Glyoxylate cycle is a modified form of tricarboxylic acid (TCA) cycle and its function as an anaplerotic pathway was shown by Kornberg (1966). The cycle operates in most plants and microorganisms but is absent in higher animals. The concept of glyoxylate pathway was developed by Kornberg to explain the importance of enzymatic reactions which compensate for the removal of intermediates from central metabolic routes during biosynthesis. The primary purpose of the cycle as shown by Krebs and H.R. Kornberg (1957) was to utilise fatty acids or acetate in the form of acetyl-CoA by plants and microorganisms. This pathway provides a bypass to the decarboxylating steps of Krebs cycle and thus provides the organisms with the gluconeogenic precursors when supplied with 2-carbon substrate as the sole carbon source. Such reactions occur in fungi mainly because several tricarboxylic acid cycle acids are precursors of biosynthetic pathway (Kornberg, 1959; Niederpreum, 1965). Wherever present, the glyoxylate cycle (Kornberg and Krebs, 1957) provides for the net synthesis of C₄ dicarboxylic acids from acetyl-CoA. The overall plan of the glyoxylate cycle is
In each turn of the cycle two molecules of acetyl-CoA enter and one molecule of succinate is formed. The overall equation of the glyoxylate cycle is

\[
2 \text{ Acetyl-CoA} + \text{NAD}^+ + 2\text{H}_2\text{O} \rightarrow \text{Succinate} + 2\text{CoA} + \text{NADH} + \text{H}^+.
\]

The two key enzymes of glyoxylate cycle, isocitrate lyase and malate synthase, are located in cytoplasmic organelles called 'glyoxysomes'. These membrane surrounded organelles lack most of the tricarboxylic acid cycle enzymes and are devoid of cytochrome system. Isocitrate lyase and malate synthase both are inducible enzymes and are synthesised when needed for oxidation of specific substrates. The occurrence of Isocitrate lyase had been established in certain fungi even before its involvement in the glyoxylate cycle was suggested by Kornberg and Krebs in 1957 (Olson, 1954). Subsequently several authors (Olson, 1957; Collins and Kornberg, 1960; Gottlieb and Ramachandran, 1960; Turian, 1961 a,b; O'Sullivan and Caselton, 1973) showed the presence of isocitrate lyase and malate synthase in cell free extracts of fungi belonging to the major taxonomic groups. In case of Aspergillus niger it was confirmed that the activities of the enzymes were adequate to account for the rate of growth of the fungus in acetate medium (Collins and Kornberg, 1960). Acetyl-CoA synthetase is also required when acetate is metabolised but has received less attention.

Since the work of Krebs and Kornberg (1957) extensive studies have been done on the role of this cycle in the metabolism of acetate and other 2-carbon compounds in different microorganisms as well as in plant metabolism during germination in fatty seeds of higher plants.

Our present investigations are mainly with the acetate metabolism in Aspergillus terreus which can be well explained by studying its acetate
non-utilising mutants.

Here a brief review of the work done on acetate metabolism in bacteria and molds is given. The review is somewhat related to the contents of the thesis so it will certainly help the reader to know the general informations that have gathered up to the present time. The enzyme isocitrate lyase was variously named by different authors as isocitratase and isocitritase.

Wong and Ajl (1956) discovered malate synthetase and isocitratase which was originally described by Campbell et al. (1953) and subsequently by Olson (1954, 1957), Saz (1954), Saz and Hillary (1956) and Smith and Günsalus (1954, 1955, 1957). These were combined by Kornberg and Madsen (1957) to formulate the so called 'Glyoxylate bypass'. The hypothesis has been advanced (Wong and Ajl, 1957; Kornberg and Krebs, 1957) and expanded in several reviews (Ajl, 1958; Kornberg, 1959) that this pathway, in conjunction with the tricarboxylic acid cycle, provides an explanation for the ability of certain bacteria to grow in a medium containing a 2-carbon compound, such as acetic acid, as the sole carbon source.

