A study material for M.Sc. Biochemistry (Semester: II) Students

on the topic (CC-6; Unit II)

Citric Acid Cycle

Or

Tri-carboxylic Acid Cycle

Or

Krebs Cycle

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Glycolysis is the basic and universal pathway for catalysis or breakdown of glucose molecule. Glucose (a 6- carbon compound) breakdown into two molecules of Pyruvate (3- carbon compound) and hence no carbon is liberated. So we can say that during glycolysis the complete breakdown or catalysis of glucose molecule does not occur. Hence, there are several fates of the Pyruvate molecule. Pyruvate also acts as a metabolic junction just like glucose molecule. It may convert into ethanol (in prokaryotes and anaerobic organisms) and into lactate (in anaerobic condition). It may also convert into oxaloacetate or it may act as precursor for the biosynthesis of several other molecules. But its complete break down into three molecules of carbon dioxide takes place only in aerobic organisms(which have electron transport chain to use $NADPH+H^+$ and $FADH_2$) in presence of oxygen (acts as the last electron acceptor). This catalysis takes place in 2 steps: Oxidative Decarboxylation or Link Reaction and Krebs Cycle or Tri-carboxylic Acid cycle (TCA).

Oxidative Decarboxylation

Formation of Acetyl Coenzyme A from Pyruvate is the process of Oxidative Decarboxylation, if sufficient oxygen is present oxidative decarboxylation of pyruvic acid to form Acetyl Coenzyme A takes place. The reaction takes place in the matrix of Mitochondria. This reaction is complex and it requires the presence of at least five essential cofactors and an enzyme Complex. The five cofactors are:

a. Thiamine Pyrophosphate (TPP),

b. Magnesium ion (Mn^{++})

c. NAD^+

d. Coenzyme - A and

e. Lipoic Acid

and it needs an enzyme complex Pyruvate Dehydrogenase.

Pyruvate Dehydrogenase complex is a sum up of three enzymes: E1, E2 and E3; where E1 is Pyruvate Dehydrogenase itself, E2 is Dihydrolipoyl Transacetylase and if 3 is Dihydrolipoyl Dehydrogenase.

The process of oxidative decarboxylation can be divided into four steps:

i. Formation of a complex between TPP and pyruvate followed by decarboxylation of pyruvate; in this process two carbons of pyruvate molecule are transferred to TPP and one CO_2 is liberated. Enzyme E1 catalyzes this reaction.

ii. In the next step Hydroxy methyl group is transferred to oxidise lipo lysine and TPP is regenerated. Now acetaldehyde unit remaining after decarboxylation reacts with lipoic acid

to form and acetyl lipoic acid Complex and aldehyde is oxidised to acid. This acid forms a thioester with lipoic acid.

iii. Release of acetyl group from lipoic acid to coenzyme A and to produce acetyl coenzyme A and reduced lipoic acid.

iv. Regeneration of oxidised lipoic acid by the transfer of electron from reduced lipoic acid to NAD^+. It favours continuous supply of oxidised lipoic acid for the formation of acetyl coenzyme A from pyruvate

The two electrons transferred to NAD^+ to form $NADH+H^+$ which eventually passed along to ETS to form 3 ATP molecules.

The Oxidative Decarboxylation process can be visualized as follows:

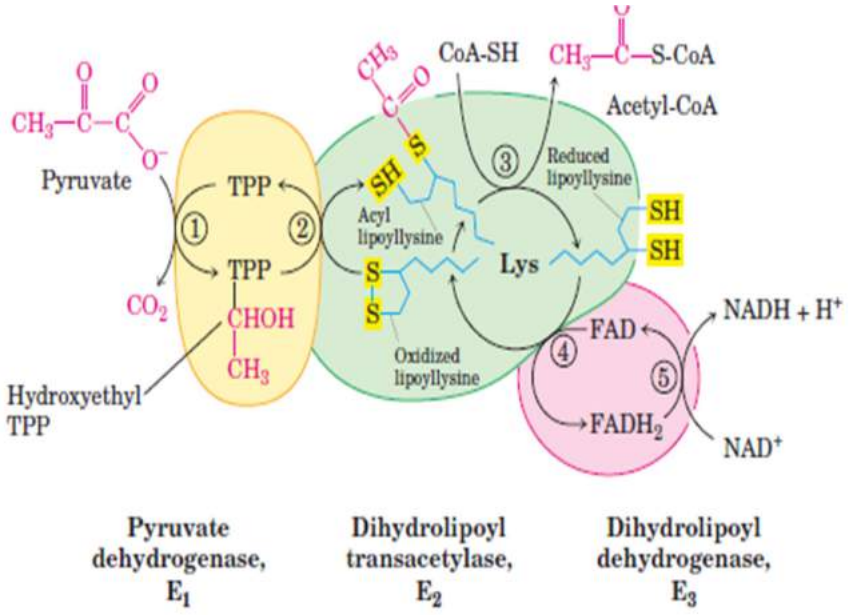


FIGURE 16-6 Oxidative decarboxylation of pyruvate to acetyl-CoA by the PDH complex. The fate of pyruvate is traced in red. In step ① pyruvate reacts with the bound thiamine pyrophosphate (TPP) of pyruvate dehydrogenase (E_1), undergoing decarboxylation to the hydroxyethyl derivative (see Fig. 14–13). Pyruvate dehydrogenase also carries out step ②, the transfer of two electrons and the acetyl group from TPP to the oxidized form of the lipoyllysyl group of the core enzyme, dihydrolipoyl transacetylase (E_2), to form the acetyl thioester of the reduced lipoyl group. Step ③ is a transesterification in which the —SH group of CoA replaces the —SH group of E_2 to yield acetyl-CoA and the fully reduced (dithiol) form of the lipoyl group. In step ④ dihydrolipoyl dehydrogenase (E_3) promotes transfer of two hydrogen atoms from the reduced lipoyl groups of E_2 to the FAD prosthetic group of E_3, restoring the oxidized form of the lipoyllysyl group of E_2. In step ⑤ the reduced $FADH_2$ of E_3 transfers a hydride ion to NAD^+, forming NADH. The enzyme complex is now ready for another catalytic cycle. (Subunit colors correspond to those in Fig. 16–5b.)

(Figure Source: Lehninger's Biochemistry; Fifth Edition)

Kreb Cycle or Citric Acid Cycle or Tricarboxylic Acid Cycle (TCA)

This cycle takes place in the mitochondrial matrix. It was discovered by German biochemist Sir Hans Krebs in 1937. Kreb's Cycle is a 8 step cycle catalyzed by different enzymes. Its entrant molecule is a two carbon compound is acetyl coenzyme A and its receptor is a four carbon molecule Oxaloacetic Acid.

The steps of the cycle are as follows:

1. **Condensation:** The acetyl coenzyme A reacts with its acceptor oxaloacetate in presence of water to form a Citric acid (which has three Carboxylic acid groups). Hence Kreb's cycle is also known as Tricarboxylic Acid cycle or TCA cycle after its first product. The reaction is catalyzed by the enzyme Citrate Sythase. Mark the name of the enzyme, it is synthase not synthetase. Synthetase enzyme uses energy (ATP), while synthase does not. The ΔG^0 value for this reaction is -32.2 KJ/mol. This reaction is irrereversible.

2. **Reorganization (Dehydration and then Hydration):** Water molecules removed from Citrate to form Cis-Aconitase in the presence of enzyme Aconitase. Again Aconitase work to add water molecule to form isocitrate from Cis-Aconitase. The ΔG^0 value for this reaction is +13.3 KJ/mol. This reaction is reversible one.

   Two consecutive Decarboxylation in next two step takes place:

3. **Oxidative Decarboxylation:** One CO_2 molecule and two Proton atoms (H^+) releases from cis - Aconitase to form 5 carbon α- Ketoglutarate. H^+ ions and electrons goes to NAD^+ to form $NADH + H^+$ which further generates ATP by transferring its electrons over the electron transport system. The oxygen required to oxidise carbon molecules to form CO_2 in step 3 and 4 comes from water molecule (H_2O).

4. **Oxidative Decarboxylation**: Coenzyme A reacts with α- Ketoglutarate (5 carbon compound) to form a four carbon Succinyl Coenzyme A and releasing CO_2 and $2H^+$ catalyzed by the enzyme α-Ketoglutarate Dehydrogenase complex. This α-Ketoglutarate dehydrogenase Complex is similar in function to Pyruvate Dehydrogenase Complex. Again 2 H+ is released and transferred to NAD^+ to form $NADH + H^+$ which further generates ATP by transferring its electrons over the electron transport system. The ΔG^0 value for this reaction is -33.5 KJ/mol. This reaction is also irrereversible.

5. **Substrate Level Phosphorylation**: Succinyl Coenzyme A splits into 4 carbon Succinate and coenzyme A with addition of water. The high energy phosphate of coenzyme is transferred to GDP in animal and ADP in plant cell to form GTP and ATP respectively this is only high energy phosphate produced in the Krebs Cycle. This is the only step of TCA, where substrate level phosphorylation takes place. The ΔG^0 value for this reaction is -2.9 KJ/mol. This reaction is reversible in nature.

