The tricarboxylic acid cycle

In many bacteria, yeasts, filamentous fungi, algae and protozoa, further catabolism of pyruvate under aerobic conditions involves its direction into the tricarboxylic
acid (TCA) cycle (Fig. 3.5).
TCA cycle enzymes are located within the mitochondrial matrix in eukaryotes,
whereas in prokaryotes they are cytoplasmic. The step immediately before the cycle involves the oxidative decarboxylation of pyruvate to form the C2 unit acetyl
coenzyme A (acetyl CoA), catalysed by the multienzyme complex, pyruvate dehydrogenase. Acetyl CoA can also be formed via breakdown of lipids and some amino acids, and from the oxidation of alkanes, which certain microorganisms can utilize.

$$\text{Pyruvate } (C_3) + NAD^+ + CoA \rightarrow$$
$$\text{acetyl CoA } (C_2) + CO_2 + NADH + H^+$$
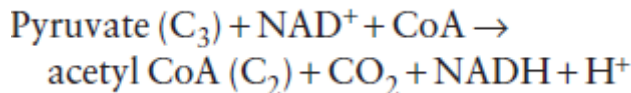
The cycle proper commences with the condensation of this two-carbon compound with oxaloacetate (C4) to form citrate (C6). During the following eight steps
of the complete TCA cycle, the two-carbon fragment is oxidized to two CO2 molecules and oxaloacetate is regenerated to accept a further two-carbon unit. Three reactions within the cycle result in NADH formation and one generates FADH2, and a single ATP molecule is formed indirectly by a substrate-level phosphorylation.
Net yield is as follows:

$$\text{acetyl CoA } (C_2) + 3NAD^+ + FAD + ADP \rightarrow$$
$$2CO_2 + 3NADH + 3H^+ + FADH_2 + ATP$$
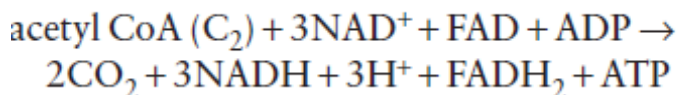
In terms of catabolism, the TCA cycle completes the oxidation of pyruvate to CO2, and reduces electron carriers to produce NADH and FADH2. These reduced
coenzymes may then be used for further ATP synthesis in respiration and importantly, are thereby reoxidized to participate in further catabolism.