Kornberg and Krebs (1957) established from the works in different laboratories, the occurrence of a metabolic cycle in microorganisms which could derive all their carbon requirements from 2-carbon compounds. The cycle is a variant of the TCA cycle and is as follows:

\[
\text{Acetate} + \text{Oxaloacetate} \rightarrow \text{Citrate} \rightarrow \text{cis-Aconitate} \rightarrow \text{Isocitrate} \rightarrow \text{Succinate} + \text{Glyoxylate}, \text{Glyoxylate} + \text{Acetate} \rightarrow \text{malate; Malate} + \frac{1}{2}O_2 \rightarrow \text{Oxaloacetate.}
\]

Acetate reacts in the form of acetyl-CoA. The stages between citrate and malate in the TCA cycle are replaced by reaction in which glyoxylate is a key metabolite. Therefore, the cycle is referred as glyoxylate cycle.
The main discoveries which led to the elaboration of the cycle were (i) isocitrate apart from undergoing dehydrogenation was split enzymatically into succinate and glyoxylate (ii) the recognition of an enzyme system by Wong and Ajl(1956) which brought about the synthesis of malate from acetyl-CoA and glyoxylate and (iii) demonstration of the occurrence of combined action of two enzyme systems in cell free extracts. The overall effect of one turn of glyoxylate cycle is the formation of one molecule of C4 dicarboxylic acid from two molecules of acetate. This together with acetate can serve as the precursor of many cell constituents. Therefore, the cycle is a stage in synthesis of cell constituents from acetate.

Kornberg and Madsen(1957) worked with the bacterial strain Pseudomonas KBI. The authors concluded that this organism when grown on acetate as sole carbon source possessed, in addition to the enzymic reactions of the TCA cycle, a mechanism offering an alternative route from isocitrate to malate. Isocitrate was cleaved by isocitratase as well as acetyl-CoA and glyoxylate was condensed by malate synthase.

Wong and Ajl(1957) suggested that the occurrence of malate synthetase which catalysed the conversion of glyoxylate and acetyl-Coenzyme A to malic acid and coenzyme A was believed to explain how bacteria grew on 3-carbon compounds. The proposed explanation was that a minimal catalytic amount of oxaloacetate combined with acetate to form citrate which was then cleaved by way of isocitrate to glyoxylate and succinate. Glyoxylate then condensed with another molecule of acetate by means of the reaction catalysed by malate synthetase to form a new 4-carbon compound. Malate synthetase was adaptively formed when cells were grown on acetate as sole carbon source.

Experiments with Pseudomonas aeruginosa strain 18, Bachrach(1957) observed that glyoxylic acid was metabolised by cell free preparation or
by dried cells in the presence of succinic acid, Mg$^{++}$ and glutathione. The author suggested that glyoxylic acid was metabolised by the isocitratase pathway.

An enzyme which catalysed the reversible aldol reaction, glyoxylate + succinate $\rightarrow$ L(+)-isocitrate, was identified in aerobic and facultative microorganisms by Smith and Gunsalus(1957). Carbohydrate containing media repressed the formation of the enzyme whereas aerobic growth in organic acid media favoured the production of the enzyme. This enzyme was purified 30 fold in Pseudomonas aeruginosa and it required the presence of Mg$^{++}$ and cysteine as activator.

Kornberg and Collins(1958) worked with Aspergillus niger and their finding suggested the operation of glyoxylate cycle, providing an explanation for the growth of A. niger and accumulation of intermediates when acetate was supplied as sole carbon source. They also identified acetyl-CoA kinase in the cell free extract of the fungus. While studying with Pseudomonas ovnilis Kornberg et al.(1958) found that isocitratase was formed when the cells needed 4-Carbon compounds from acetate and this formation of isocitratase precedes growth on acetate.

Kornberg and Hudson(1958) found that the cell free extracts of acetate grown Pseudomonas fluorescens K61 were capable of catalysing the synthesis of acetyl-CoA from acetate. The acetyl-CoA reacted both with oxaloacetate and glyoxylate to form citrate and malate respectively.

Takahiro Moriyama et al. (1958) suggested the occurrence of isocitratase in Mycobacterium gryns, as it catalysed the enzymic breakdown of isocitric acid to form succinic and glyoxylic acids.