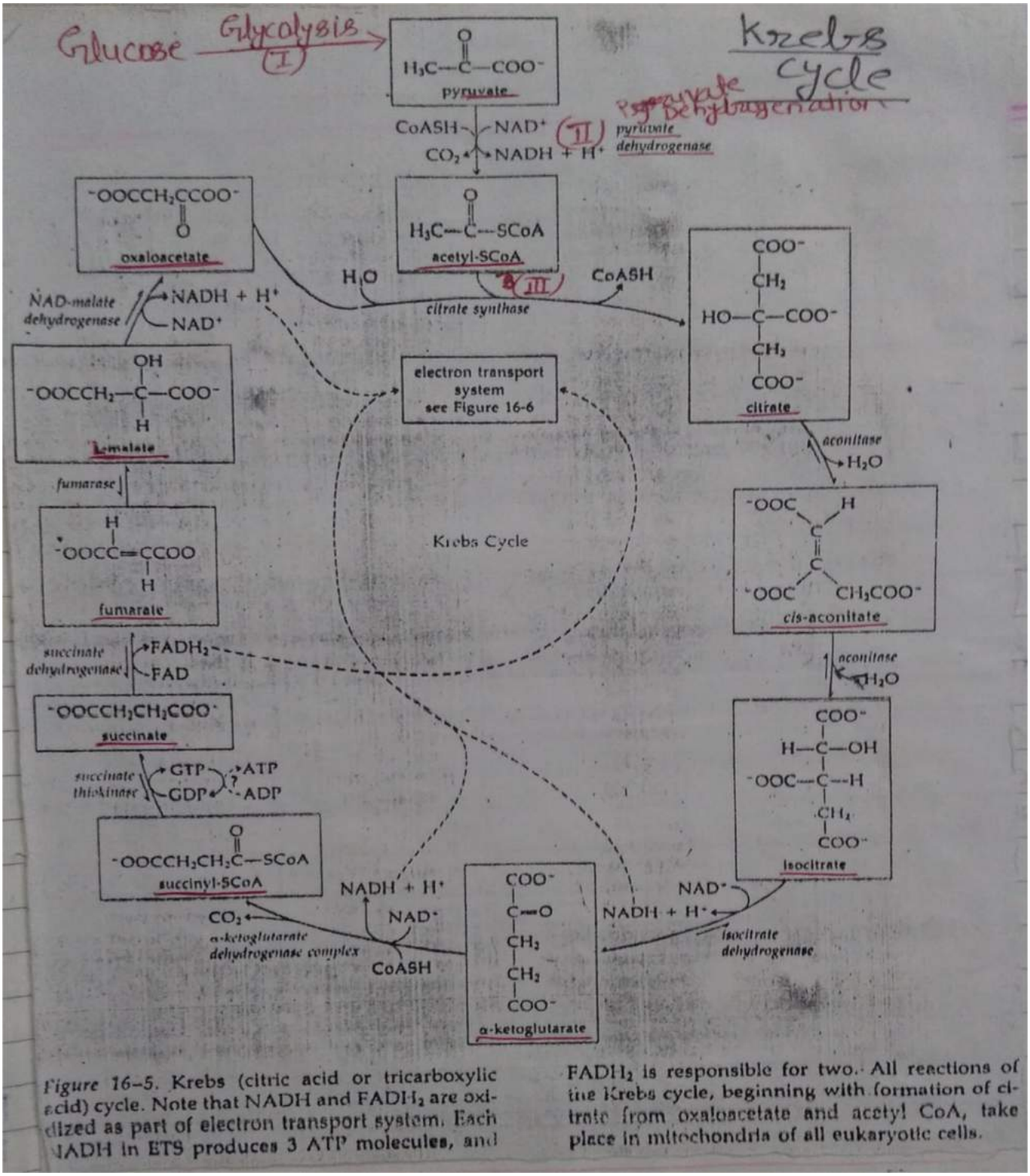


Figure 16–5. Krebs (citric acid or tricarboxylic acid) cycle. Note that NADH and $FADH_2$ are oxidized as part of electron transport system. Each NADH in ETS produces 3 ATP molecules, and $FADH_2$ is responsible for two. All reactions of the Krebs cycle, beginning with formation of citrate from oxaloacetate and acetyl CoA, take place in mitochondria of all eukaryotic cells.

6. **Dehydrogenation:** Succinate is converted into Fumarate (4 Carbon) and liberates two H^+, which later pass to FAD (Flavin Adenine Dinucleotide) to form $FADH_2$ (which carry it as whole

atom). This reaction is catalysed by the enzyme Succinate Dehydrogenase. This Succinate Dehydrogenase is only enzyme of the Krebs Cycle, which is membrane bound. We have already learnt earlier that Succinate Dehydrogenase is Complex II in Electron Transport System (ETS). It is a membrane bound enzyme which remains embedded in the inner mitochondrial membrane. Hence it acts as a connecting link between TCA cycle and electron transport system. The ΔG^0 value for this reaction is 0 KJ/mol. This reaction is also reversible.

