complete oxidation of pyruvic acid to CO_2 and H_2O. All enzymes of TCA cycle are found in the mitochondrial matrix. A summary respresentation of the scheme can be

$$\text{Pyruvate} \xrightarrow{\text{Krebs cycle}} CO_2 + \text{electrons} + \text{protons}$$

$$\text{Pi} + \text{ADP} + \text{electrons} + \text{protons} + \text{oxygen} \xrightarrow{\text{Respiratory chain}} \text{Water} + \text{ATP}$$

Formation of Acetyl CoA

The oxidative decarboxylation of Pyruate into Acetyl CoA involves the presence of at least five essential cofactors and a complex enzyme. The cofactors involved are Mg ions, thiamine pyrophosphate (TPP), NAD$^+$, Coenzyme A(CoA) and Lipoic acid. Various steps in the oxidative decarboxylation of Pyruvic acid to Acetyl CoA are summarised as follows and in Fig. 16.4.

$$\text{Pyruvic acid} + \text{TPP} \longrightarrow \text{TPP-complex} + CO_2$$

$$\text{TPP complex} + \text{Lipoic acid (oxidised form)} \longrightarrow \text{Acetyl lipoic acid complex} + \text{TPP}$$

$$\text{Acetyl lipoic acid complex} + \text{CoA} \longrightarrow \text{Acetyl CoA} + \text{Lipoic acid (reduced form)}$$

$$\text{Lipoic acid + NAD (reduced form)} \longrightarrow \text{Lipoic acid} + NADH + H^+ (NADH_2) \text{ (oxidised form)}$$

The summary representation of the reactions can be

$$\text{Pyruvate} + \text{CoA} + NAD^+ \longrightarrow \text{Acetyl CoA} + CO_2 + NADH + H^+$$

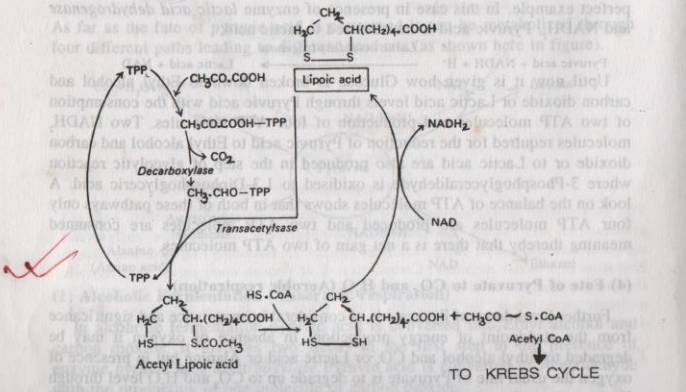


Fig. 16.4. Formation of acetyl CoA from Pyruvic acid.

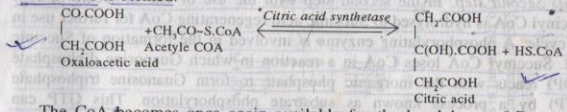
KREBS CYCLE

(Named *after* H.A. Kreb)

(Tricarboxylic acid cycle, TCA cycle, Organic acid cycle, Mitochondrial respiration, Oxidation of pyruvate, Citric acid cycle)

The cycle consists of following important steps.

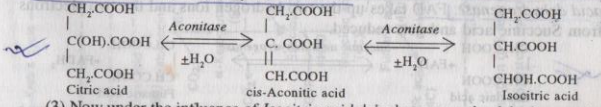
(1) The 2-carbon compound Acetyl CoA is the connecting link between EMP pathway and Krebs cycle. The reactions of EMP pathway occur in the cytoplasm and the respiratory substrate enters for Krebs cycle inside the mitochondria in the form of pyruvic acid. Pyruvic acid is first converted into Acetyl CoA. Acetyl CoA reacts with a 4-carbon compound Oxaloacetic acid with the use of one molecule of water and as a result a 6-carbon compound Citric acid is formed.