Kornberg et al.(1959) demonstrated from their experiments using C$^{14}$O$_2$ and acetate-1-C$^{14}$ that Escherichia coli strain W growing on acetate utilised acetate via the TCA and glyoxylate cycle. Kornberg and Lund(1959)
found that the growth of *Pseudomonas ovalis* on substrates (butyrate, malonate, lysine, tyrosine and phenylalanine) which yielded acetate or acetoacetate as principal products in mammalian systems was also accompanied by isocitratase formation. Both isocitratase formation and growth were abolished or inhibited by addition of chloromphenicol (50 μg/ml) to succinate grown cells adapting to acetate. So they suggested that the isocitratase formation appeared to be associated with de novo synthesis of protein. Malate synthetase and condensing enzyme were present under all conditions of growth in *P. ovalis* in high activity. Isoeitratase might be the only enzyme of the glyoxylate cycle under adaptive control and malate synthetase was constitutive to *P. ovalis*.

While working with *E. coli* Bolcato (1959) suggested that the glyoxylate was formed through the action of malate synthetase on malate or of isocitratase on isocitrate. He concluded that glyoxylate was an intermediate in the oxidation of acetate and glycolate and deserved its place in the monocarboxylic acid scheme.

Experimental evidence with *E. coli* provided the hypothesis that the function of isocitratase and malate synthetase was to permit *E. coli* to grow and multiply on acetic acid or other C₂ molecules as sole carbon source (Reeves and Ajl, 1960). Both these two enzymes were formed when *E. coli* was grown aerobically on a variety of substrates. Kornberg et al. (1960) obtained high activity of isocitratase in the acetate grown cells of *Micrococcus denitrificans*. The activity of glyoxylate cycle enzymes in these cells was high enough to account for the growth of the organism on acetate as sole source of carbon. McCurdy and Cantino (1960) purified the enzyme isocitratase about 50 fold from the extracts of *Blastocladiella emersonii* which reversibly catalysed the conversion of D-isocitrate to glyoxylate and succinate.
MacLeod et al. (1960) obtained malate synthase and isocitratase from a marine bacterium when the cells were grown in the medium with acetate as sole carbon source. Collins and Kornberg (1960) found that the isotope from acetate-2-C\(^{14}\) when added to Aspergillus niger growing on acetate as sole carbon source was rapidly incorporated into cellular components. Isotope from acetate entered the TCA cycle at 2 sites, to form citrate at one and malate at the other. They found that two key enzymes of the glyoxylate cycle, isocitritase and malate synthetase, were present in cell-free extracts. Cell-free extracts of the mold grown on glucose or succinate contained malate synthetase but only traces of isocitritase. Bullin and Hassall (1960) found that the cell-free extracts of Pseudomonas fluorescens grown on butane 2,3-diol possessed isocitritase activity as high as when the cells were grown on acetate, and about 20 times as high as when the cells were grown on succinate. This extract also exhibited the malate synthetase activity. Gottlieb and Ramachandran (1960) found an operative glyoxylate cycle during germination of the spores of Penicillium oxalicum (P-56 strain) in the media containing either acetate or glucose. Both isocitritase and malate synthetase activities were present in the cell-free extracts when the spores were germinated in the presence of either glucose or acetate as substrate, but higher concentration was produced by acetate. The results indicated that isocitritase and malate synthetase were constitutive enzymes in germinating spores of P. oxalicum.

While studying with Neurospora crassa Turian (1961a) found high isocitritase activity when it was cultivated in the presence of acetate but did not show this marked activity if cultured on sucrose. The induced hyper isocitritase activity suggested adaptive enzymic synthesis.

Ghosh and Vinze (1961) showed that acetate was rapidly oxidised by the spores of Penicillium chrysogenum by way of glyoxylate.
Experimental evidence of the absence of isocitratase and malate synthetase in the anaerobically grown *E. coli* in glucose-citrate medium was shown by Reeves and Ajl (1962a). They demonstrated that the adaptation of such cells to aerobic growth in a medium containing acetate as a sole carbon source involved at least two separate events. The first appeared to be an adaptation which permitted the oxidative dissimilation of acetate and synthesis of condensing enzyme. The second was the synthesis of isocitratase and malate synthetase. Finally the authors concluded that the enzymes of glyoxylate bypass were essential for the bacteria to grow on acetate as sole carbon source and suggested that these enzymes had no major function in the aerobic oxidation of acetate by resting cells.