7. **Hydration:** Fumarate changes to Malate in presence of water. This reaction is catalysed by the enzyme Fumarase. The ΔG^0 value for this reaction is -3.8 KJ/mol. This reaction is also reversible.

8. **Dehydrogenation**: Restoring of Oxaloacetate by removal of $2H^+$ from Malate. H^+ ions and electrons goes to NAD^+ to form NADH + H^+, which further generates ATP by transferring its electrons over the electron transport system. The ΔG^0 value for this reaction is +29.7 KJ/mol. This reaction is also reversible.

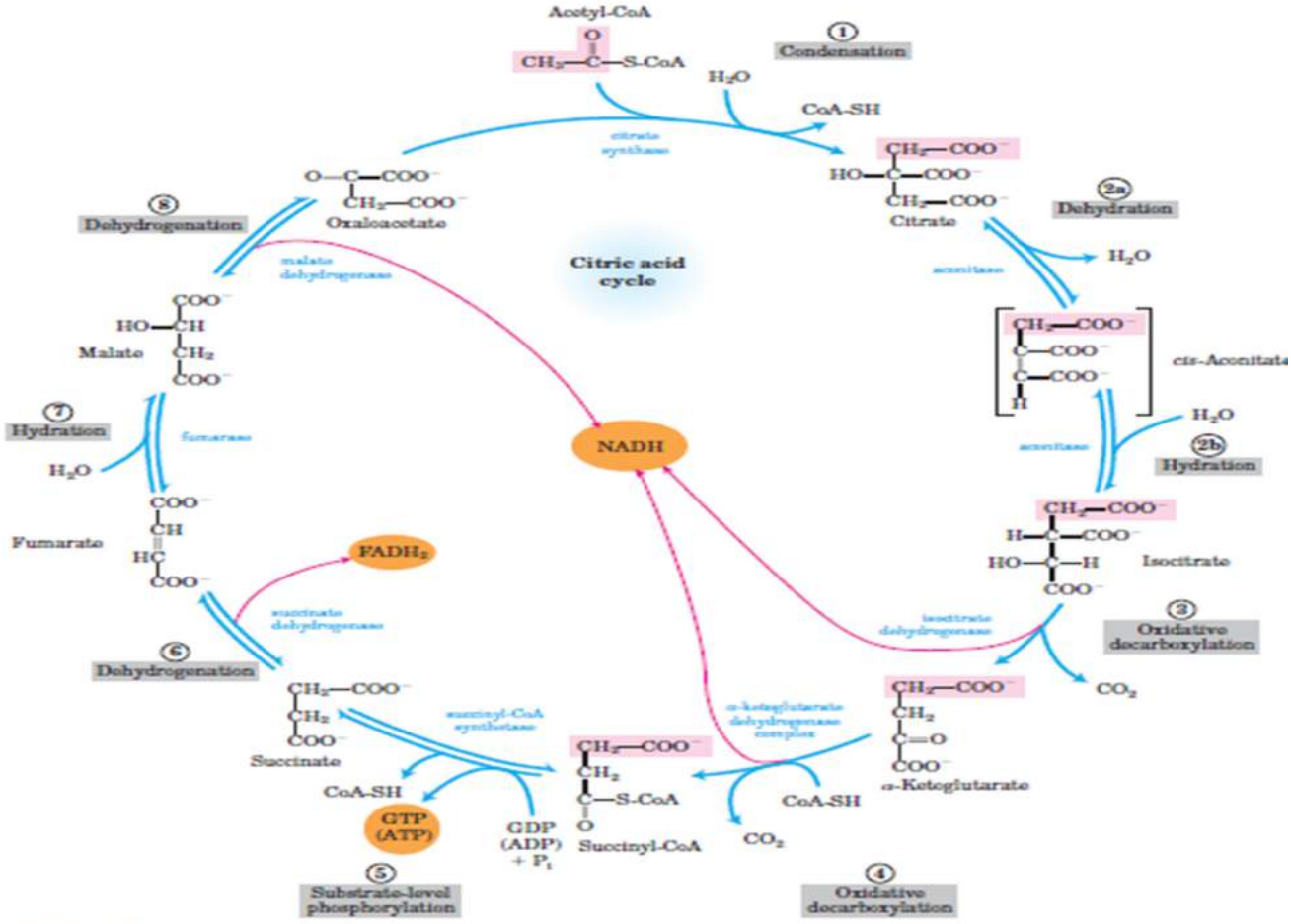


FIGURE 16–7 Reactions of the citric acid cycle. The carbon atoms shaded in pink are those derived from the acetate of acetyl-CoA in the first turn of the cycle; these are *not* the carbons released as CO_2 in the first turn. Note that in succinate and fumarate, the two-carbon group derived from acetate can no longer be specifically denoted; because succinate and fumarate are symmetric molecules, C-1 and C-2 are indistinguishable from C-4 and C-3. The number beside each reaction step corresponds to a numbered heading on pages 608–612. The red arrows show where energy is conserved by electron transfer to FAD or NAD^+, forming $FADH_2$ or NADH + H^+. Steps ①, ③, and ④ are essentially irreversible in the cell; all other steps are reversible. The product of step ⑤ may be either ATP or GTP, depending on which succinyl-CoA synthetase isozyme is the catalyst.