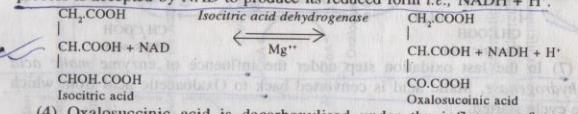
The CoA becomes once again available for the breakdown of Pyruvate to Acetyl CoA. At this stage CoA has a controlling influence on respiration. When intermediates of TCA cycle are used in other processes, the oxaloacetic acid is made available by an anaplerotic reaction catalysed by *PEP carboxylase* found in cytosol.

$$\text{Phosphoenol pyruvate} + HCO_3^- \longrightarrow \text{Oxaloacetate} + H_2PO_3^-$$

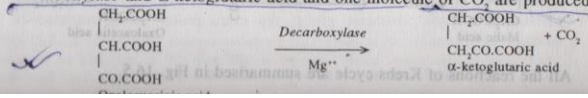
(2) In the next step Citric acid loses one molecule of water to form Cis-aconitic acid which acts as an intermediate in the isomerisation of Citric acid to Isocitric acid after taking back the molecule of water. Both the reactions of this step are catalysed by the enzyme *aconitase*.



(3) Now under the influence of *Isocitric acid dehydrogenase*, the dehydrogenation of Isocitric acid to Oxalosuccinic acid occurs and the hydrogen released in the process is accepted by NAD to produce its reduced form *i.e.*, NADH + H$^+$.



(4) Oxalosuccinic acid is decarboxylised under the influence of enzyme *decarboxylase* and α-ketoglutaric acid and one molecule of CO_2 are produced.