Reeves and Ajl (1962b) isolated an auxotroph of *E. coli* (E26-6) which was unable to grow on acetate mineral salts medium but did grow on this medium when supplemented with glutamate, isocitrate or any one of the C4 acids of the TCA cycle. It was suggested that the malate synthetase and isocitratase reactions provided a mechanism by which the bacteria could grow on a medium containing acetate as the sole source of carbon.

McFadden and Howes (1962a) found that intact cells of *Pseudomonas indigofera* oxidised acetate, ethanol, fumarate, glyoxylate, α-keto glutarate, malate, oxaloacetate, pyruvate and succinate to greater than 35% of completion. They investigated the levels of isocitrate lyase and malate synthetase as functions of growth substrates. Assays for these in soluble preparations were performed under optimal conditions for catalysis. Furthermore, these authors also showed that provision of succinate to cultures grown on acetate apparently inhibited isocitrate lyase synthesis during further cell division.

Howes and McFadden (1962) studied the specific activities of isocitrate lyase and malate synthetase from *Pseudomonas indigofera* as a function of
growth. Activity of each enzyme was maximum at the mid of the late exponential phase. Higher concentrations of glucose, malate and yeast extract suppressed both enzymic activities. Glyoxylate at higher concentration suppressed isocitrate lyase but substantially stimulated malate synthase. Rosenberger (1962) showed high isocitrate lyase activity in _Achromobacter_, strain D15 during its growth on media containing both succinate and acetate or L-malate and acetate. Cells grown on L-malate or D-tartrate or succinate had approximately one-tenth isocitrate lyase content than that of acetate grown cells. Cells grown on dicarboxylic acids plus acetate had the same activity as those grown in acetate alone. Goldman and Wagner (1962) partially purified isocitrate lyase and malate synthase from the cell free extracts of _Mycobacterium tuberculosis_ H 37 Ra. Isocitratelyase was optimally active at pH 7.4 and required Mg$^{++}$ and cysteine. Malate synthase was optimally active at pH 8.5 and showed partial requirement of Mg$^{++}$.

Kennedy and Dilworth (1963) reported isocitrate lyase activity in _Azotobacter vinelandii_ when it was grown on acetate. The authors also reported the requirement of Mg$^{++}$ and cysteine for isocitrate lyase and found EDTA could replace the requirement of cysteine more effectively. McFadden and Howes (1965) crystallised the enzyme isocitrate lyase from _Pseudomonas indigofera_. They found pH optima of this enzyme to be 7.7 and requirement of a thiol group either glutathione or cysteine as well as metal ion, Mg$^{++}$ for catalysis. Kornberg (1963) presented evidence for the hypothesis that acetate might combine with some other metabolites to relieve repression of isocitrate lyase synthesis in _E. coli_. The author found that when the bacteria were grown in media containing 20 mM of any one of glycerate, glycerol, malate, fumarate, alanine, pyruvate, lactate, succinate, aspartate or glycolate as carbons source, had the identical
specific activity when grown in media which contained 20 mM of acetate in addition. It showed that acetate was unable to induce synthesis of isocitrate lyase in presence of these substrates.

Vanderwinkel et al. (1963) after experiments with E. coli K12 strain VG concluded that the growth of this organism on acetate or glycolate was possible through the occurrence of inducible metabolic pathways. A high level of isocitratase and malate synthase were found after growth on acetate and there was inhibition if glucose or succinate were present in addition to acetate. Growth on glycolate induced the formation of malate synthase but there was little change in isocitratase. Aj1 and Reeves in the year 1963 supported the hypothesis that isocitratase and malic synthase were adaptive in nature and essential for aerobic growth of bacteria on 2-Carbon compounds. Shah and Ramakrishnan (1963a,b) partially purified the enzyme isocitrate lyase from Aspergillus niger grown on citrate. This enzyme was activated by MgCl₂. They also purified the enzyme acetyl-CoA kinase from the cell free extracts of A. niger.