(Figure Source: Lehninger's Biochemistry; Fifth Edition)

ATP Balance Sheet for Tricarboxylic Acid Cycle

There are three steps in tricarboxylic acid cycle where NADH + H^+ are produced. The first step is the conversion of Isocitrate to α- Ketoglutarate, second step is the conversion of α- Ketoglutarate into Succinyl Coenzyme A and the third step is the last step of the cycle, i.e., the conversion of Malate into Oxaloacetate and hence 3 NADH + H^+ are produced per cycle.

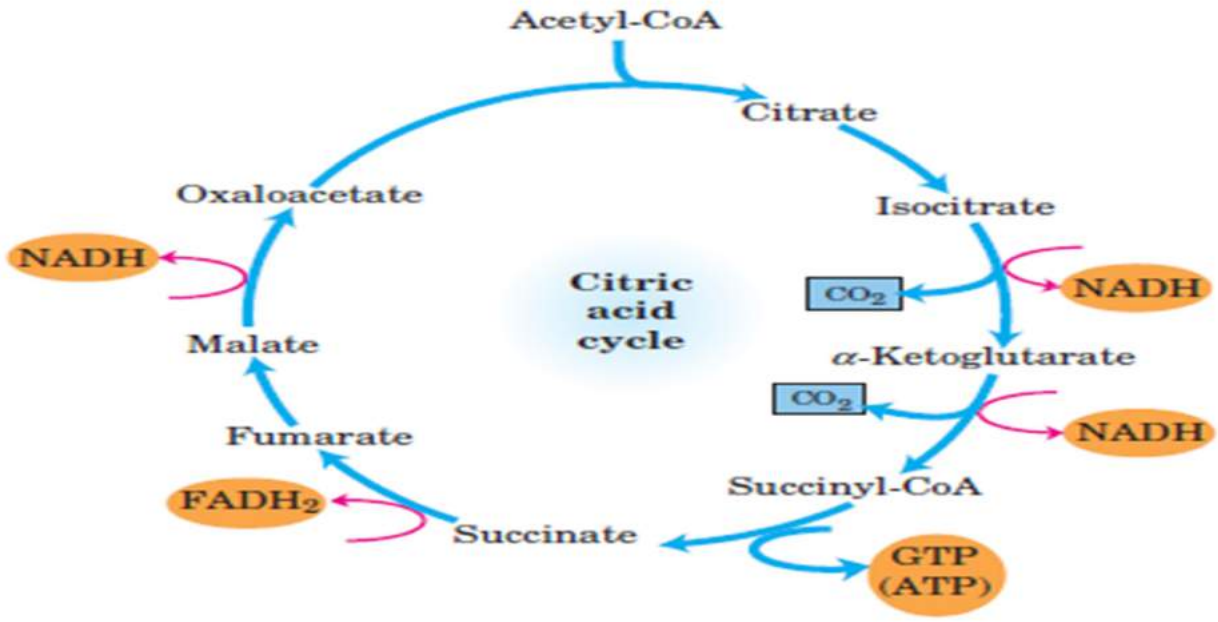


FIGURE 16–13 Products of one turn of the citric acid cycle. At each turn of the cycle, three NADH, one $FADH_2$, one GTP (or ATP), and two CO_2 are released in oxidative decarboxylation reactions. Here and in several following figures, all cycle reactions are shown as proceeding in one direction only, but keep in mind that most of the reactions are reversible (see Fig. 16–7).

(Figure Source: Lehninger's Biochemistry; Fifth Edition)