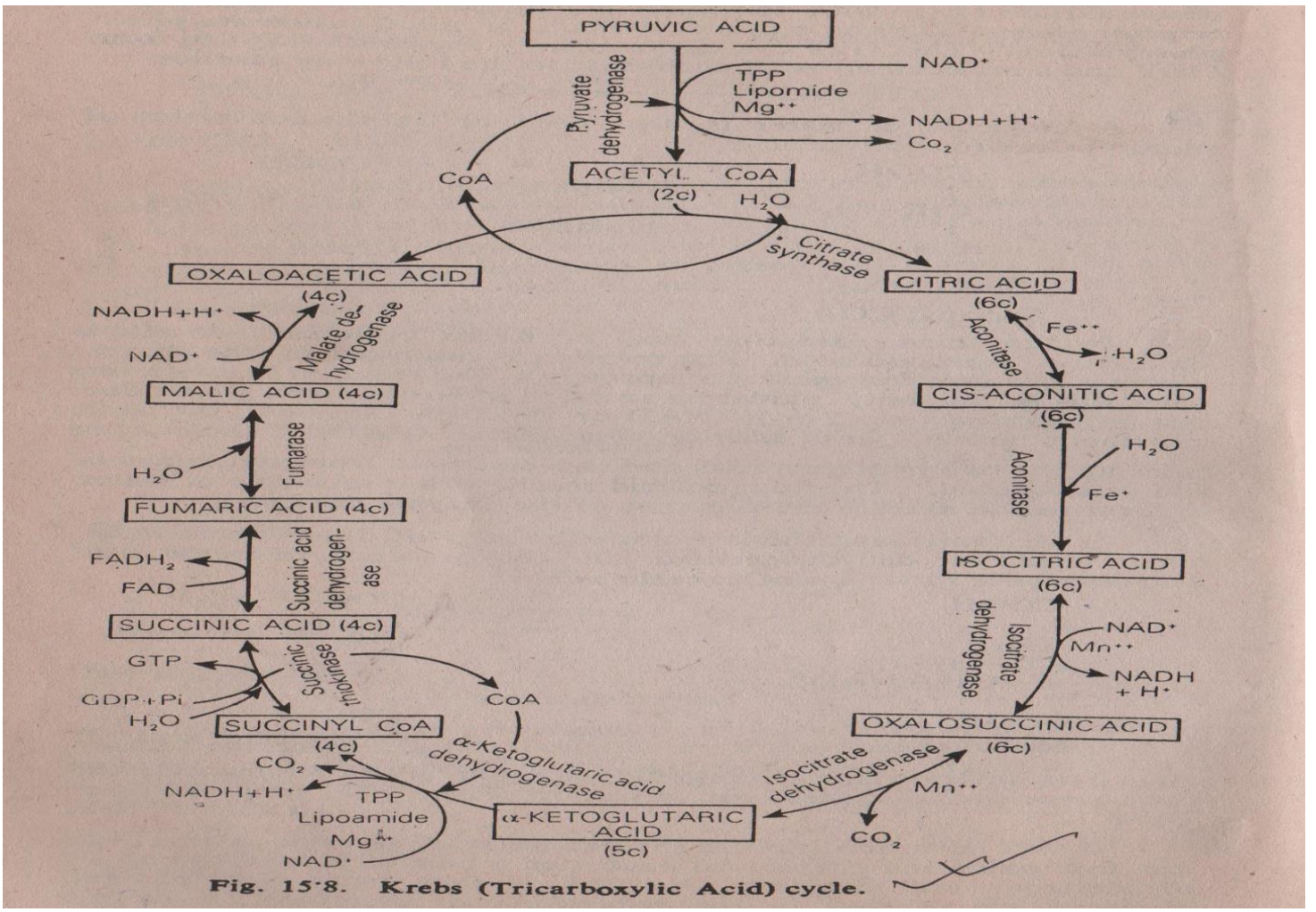


Fig. 15·8. Krebs (Tricarboxylic Acid) cycle.

(5) The next step involves the oxidation of α-ketoglutaric acid which takes place in two steps.

(i) First step. In the first step, α-ketoglutaric acid is converted into Succinyl CoA and the reaction is catalysed by the enzyme *a-ketoglutaric acid dehydrogenase* which requires TPP, Mg^{++}, NAD, FAD, lipoic acid and coenzyme A(CoA) as cofactors. The step is analogous to that of oxidation of pyruvic acid to Acetyl CoA. In this step one molecule of NAD is also reduced to $NADH_2$ and one molecule of CO_2 is liberated.

$$\begin{array}{l} CH_2.COOH \\ | \\ CH_2.CO.COOH \\ \alpha\text{-ketoglutaric acid} \end{array} + HS.CoA + NAD^+ \xrightarrow[Mg^{++},\ TPP,\ \text{lipoic acid, FAD}]{\alpha\text{-ketoglutaric acid dehydrogenase}} \begin{array}{l} CH_2.COOH \\ | \\ CH_2.CO \sim S.CoA \\ \text{Succinyl CoA} \end{array} + NADH_2 + CO_2$$

(ii) Second step. In the second step with the use of one molecule of H_2O Succinyl CoA is hydrolysed to Succinic acid regenerating CoA for further use in the cycle. A phosphorylating enzyme is involved in the formation of Succinic acid. Succinyl CoA loses CoA in a reaction in which Guanosine diphosphate (GDP) reacts with the inorganic phosphate to form Guanosine triphosphate (GTP) by a process known as substrate phosphorylation. This GTP can enzymatically react with ADP to produce ATP.

$$\begin{array}{l} CH_2.COOH \\ | \\ CH_2.CO \sim S.CoA \\ \text{Succinyl CoA} \end{array} + GDP + Pi \xrightleftharpoons{\text{Succinyl thiokinase}} \begin{array}{l} CH_2.COOH \\ | \\ CH_2.COOH \\ \text{Succinic acid} \end{array} + GTP + HS.CoA$$

$$GTP + ADP \rightleftharpoons GDP + ATP$$

(6) The Succinic acid is oxidised to Fumaric acid. The enzyme involved is *succinic acid dehydrogenase*. This is the only reaction of Krebs cycle which does not involve the utilisation of Co I or II (NAD or NADP) and the place of which is taken by Flavin adenine dinucleotide (FAD) of the enzyme *succinic acid dehydrogenase*. FAD takes up the two hydrogen ions and the two electrons from Succinic acid and gets reduced.