Stouthamer et al. (1963) found isocitrate lyase and malate synthase in the cell free extracts of Acetobacter acetii grown on the mineral medium with acetate as sole carbon source. Isocitrate lyase was absent in glycerol and lactate grown cells. Innami et al. (1963) found high isocitratase activity in the cell free extracts of Aerobacter mannanolyticus grown on a medium containing either Na-citrate, Na-acetate and Na-glyoxylate or Na-malate, but cells grown on glucose media were devoid of isocitratase activity. They suggested that a pathway through glyoxylate seemed to exist in this organism on Na-citrate or Na-acetate-Na-glyoxylate media.

Kazuo Kimura et al. (1962) observed high activity of isocitratase in the cells of Micrococcus glutamicus when acetate was supplied as sole source...
of carbon. High activity of isocitratase was observed when cells were cultivated on glucose medium rich in biotine (10-20Y/l), while activity of this enzyme was barely detected in biotine deficient medium which was favourable for glutamate synthesis. It was noted that in biotine rich medium isocitrate was oxidised through a glyoxylate by path, while in biotine deficient medium isocitrate was completely converted to glutamate because of the lack of isocitratase.

Kornberg et al. (1964) found that the strain Achromobacter d-15 formed large quantity of isocitrate lyase when acetate was added in the culture growing on succinate. Isocitrate lyase was also formed during growth of the organism on 2-Carbon compound such as lactate, alanine or pyruvate. French et al. (1964) reported the occurrence of isocitrate lyase and malate synthase in the cell free extracts of acetate grown cells of Micrococcus denitrificans. The findings led the authors to conclude the occurrence of an unknown pathway of acetate utilisation.

Polakis and Bartley (1965) observed that during aerobic growth both glucose and galactose were equally effective in repressing almost completely the enzymes of glyoxylate bypass in Saccharomyces cerevisiae. Kobr et al. (1965) assayed the enzymes, regulating the breakdown of isocitrate in Neurospora crassa grown on various concentrations of sucrose and sodium acetate. Isocitrate lyase, NADP isocitrate dehydrogenase, NAD isocitrate dehydrogenase were detected and their activities increased with decreasing sucrose concentration or by supplementing sodium acetate. According to the authors the physiological adaptation of N. crassa to utilise sodium acetate as carbon source involved an increase in the breakdown of isocitrate by oxidation to α-ketoglutarate or by cleavage to succinate and glyoxylate.
Darson (1967) isolated a thermophilic spore forming bacteria from soil on a medium containing acetate as carbon source. The enzyme isocitrate lyase was found in the cell free extracts of the bacteria grown on acetate but specific activity decreased by a factor of 400 when the organism utilised glucose as carbon source. The enzyme was more heat stable than pure isocitrate lyase from Pseudomonas indigofera and its activity was stimulated by Mg++ but cysteine and EDTA had little effect. Wegener et al. (1967) detected high malate synthase activities in E. coli grown on acetate. Duntze et al. (1967) detected greater activity of isocitrate lyase and malate synthase in the acetate grown cells than in the glucose grown cells of three species of yeast - Saccharomyces cerevisiae var ellipsoideus, Hansenula anomala W-10 and Rhodotorula glutinis RH 1413. The extensive increase in the enzymes of the glyoxylate bypass during incubation with acetate emphasised the importance of this pathway for gluconeogenesis in yeast. Wegener et al. (1967) reported a more or less similar control mechanism of malate synthase formation in a fumaric acid producing strain of Rhizopus nigricans to that of isocitrate lyase. Both acetate and glycolate acted as apparent inducers of malate synthase but glycolate was more effective of the two when added alone. The authors concluded that acetate stimulated the formation of glyoxylate bypass enzymes by derepression mechanism whereas glycolate or a product derived from it acted directly as an inducer.

Flavell and Fincham (1968b) measured the level of Krebs cycle, glyoxylate cycle enzymes and certain other enzymes in the wild type strain and in 7 groups of acetate non-utilising mutants of Neurospora crassa both after growth on a medium containing sucrose and after a subsequent 6 h incubation in a similar medium containing acetate as the sole carbon source. In the wild strain incubation in acetate medium caused a rise in
the level of isocitrate lyase, malate synthase, acetyl-CoA synthase, phosphoenol pyruvate carboxykinase, NADP linked isocitrate dehydrogenase, citrate synthase and fumarate hydratase. They found the absence of isocitrate lyase activity in \textit{acu-3} mutants and acetyl-CoA synthase activity in \textit{acu-5} mutants. Oxoglutarate dehydrogenase activity could not be detected in \textit{acu-2} and \textit{acu-7} mutants and \textit{acu-6} mutants lacked phosphoenol pyruvate carboxykinase activity. Vanderwinkel and Devlieghere (1968) found two types of malate synthase in \textit{E. coli}. One of them was malate synthase A, produced with isocitratase during growth on acetate and other was malate synthase G, found when grown on glycolate as sole carbon source. The mutants they obtained were impaired in growth on acetate (\textit{acu A, acu B}) and were deficient in isocitratase and malate synthase A respectively.