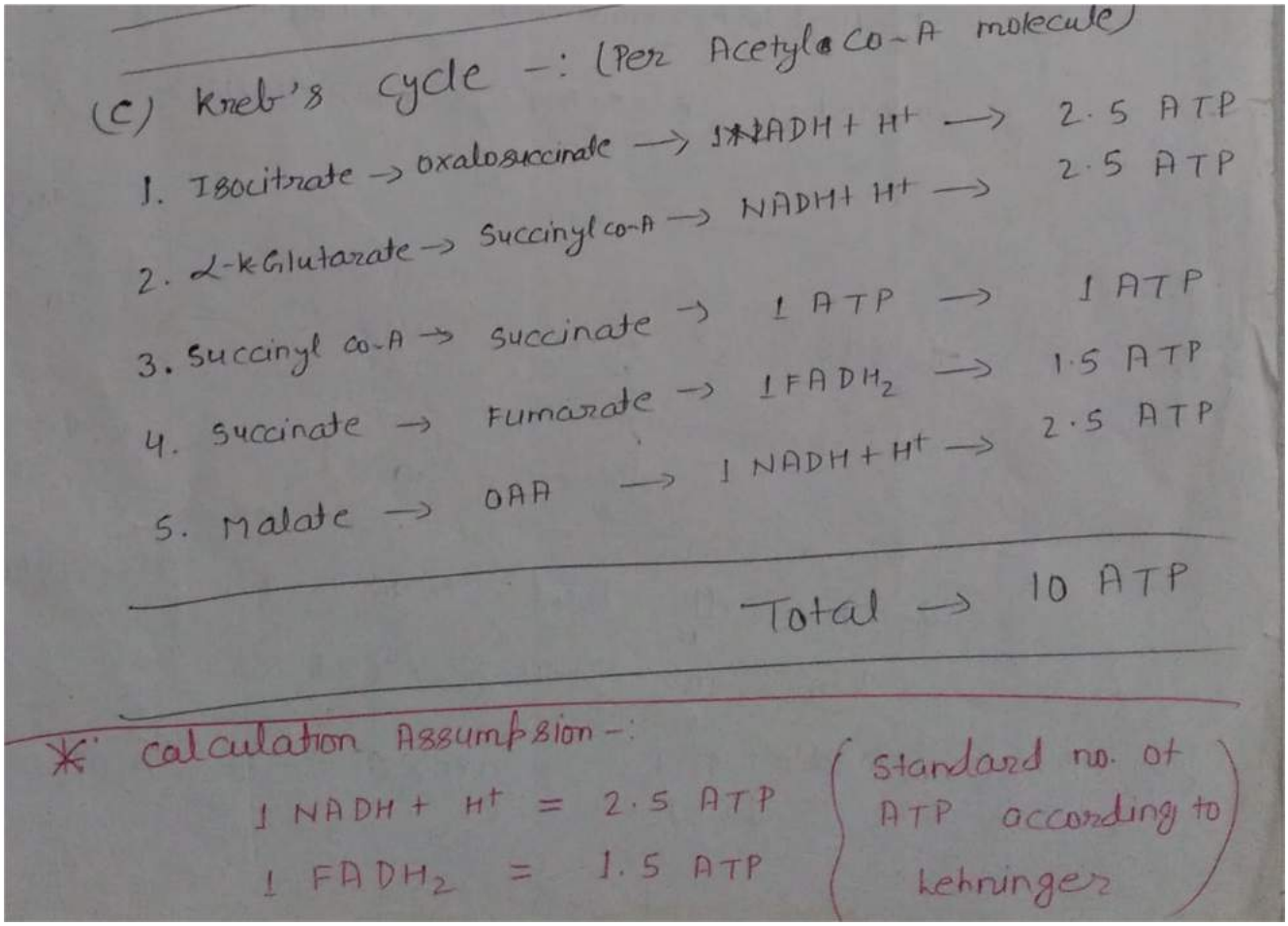
There is only one step, i.e., the conversion of Succinyl Coenzyme A into Succinate where direct ATP is produced. It is the only substrate level phosphorylation step of the cycle.

The only step where $FADH_2$ is produced is the conversion of Succinate into Fumarate with the help of enzyme Succinate Dehydrogenase.

In this way we can see that 3 NADH + H^+ and 1 $FADH_2$ is produced along with one direct ATP per cycle for one molecule of Acetyl Coenzyme A.

In	Out
Acetyl Coenzyme A (2 Carbon)	2 CO_2
1 ADP or 1 GDP	1 ATP or 1 GTP
1 FAD+	1 $FADH_2$
3 NAD^+	3 NADH + H^+

Now, if we consider 1 NADH + H^+ is equals to 2.5 ATP and one $FADH_2$ is 2 equal to 1.5 ATP (according to Lehninger), so we can calculate that after one Kreb's cycle total 10 ATP are produced.



Regulation of TCA cycle

There are four steps, where this TCA cycle can be regulated. The first step is the conversion of Pyruvate into Acetyl Coenzyme A i.e., where Pyruvate Dehydrogenase Complex is involved. Second step is conversion of oxaloacetate into citrate with the help of enzymes Citrate Synthase. The conversion of Isocitrate into α-Ketoglutarate is also one of the regulating steps and the last one is the conversion of α-Ketoglutarate into Succinyl Coenzyme A.

The regulation takes place with the help of the concentration of several regulator molecules such as ATP, AMP, Acetyl Coenzyme A, Fatty acid, Citrate and NADH. The elevated level of some the molecules accelerates the cycle, whereas the decrease level of some of the molecules accelerates the cycle.

