(2.2) Citric Acid Cycle (TCA Cycle) or Kreb's Cycle:-

✓Overview of Kreb's Cycle:

* The tricarboxylic Acid cycle, also known as Kreb's or citric acid cycle, is the final pathway for the metabolism of molecules like Carbohydrates, Fatty Acids and Amino acid.

* All reactions of the TCA Cycle occurs entirely within the Mitochondrial Matrix

* The Main role of the cycle is to extract electrons from carbon skeleton that are derived from fuel Molecules. The electrons are captured in NADH and $FADH_2$ forms and are transported to electron transport chain

* The cycle is a cyclic process where a small amount of Oxaloacetate facilitates complete oxidation of many acetyl-CoA molecules.

* The entire process is aerobic and requires oxygen as the final Oxidant.

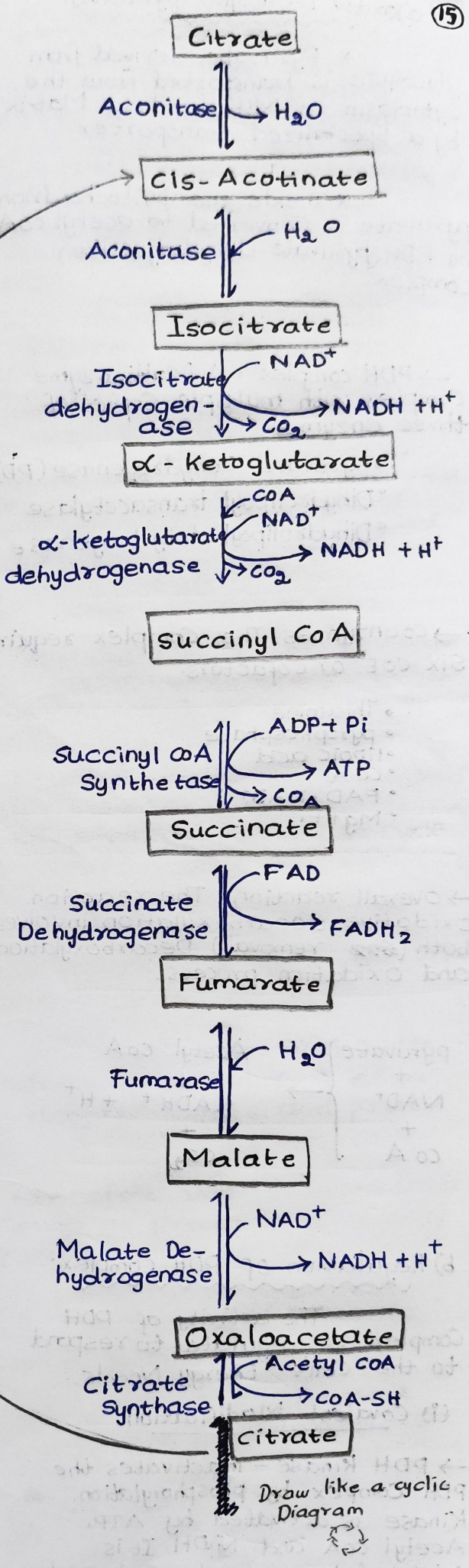
✓Biosynthesis of Acetyl CoA:

* The Main entry in the TCA Cycle is the Acetyl CoA a two carbon unit.

* It is followed by two steps.

a) Oxidative Decarboxylation of pyruvate

b) PDH Complex Regulation.



a) Oxdtv D.Crbxylt̃n (pyruvate):

* Pyruvate, derived from glycolysis is transported from the Cytoplasm to Mitochondrial Matrix by a Specialized transporter.

* Inside the Mitochondrion, pyruvate is Converted to acetyl coA by PDH (pyruvate dehydrogenase) Complex.

→ PDH complex : A Multienzyme Complex with multiple Copies of three enzymes

- pyruvate Dehydrogenase (PD)
- Dihydrolipoyl transacetylase
- Dihydrolipoyl dehydrogenase

→ Coenzymes: The Complex require Six CoE. or Cofactors.

- Thiamine
- pyrophosphate
- lipoic acid
- CoA
- FAD, NAD^+
- Mg^{++}

→ Overall reaction: The reaction oxidative decarboxylation, involves both (~~the~~ removal) Decarboxylation and Oxidation process.

pyruvate + NAD^+ + coA → Acetyl coA + $NADH^+ + H^+$ + CO_2

b) Regulation of PDH Complex:

The activity of PDH Complex is regulated to respond to the Cell's Energy Needs.