Klein and Jahnke (1968) detected highest specific activity of acetyl-CoA synthase in the microsomal fraction of \textit{Saccharomyces cerevisiae} when grown with glucose in standing culture. They found that addition of acetate did not affect the distribution of the enzyme. Brice and Kornberg (1968) isolated four mutants (\textit{icl}) of \textit{E. coli} which were unable to form isocitrate lyase and found that isocitrate lyase was necessary for growth of \textit{E. coli} on acetate as carbon source. All these \textit{icl} mutants were cotransducible with \textit{met A}. Since the gene specifying the second enzyme of the glyoxylate cycle, malate synthase (\textit{mas A}) was also cotransducible at high frequency with \textit{met A} and \textit{icl}, according to the authors, it was possible that the structural and regulator genes of the glyoxylate cycle formed an operon.

Isamu \textit{et al.} (1969) isolated acetate kinase negative, phosphate acetyl transferase negative and isocitrate lyase negative mutants of \textit{Brevibacterium flavum}. All these strains grew on glucose but not on acetate. These facts led the authors to conclude that these enzymes were
essential for the acetate utilisation by *B. flavum* but not for glucose utilisation. Aitken and Brown (1969) obtained two key enzymes of glyoxylate cycle, isocitrate lyase and malate synthase, in a strain of *Halobacterium salinarium* only when citrate was replaced by acetate in the growth medium. Casselton *et al.* (1969) detected isocitrate lyase activity in the cell free extracts of both monokaryon and dikaryon of *Coprinus lagopus* grown in a medium containing either glucose or acetate as carbon source. But greater activity was found with acetate.

Duntze *et al.* (1969) studied the regulation of the enzymes of glyoxylate cycle under the influence of the carbon source of the medium. These authors were interested in whether the glyoxylate cycle in yeast was regulated and operated as a functional unit, independently from the citric acid cycle. They investigated the distribution of the enzyme activities in cell free fractions obtained by differential centrifugation. They indicated that there was a partial separation of the glyoxylate cycle and the citric acid cycle in different components of the cell but there was a close functional relationship between them.

Combepine and Turian (1970) obtained higher malate synthase activity in the acetate grown cell extracts of *Neurospora crassa*. While working with *A. nidulans* Amitt *et al.* (1970) found that the level of isocitrate lyase activity in the cell free extract was low during growth on sucrose, glucose, glycerol and glutamate but high during growth on acetate and ethanol. Isocitrate lyase could be induced by transferring the mycelium from a sucrose to an acetate medium. The investigators also found that isocitrate lyase was no longer formed when mycelium was transferred from acetate to sucrose and that enzyme formed in acetate was not destroyed but gradually diluted out during growth. Mutants devoid of isocitrate lyase activity which did not grow on acetate were isolated by the authors.
Cotter et al. (1970) found that the activity of isocitrate lyase was repressed in glucose cultures of Schizophyllum commune and derepressed in acetate grown mycelia of this basidomycetous fungus.

Herman and Bell (1970) observed a rapid induction of isocitrate lyase by addition of acetate in the culture of Acinetobacter sp. growing in a medium containing either succinate or l-malate (C₄ intermediates). Trust and Millis (1970) identified acetyl-CoA synthetase (Ec 6.2.1.1) activating C₁–C₄ fatty acids and an acetokinase which was specific for acetate in the yeast Torulopsis grown on n-hexadecane. This was the first report of acetokinase in yeast. Benveniste and Munkres (1970) found the presence of glyoxylate cycle enzymes by transferring the mycelia of Neurospora crassa from glucose to acetate as carbon source. Donawa and Inniss (1970) found high isocitrate lyase and malate synthase activities in acetate grown cells of Bacillus megaterium when compared with glucose grown cells. Chloramphenicol prevented the increase in isocitrate lyase activity when cells were transferred from glucose to acetate media, indicating that such an increase in activity was probably due to de novo protein synthesis.