Different regulating molecules and their regulation at different sites are shown in the figure

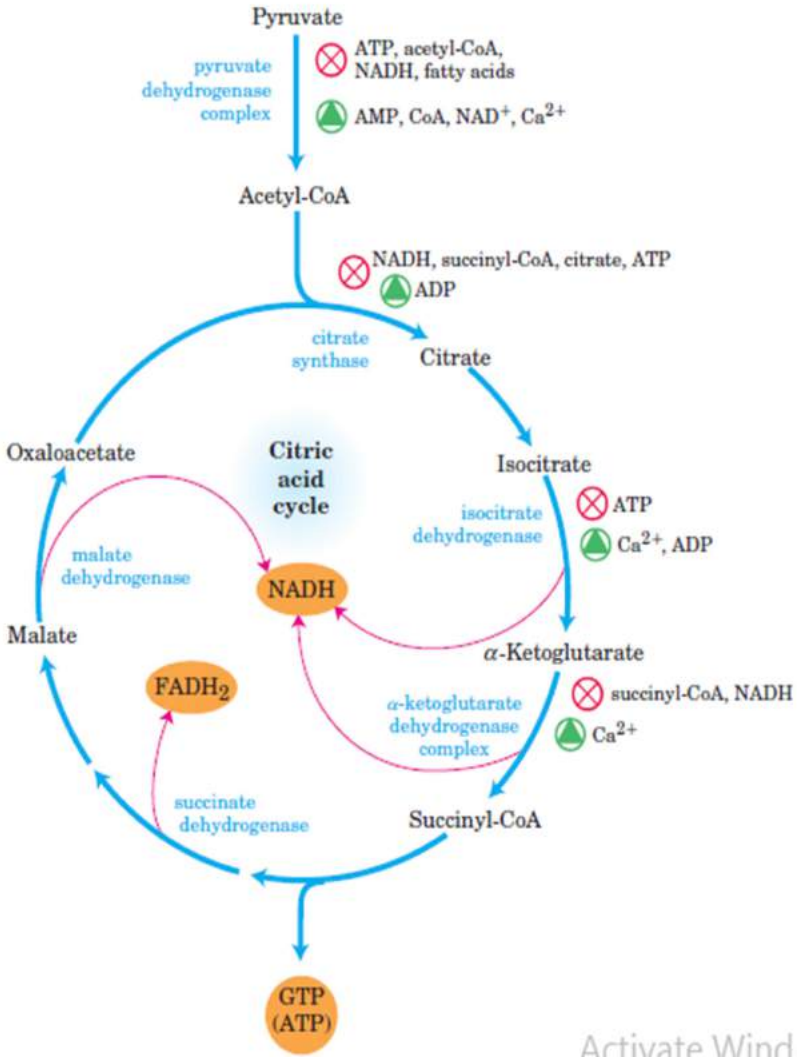


FIGURE 16-18 Regulation of metabolite flow from the PDH complex through the citric acid cycle. The PDH complex is allosterically inhibited when [ATP]/[ADP], [NADH]/[NAD^+], and [acetyl-CoA]/[CoA] ratios are high, indicating an energy-sufficient metabolic state. When these ratios decrease, allosteric activation of pyruvate oxidation results. The rate of flow through the citric acid cycle can be limited by the availability of the citrate synthase substrates, oxaloacetate and acetyl-CoA, or of NAD^+, which is depleted by its conversion to NADH, slowing the three NAD-dependent oxidation steps. Feedback inhibition by succinyl-CoA, citrate, and ATP also slows the cycle by inhibiting early steps. In muscle tissue, Ca^{2+} signals contraction and, as shown here, stimulates energy-yielding metabolism to replace the ATP consumed by contraction.

(Figure Source: Lehninger's Biochemistry; Fifth Edition)

TCA Cycle as an Amphibolic Pathway

As we already know that TCA cycle is the catabolic cycle for Acetyl Coenzyme A. There are several Pathways which produce Acetyl Coenzyme A. One of the pathway is from glucose to Pyruvate and then from Pyruvate to Acetyl Coenzyme A, as we have seen earlier in case of aerobic respiration. There are several other molecules which convert into Acetyl Coenzyme A, after their catabolism such as Fatty acids. The precursor molecule for Fatty Acid synthesis is Acetyl Coenzyme A and the catabolic product of Fatty acids is Acetyl Coenzyme A.. Several amino acids also produce Acetyl Coenzyme A after their catabolism. Hence this Kreb's cycle acts as the main catabolic pathway for those molecules which produce Acetyl Coenzyme A. This is one face of the cycle

There is also another face of the cycle too, which prove that cycle as anabolic pathway. Several intermediate of the cycle plays the role of precursor for several molecule, such as Citrate acts as precursor for fatty acid and steroid molecules, while α-Ketoglutarate acts as precursor for glutamate. Glutamate is an amino acid. This glutamate further synthesizes several amino acids such as Glutamine, Arginine and Proline. Glutamate also acts as the precursor for Purine biosynthesis pathway. Succinyl Coenzyme A acts as precursor for the synthesis of Porphyrins. Porphyrin rings are found in case of hemoglobin in humans and chlorophyll in case of plants.