The TCA cycle is not only a catabolic pathway, but also provides intermediates, C4 and C5 compounds, for the biosynthesis of amino acids, purines and pyrimidines. Under anaerobic conditions it does not function as a cycle. However, as several intermediates are still required for biosynthesis, it operates as a branched
biosynthetic pathway. This facility to produce biosynthetic intermediates is also present in other microorganisms which lack a complete TCA cycle.
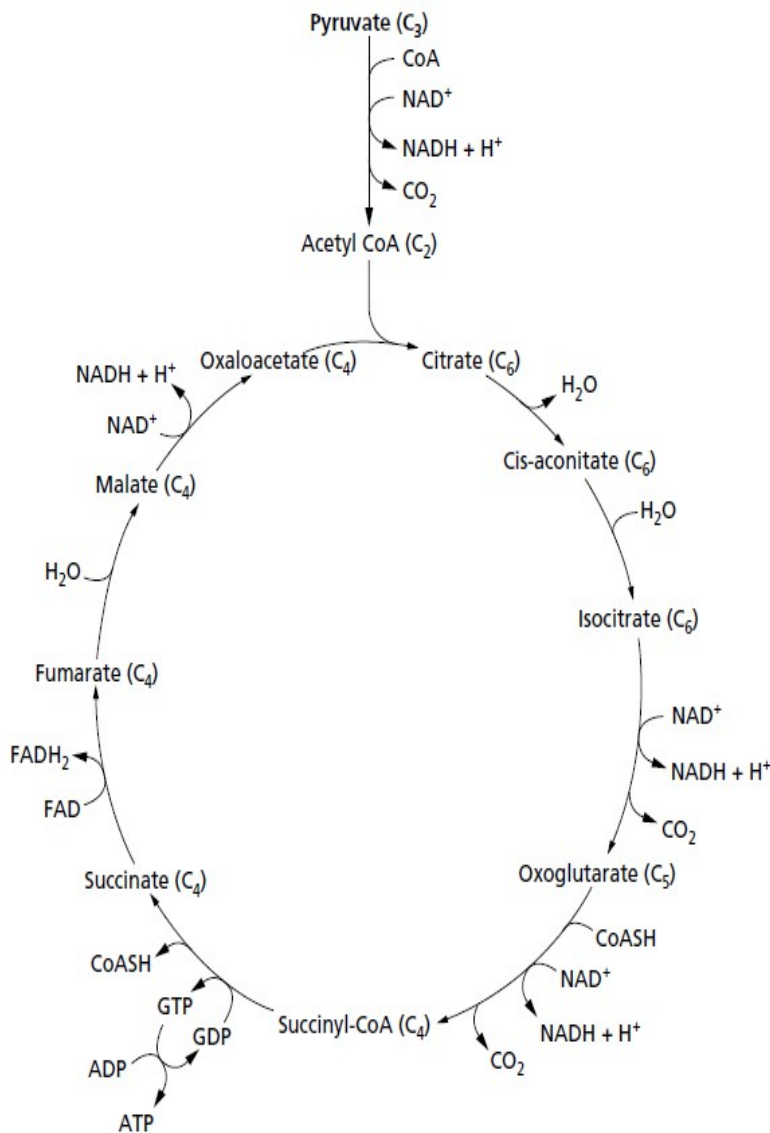

Fig. 3.5 The tricarboxylic acid cycle.

The glyoxalate cycle

As a consequence of removing intermediate compounds from these amphibolic pathways for biosynthesis, their levels may become depleted. For example, oxaloacetate is taken from the TCA cycle to furnish the demand for carbon skeletons in amino acid biosynthesis. Hence, these intermediates have to be replenished via an alternate route, referred to as an **anaplerotic pathway**, in order to maintain operation of this cycle. One such anaplerotic route is the **glyoxalate cycle** (Fig. 3.8). This cycle operates in many organisms for the replenishment of oxaloacetate, particularly for gluconeogenesis (see below) from lipids and during growth on C2 compounds. It involves **isocitrate lyase**, which cleaves isocitrate to succinate and glyoxalate. Acetyl CoA is then condensed with glyoxylate to generate malate and coenzyme A (CoASH) by the action **of malate synthase**. The malate can then be transformed to oxaloacetate to allow the continued operation of the TCA cycle.
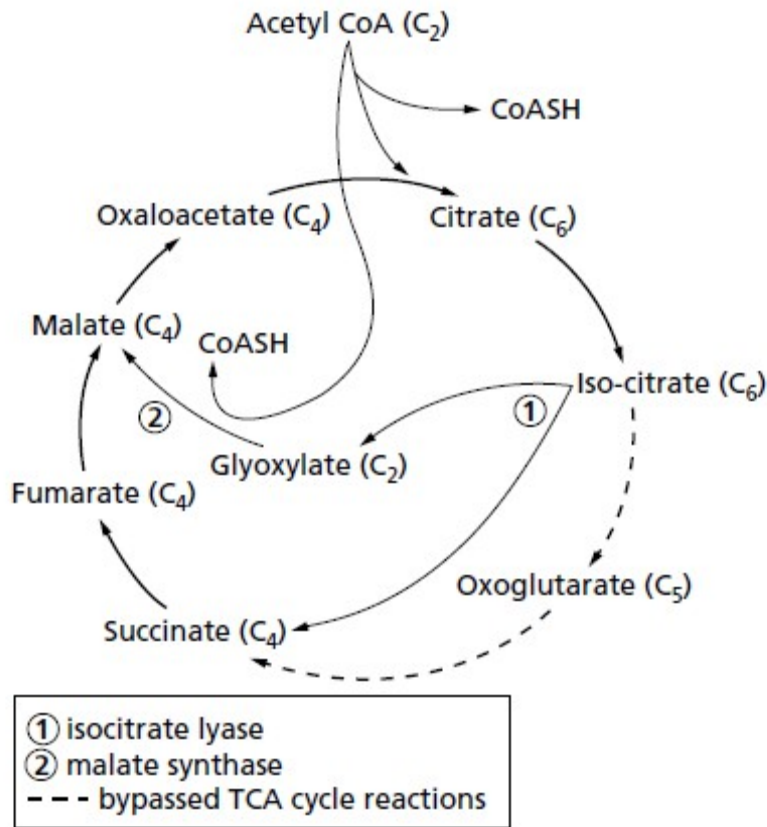
Fig. 3.8 The glyoxylate cycle.