Kornberg (1970) showed that the glyoxylate cycle acted as an anaplerotic way during growth of E. coli on acetate and its key enzymes constituted an operon. Krasilnikova (1970) found that with acetate as sole carbon source, purple bacteria (Rhodopseudomonas palustris, R. spheroides, Ectothiorhodospira shaposhnikovii and Chromatium minutissimum) form isocitrate lyase, an enzyme essential to the glyoxylate cycle. Kleber and Mueller (1970) observed an induction of isocitrate lyase in Pseudomonas aeruginosa under aerobic condition when acetate or L-carnitine was supplied as sole carbon and nitrogen source. The isocitrate lyase activity induced by acetate resulted from the decomposition of L-carnitine where as succinate, pyruvate and glucose inhibited it. Malate synthase, another enzyme of the glyoxylate cycle of P. aeruginosa was constitutive and independent of carbon source.
Flavell and Woodward (1971) suggested that the synthesis of isocitrate lyase (EC 4.1.3.1) and malate synthase (EC 4.1.3.2) in Neurospora crassa was apparently regulated by a glycolytic intermediate or derivative. These glyoxylate shunt enzymes were present comparatively at high levels in the mycelia grown on acetate as sole carbon source than the mycelia grown on sucrose as the sole carbon source. While working with Thiobacillus novellus, Michael (1971) found high activities of the enzymes of citric acid and glyoxylic acid cycles in the acetate grown cells. Ermakova and Finogenova (1971) obtained 6-10 times higher isocitrate lyase activities in the cells of Candida lipolytica grown on acetate or hexadecane than in the cells grown on glucose. Gosling and Duggen (1971) found that baker’s yeast oxidised acetate at a high rate only after an adaptation period during which the capacity of the glyoxylate cycle increased. They also found no necessity of the activity of acetyl-CoA synthase for this adaption. Arnaud et al. (1971) observed about 29% greater activities of isocitratase in Saccharomyces cerevisiae when grown with 0.1 to 0.2 M. Potassium acetate at pH 4.5 for 16 h than in normally grown cells. Incubation of the cells for 16 h without Potassium acetate in phosphate or bicarbonate buffer at pH 7.0 caused a 50% fall in isocitratase, but addition of Potassium acetate caused a 35% increase of activity.

Kitano et al. (1972) isolated mutants from Brevibacterium thiogenitalis defective in glutamate dehydrogenase (GDH), isocitrate dehydrogenase (ICDH), isocitrate lyase (ICL) or aconitate hydratase (AH) activity. In ICL and ICDH, acetate was practically completely oxidised. However AH lost the ability to oxidise acetate. The authors suggested that the present strain had two alternative pathways of acetate oxidation via the TCA and the glyoxylic acid cycles and it failed to oxidise acetate without aconitate hydratase; that is, acetate could never be metabolised without passing through the citrate-cis-aconitate-isocitrate system. Bellione and
Hersh (1972) found that when the cells of a *Pseudomonas* sp. were grown on acetate or methylamine, but not on succinate, contained the enzyme isocitrate lyase; while cells grown on acetate or succinate but not methylamine contained significant levels of malate synthase.

McCarthy and Michael (1973) purified the enzyme isocitrate lyase by about 8 folds from the crude extract of acetate grown cells of *Thiobacillus novellus*, a facultative autotroph. Kobr and Vanderhaeghe (1973) observed that the density of glyoxysome like particles (GLPs) was affected by growth conditions and by the isolation procedure. The equilibrium density of both mitochondria and GLP was affected by the nature of the carbons source. Growth conditions which derepressed the glyoxylate cycle enzymes and some Kreb cycle enzymes also increased the density of both the organelles. It was found from 50% sucrose density gradient result that all of the II activity was confined to a single peak with a mean density of 1.219 g/cm³. Regardless of sucrose concentration the NAD isocitrate dehydrogenase peak was consistently at around 1.195 g/cm³.