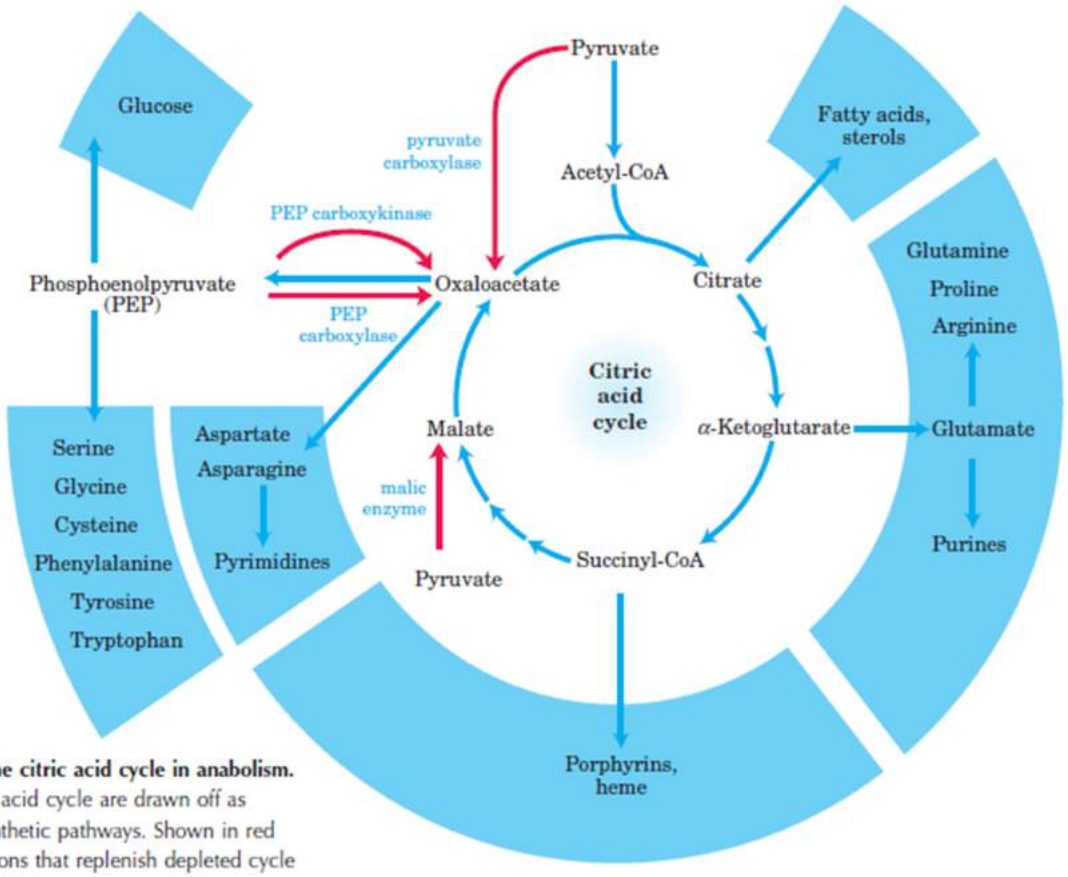


FIGURE 16-15 **Role of the citric acid cycle in anabolism.** Intermediates of the citric acid cycle are drawn off as precursors in many biosynthetic pathways. Shown in red are four anaplerotic reactions that replenish depleted cycle intermediates (see Table 16-2).

(Figure Source: Lehninger's Biochemistry; Fifth Edition)

One of the the most important intermediate of this TCA cycle is Oxaloacetate. Oxaloacetate is also the precursor molecule of the cycle, i.e., it acts as acceptor molecule of the Kreb's Cycle. It plays an important role in the synthesis of Aspartate and Asparagine and it also acts as a precursor molecule for the biosynthesis of Pyrimidines. Oxaloacetate convert into Phosphoenolpyruvate and this Phosphoenolpyruvate can synthesise glucose through gluconeogenesis or it can play a role as precursor for several amino acids such as Serine, Glycine, Cysteine, Phenylalanine, Tyrosine and Tryptophan.

Suppose the cell has to synthesise porphyrin ring, so it has need to synthesize its precursor firstly, i.e., Succinyl Coenzyme A, and for the synthesis of Succinyl Coenzyme A, TCA cycle is one of the pathway. Hence, on the above basis's, we can say that TCA cycle also acts as an Anabolic pathway.

So, now we can say that TCA cycle is catabolic as well as anabolic hence it can be considered as an Amphibolic pathway

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