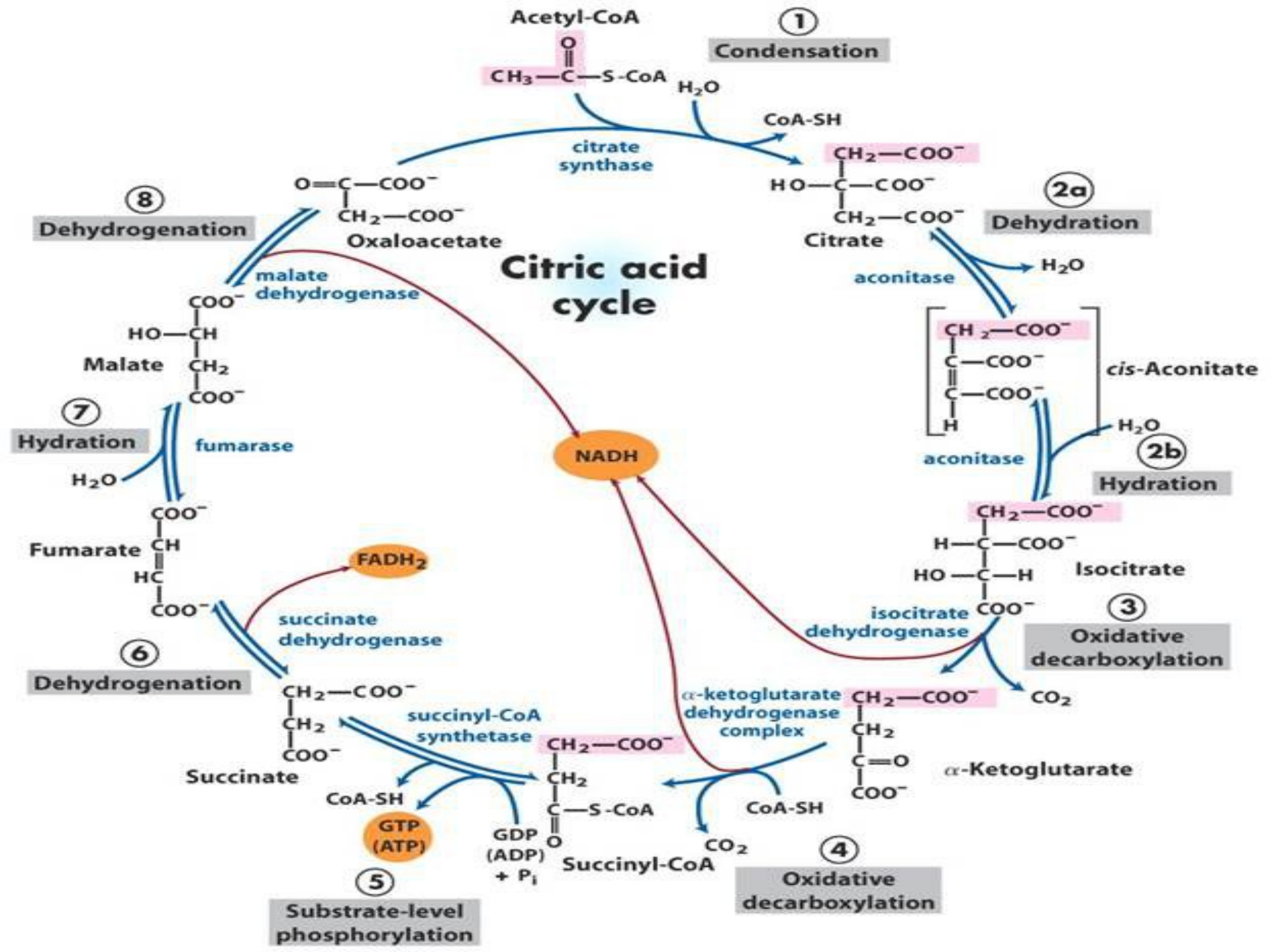
6. Succinate Dehydrogenase: Oxidation of succinate to fumarate. This is the only citric acid cycle enzyme that is tightly bound to the inner mitochondrial membrane. It is an FAD dependent enzyme. Malonate has similar structure to Succinate, and it competitively inhibits SDH.