$$\begin{array}{l} CH_2.COOH \\ | \\ CH_2.COOH \\ \text{Succinic acid} \end{array} + FAD \xrightleftharpoons{\text{Succinic acid dehydrogenase}} \begin{array}{l} CH.COOH \\ \| \\ CH.COOH \\ \text{Fumaric acid} \end{array} + FADH_2$$

Now with the addition of one molecule of water in the presence of enzyme *fumarase*, Fumaric acid is converted into Malic acid.

$$\begin{array}{l} CH.COOH \\ \| \\ CH.COOH \\ \text{Fumaric acid} \end{array} + H_2O \xrightleftharpoons{\text{Fumarase}} \begin{array}{l} CHOH.COOH \\ | \\ CH_2.COOH \\ \text{Malic acid} \end{array}$$

(7) In the last oxidation step under the influence of enzyme *malic acid dehydrogenase*, Malic acid is converted back to Oxaloacetic acid from which the cycle started.

$$\begin{array}{l} CHOH.COOH \\ | \\ CH_2.COOH \\ \text{Malic acid} \end{array} + NAD \xrightleftharpoons{\text{Malic acid dehydrogenase}} \begin{array}{l} CO.COOH \\ | \\ CH_2.COOH \\ \text{Oxaloacetic acid} \end{array} + NADH_2$$

All the reactions of Krebs cycle are summarised in Fig. 16.5.

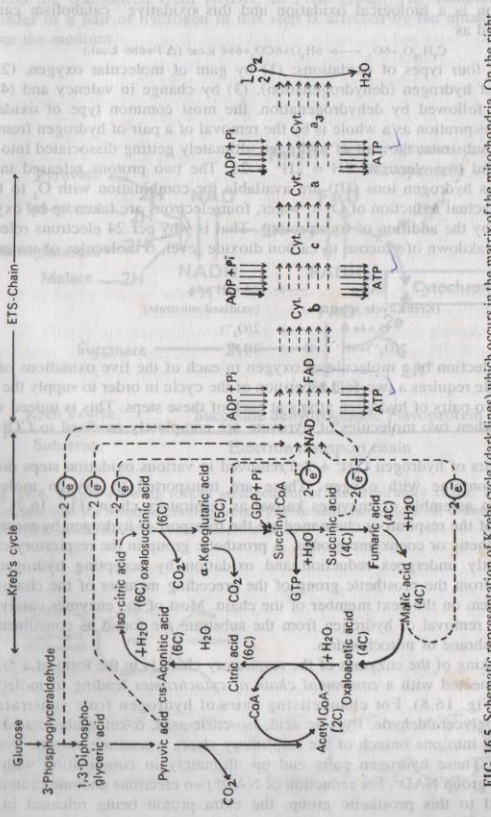


FIG. 16.5. Schematic representation of Krebs cycle (dark lines) which occurs in the matrix of the mitochondria. On the right side of figure (dotted lines) ETS system and origin of electrons are shown. Enzymes of ETS chain are the constituent of the cristae (inner membrane).

TABLE 15.5. Showing the number of ATP formed in the various reactions of respiration.

Compounds to be oxidized	*Compounds after Oxidation*	*Acceptor*	*Number of ATP formed*
Pyruvic acid	Acetyl CoA	NAD^+	$2\times3=6$
Isocitric acid	Oxalosuccinic acid	NAD^+	$2\times3=6$
α-Ketoglutaric acid	Succinic acid	NAD^+	$2\times4=8$
Succinic acid	Fumaric acid	FAD	$2\times2=4$
Malic acid	Oxaloacetic acid	NAD^+	$2\times3=6$
Net gain of ATP in glycolysis=8			30

Total=38 ATP molecules