Production of Citric Acid
Citric acid is a tribasic acid with the structure shown in Fig. 20.1.
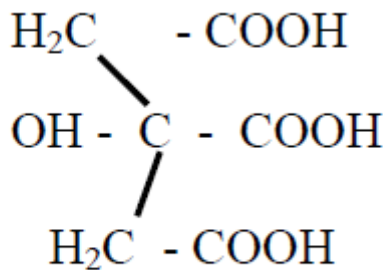
$$H_2C \quad - COOH$$
$$OH - C - COOH$$
$$H_2C - COOH$$

Fig. 20.1 Structure of Citric Acid

Uses of Citric Acid

Citric acid is used in the food industry, in medicine, pharmacy and in various other
industries.
For a long time the production of citric acid has been based on the use of molasses and various strains of *Aspergillus niger* and occasionally *Asp. wenti.* Although several reports of citric acid production by *Penicillium* are available, in practice, organisms in this group are not used because of their low productivity. In recent times yeasts, especially *Candida* spp. (including *Candida quillermondi)* have been used to produce the acid from sugar. Paraffins became used as substrate from about 1970. In the processes described mainly by Japanese workers bacteria and yeasts have been used. Among the bacteria were *Arthrobacter paraffineus* and corynebacteria; the yeasts include *Candida lipolytica* and *Candida oleiphila.*
Fermentation with molasses and other sugar sources can be either surface or
submerged. Fermentation with paraffins however is submerged.

(a) *Surface fermentation*:
Surface fermentation using *Aspergillus niger* may be done on rice bran as is the case in Japan, or in liquid solution in flat aluminium or stainless steel pans. Special strains of *Asp. niger* which can produce citric acid . The citric acid is extracted from the bran by leaching and is then precipitated from the resulting solution as calcium citrate.

(b) *Submerged fermentation*:
As in all other processes where citric acid is made the fermentation the fermentor is made of acid-resistant materials such as stainless steel. The carbohydrate sources are molasses, sucrose or glucose. $MgSO_4, 7H_2O$ and KH_2PO_4 at about 1% and 0.05-2% respectively are added (in submerged fermentation phosphate restriction is not necessary). The pH is never allowed higher than (3.5) . As high aeration is deleterious to citric acid production.
The fungus occurs as a uniform dispersal of pellets in the medium. The fermentation lasts for five to fourteen days.

Extraction

The broth is filtered until clear. Calcium citrate is precipitated by the addition of
magnesium-free (Ca(OH) 2. Since magnesium is more soluble than calcium, some acid may be lost in the solution as magnesium citrate if magnesium is added. Calcium citrate is filtered and treated with sulfuric acid to precipitate the calcium.
The dilute solution containing citric acid is purified by treatment with activated carbon and passing through iron exchange beds. The purified dilute acid is evaporated to yield crystals of citric acid. Further purification may be required to meet pharmaceutical stipulations.