7. **Fumarase**: Hydration of Fumarate to malate: It is a highly stereospecific enzyme. Cis-Maleate (the cis form of fumarate is not recognized by this enzyme.



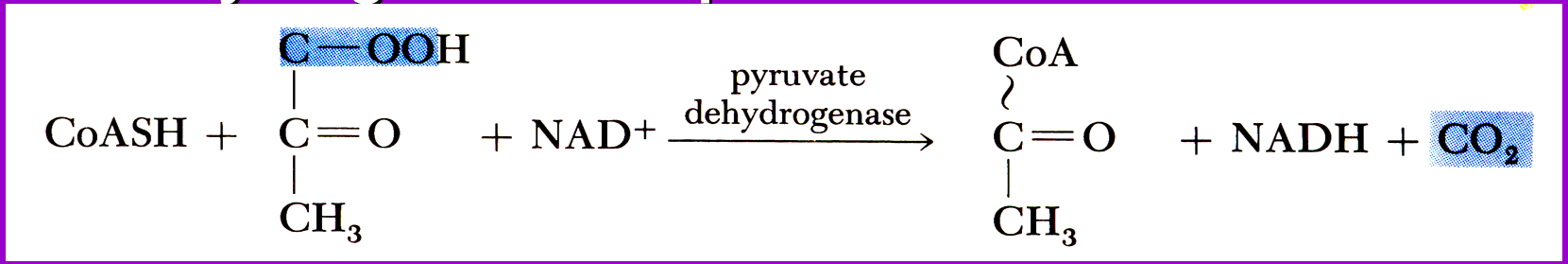
8. **L-Malate dehydrogenase**: Oxidation of malate to oxaloacetate: It is an NAD^+dependent enzyme. Reaction is pulled in forward direction by the next reaction (citrate synthase reaction) as the oxaloacetate is depleted at a very fast rate.




Citric acid cycle
Acetyl-CoA
1
Condensation
citrate synthase
CoA-SH
Citrate
2a
Dehydration
aconitase
cis-Aconitate
2b
Hydration
Isocitrate
3
Oxidative decarboxylation
isocitrate dehydrogenase
α-ketoglutarate dehydrogenase complex
α-Ketoglutarate
4
Oxidative decarboxylation
Succinyl-CoA
5
Substrate-level phosphorylation
GTP (ATP)
GDP (ADP) + Pi
succinyl-CoA synthetase
Succinate
6
Dehydrogenation
succinate dehydrogenase
FADH2
Fumarate
7
Hydration
fumarase
Malate
8
Dehydrogenation
malate dehydrogenase
Oxaloacetate
NADH
H2O
CO2

Pyruvate → Acetyl CoA

- **Pyruvate produced in cytosol and transported into mitochondria**
  - → 2 per glucose (all of Kreb's)
  - → Oxidative decarboxylation
  - → Makes NADH and -33.4kJ
- **Cannot directly enter KC**
  - **First converted to acetyl CoA by pyruvate dehydrogenase complex**



From: Summerlin LR (1981) Chemistry for the Life Sciences. New York: Random House p 548.