(i) Covalent Modification:

→ PDH kinase - inactivates the PDH Complex by phosphorylation. Kinase is activated by ATP, Acetyl coA and NADH. It is inhibited by pyruvate, CoA and NAD^+

~~Phosphate~~ by removing ~~P~~ group. It is stimulated by Insulin, Mg^{++} and Ca^{++}

(ii) Allosteric Regulation:

Unphosphorylated, active form of PDH is subjected to direct allosteric inhibition by its products, NADH and Acetyl coA

✓ Steps of TCA Cycle:

Each cycle involves the following

* 1 Acetyl CoA molecule
* 2 CO_2 molecules
* 3 NADH » (1 NADH = 3 ATP)
* 1 $FADH_2$ » (1 $FADH_2$ = 2 ATP)
* 1 GTP » (1 GTP ≈ 1 ATP)
* Regeneration of Oxaloacetate

a) Citrate Synthesis:

Acetyl coA (2c) Condenses with oxaloacetate (4c) to form citrate (6c) The reactn is Catalyzed by Citrate Synthase.

b) Isocitrate Formation:

Citrate is isomerized with the intermediate cis-aconitate Aconitase enzyme requiring Fe^{++} catalyze both steps.

c) Oxdtv D.Crbxylt̃n (1st)

Isocitrate Dehydrogenase Convert isocitrate to α-ketogluta-rate. It is a major regulatory step involving Oxdtn (NADH prdctn) and decrbxylt̃n (CO_2 release).

d) 2nd Oxdtv D.Crbxylt̃n:

α-Ketoglutarate dehydro-genase converts α-ketoglutarate to Succinyl CoA. This rtn produces the 2nd NADH & releases the 2nd CO_2 Molecule.

e) Substrate Level phosphorylation:

Succinyl coA Synthetase hydrolyzes succinyl coA to Succinate and coA. Energy released is used to form GTP. This GTP is further converted as ATP.

f) Dehydrogenation of Succinate:

Succinate dehydrogenase, an enzyme bound to inner mitochondrial membrane (Complex II) Oxidizes succinate to fumarate. This transfers electrons to FAD, forming $FADH_2$

g) Hydration of Fumarate:

Fumarase catalyzes the hydration of fumarate to form L-malate.

h) Dehydrogenation of Malate:

Malate dehydrogenase oxidizes L-malate back to oxalo-acetate Generating third and final NADH.

The cycle is now Complete and the regenerated oxaloacetate can react with another Molecule of Acetyl coA.

✓ Energy Yield:

For each Acetyl coA molec.,

3 NADH ⟶ 3x3 = 9 ATP
1 $FADH_2$ ⟶ 1x2 = 2 ATP
1 GTP ⟶ 1x1 = 1 ATP
} For a Acetyl coA molec.

∴ Total ATP per turn is 12 ATP per molecule.

So, On a Complete oxidation of one pyruvate molecule, it yields 1 GTP, 4 NADH and 1 $FADH_2$ molecule. That is roughly 15 ATP.

1 GTP ⟶ 1x1 = 1 ATP
4 NADH ⟶ 4x3 = 12 ATP
1 $FADH_2$ ⟶ 1x2 = 2 ATP
15 ATP

✓ TCA Cycle Regulation:

→ The cycle's rate is Controlled by the cell's energy status & substrate availability.

(PDH ⟶ pyruvate Dehydrogenase)

→ The Regulation of PDH, the main entry point is Critical & is inhibited by NADH, acetyl coA & ATP.

→ Citrate synthesis is also inhibited by ATP and long chain fatty acetyl coA

→ The rate limiting step, Isocitrate Dehydrogenase is activated by ADP & Ca^{2+} while it is inhibited by ATP & NADH.

→ α-Ketoglutarate Dehydrogenase is inhibited by its products, ATP Succinyl coA & NADH.

✓ Amphibolic Role - TCA Cycle:

TCA cycle is Amphibolic in nature. ie) It is both Anabolic and catabolic in mode of Participation.

→ catabolic role: Final Oxdtn Pathway of acetyl coA from Carbohydrates, Fats, and protein

→ Anabolic role: Intermediates Serves as precursor of Various biosynthesis pathways.

Synthesis	Precursor
* Heme Synth.	Succinyl coA
* Amino acid Syn.	Oxaloacetate & α-keto glutarate
* Fatty acid Synthesis	Citrate
* Gluconeogenesis	TCA Cycle intermediate, Oxaloacetate.

(F) Anaplerotic (filling up) Reactns:

The most imp. anaplerotic reaction is Carboxylation of pyruvate to form oxaloacetate, Catalyzed by Pyruvate Carboxylase.

Clinical Correlations:

*PDH deficiency - Defective PDH causes accumulation of pyruvate that is converted to lactate leading to lactic acidosis. Mainly affects brain causing weakness, neuro developmental delay & "ataxia" → (Loss of voluntary movement Crdntn)

* Thiamine deficiency - It is a vit. B1 deficiency, reducing TPP (Thiamine pyrophosphate) impairing PDH & α-Ketoglutarate Dehydrogenase activity in TCA cycle, leading to Low ATP prdctn.

* Pyruvate Carboxylase Deficiency - Reduces oxaloacetate formation, limiting TCA Cycle and gluconeogenesis; Resulting in fasting hypoglycemia, lactic acidosis and impaired energy dependent functions.