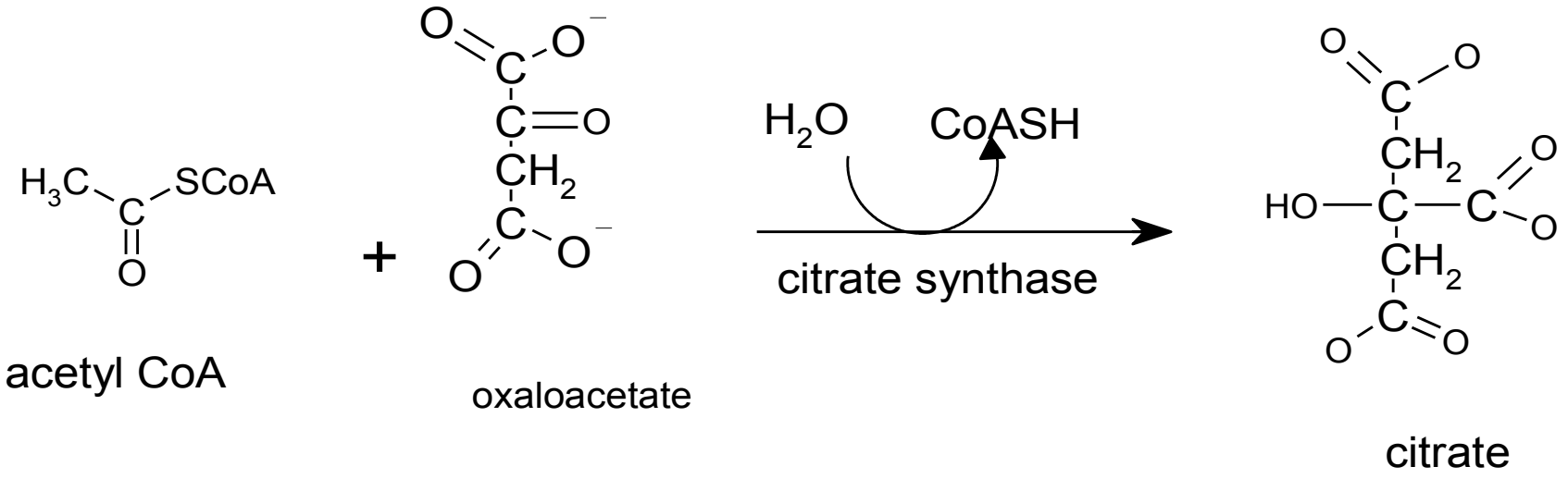
Regulation of Pyruvate → Acetyl CoA

- PDH reaction regulated to spare pyruvate from being irreversibly lost
  - Glucose important for brain and once converted to Acetyl CoA cannot be used for glucose synthesis
- PDH regulated by phosphorylation and allosteric control
  - Dephosphorylation activates PDH
    - Phosphatase enzyme activated by high $Ca2^{+}$
  - Phosphorylation inactivates PDH
    - Kinase activated by acetyl CoA and NADH
  - PDH allosterically inhibited by:
    - ATP
    - Acetyl CoA
    - NADH

Citrate synthase mechanism

Citrate synthase catalyzes the aldol condensation of oxaloacetate with acetyl-CoA to form citrate.

- The reaction is very favorable due to hydrolysis of the high energy citroyl-CoA thioester bond.
- High energy release is required because the concentration of oxaloacetate is normally very low.



acetyl CoA

oxaloacetate

citrate

Citrate ↔ isocitrate

- Citrate isomerised to isocitrate in two reactions (dehydration and hydration)
- Equilibrium reactions catalysed by aconitase
- Results in interchange of H and OH
  - Changes structure and energy distribution within molecule
    - Makes easier for next enzyme to remove hydrogen

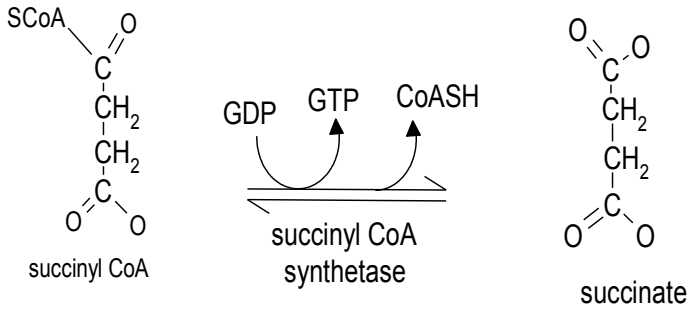
isocitrate → α-ketoglutarate

- Isocitrate dehydrogenated and decarboxylated to give α-ketoglutarate
- Non-equilibrium reactions catalysed by isocitrate dehydrogenase
- Results in formation of:

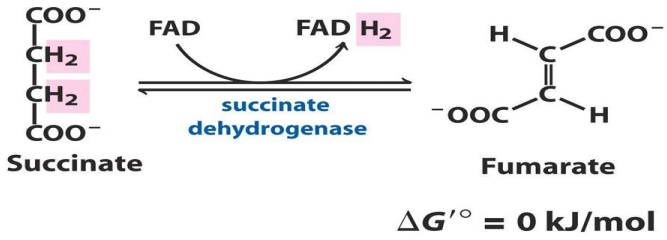
  $NADH + H^+$
  - CO_2
- Stimulated (cooperative) by isocitrate, NAD^+, Mg^{2+}, ADP, Ca^{2+} (links with contraction)
- Inhibited by NADH and ATP

α-ketoglutarate → succinyl CoA

- Series of reactions result in decarboxylation, dehydrogenation and incorporation of CoASH
- Non-equilibrium reactions catalysed by α-ketoglutarate dehydrogenase complex
- Results in formation of:
  - CO_2
  - $NADH + H^+$
  - High energy bond
- Stimulated by Ca^{2+}
- Inhibited by NADH, ATP, Succinyl CoA (prevents CoA being tied up in matrix)



Succinate ↔ fumarate

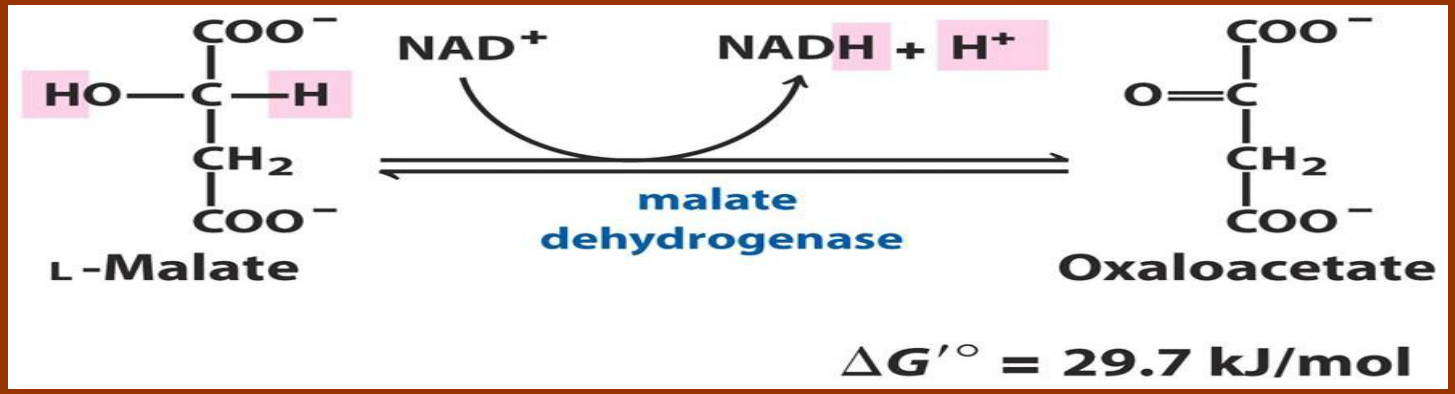


Fumarate ↔ malate

- Fumarate hydrated to form malate
- Equilibrium reaction catalysed by fumarase
- Results in redistribution of energy within molecule so next step can remove hydrogen

Malate ↔ oxaloacetate

- Malate dehydrogenated to form oxaloacetate
- Equilibrium reaction catalysed by malate dehydrogenase
- Results in formation of NADH + H+



ATP from Citric Acid Cycle

Reaction Pathway	ATP for One Glucose
ATP from Citric Acid Cycle	
Oxidation of 2 isocitrate (2NADH)	6 ATP
Oxidation of 2 α-ketoglutarate (2NADH)	6 ATP
2 Direct substrate phosphorylations (2GTP)	2 ATP
Oxidation of 2 succinate ($2FADH_2$)	4 ATP
Oxidation of 2 malate (2NADH)	6 ATP

Summary: $2\text{Acetyl CoA} \longrightarrow 4CO_2 + 2H_2O + 24\ \text{ATP}$

Regulation of the Citric Acid Cycle

- The citric acid cycle is under tight regulation at two levels:
  - The conversion of pyruvate to acetyl-CoA (the pyruvate dehydrogenase complex).
  - The entry of acetyl-CoA into the cycle (citrate synthase).
- The cycle is also regulated at the isocitrate dehydrogenase and a-ketoglutarate dehydrogenase reactions.
- Oxidation of acetate appears to be a complicated process.
- The role of the citric acid cycle is not confined to oxidation of acetate, it serves as the hub of intermediary metabolism.
- Intermediates such as oxaloacetate and a-ketoglutarate can be drawn out of the cycle and used as precursors in biosynthetic pathways of most amino acids.
- Succinyl-CoA serves as a precursor of the porphyrin ring of heme groups.
- Oxaloacetate is also a precursor in glucose synthesis

References

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There's always
Hope....
THANK YOU