Palmagne and Wiame (1973) isolated and purified both malate synthase isoenzymes A and G, involved in the utilisation of acetate and glycolate respectively from an *E. coli* strain grown on glycolate. They also found out the molecular weight of malate synthase A and G which were 54000 and 52000 respectively and their N-terminal residues were threonine and serine respectively. Griffiths and Sundaram (1973) obtained isocitrate lyase from a thermophilic *Bacillus* which was activated about 3 fold by a variety of salts. Such strong stimulation was not obtained with isocitrate lyase from mesophiles *B. licheniformis*, *B. megaterium*, *E. coli* and *Aspergillus nidulans*. This salt activation was strongly pH dependent.

Hildebrandt and Weide (1973) purified the enzyme isocitrate lyase by 3.7 fold *Candida guilliermondii* strain H17 cultured on acetate. The
optimum pH in phosphate buffer was 6.8, the Km for threo-D-3-isocitrate was 0.3 mM and enzyme showed one active site/enzyme molecule. Kleber and Aurich (1973) found that isocitrate lyase was inducibly formed when *Acinetobacter calco-aceticus* was cultivated on media containing aliphatic hydrocarbons or acetate. Actinomycin C, chloramphenicol, pyruvate, succinate and glucose repressed the synthesis of this enzyme. Malate synthase activity in *A. calco-aceticus* was twice as great if the microorganism was cultivated on n-alkanes and acetate as opposed to cultivation on yeast extracts, malate or succinate media. Krainova and Bonartseva (1973) identified isocitrate lyase in the bacteria *Propionibacterium petersonii* and *P. shermanii* when they were grown for three days in buffered medium with glucose and sodium acetate. Kraisilnikova et al. (1973) reported about several phototrophic bacteria which while growing on an acetate containing medium manifested an appreciable isocitrate lyase activity suggesting the availability of the glyoxylate shunt. Samuel and Ailhaud (1973) found an acetyl-CoA synthase in the supernatant of *Pseudomonas putida*.

Satyanarayana et al. (1974) indicated the presence of two types of acetyl-CoA synthetase in a strain of *Saccharomyces cerevisiae* grown under aerobic and anaerobic conditions. Isocitrate lyase, the first enzyme of the glyoxylate bypass, was isolated by Capalna (1974) in the partially purified state from *Achromobacter* sp. (IV-S). Casselton and Casselton (1974) selected acetate non-utilising mutants of *Coprinus lagopus* on a glucose medium containing fluoroacetic acid. The mutants were mapped at 3 different loci designated acu-1, acu-11 and acu-12. Mutation at acu-1 locus led to loss of acetyl-CoA synthetase activity but the function of other two loci were not resolved and did not involve either this enzyme or isocitrate lyase. Johanson et al. (1974) purified isocitrate lyase of
Neurospora crassa and obtained homogeneous sample of the enzyme. The enzyme was found to be inhibited by its product succinate and glyoxylate and phosphoenol pyruvate, fumarate, malate and fructose-1,6-diphosphate. Isocitrate lyase from both N. crassa and P. indigofera was found to be completely inhibited by the inorganic anions $\text{SO}_4^{2-}$, $\text{HPO}_4^{2-}$, $\text{Cl}$ and $\text{NO}_3$.

Shih et al. (1975) demonstrated the occurrence of a high level of isocitrate lyase and malate synthase when the cells of Pseudomonas putida were grown on acetate, lipoate or acetoate and lower level when grown on glucose. But levels of acyl-CoA synthase were not affected by the source of carbon. The authors suggested the induction of glyoxylate cycle to facilitate utilization of acetyl-CoA derived from lipoate. Beever (1975) concluded that synthesis of both 2-phosphoenol pyruvate carboxykinase (PEP carboxykinase) and isocitrate lyase was regulated by repression rather than induction. He obtained the conditions which showed that PEP carboxykinase was regulated through repression by a glycolytic intermediate and that isocitrate lyase was regulated through repression by a 4-Carbon acid common to the glyoxylate and tricarboxylic acid cycles. Bellion and Woodson (1975a) isolated and studied isocitrate lyase of acetate and methyamine grown Pseudomonas MA (shaw strain). Ignatov and Stupnikova (1975) isolated acetyl-CoA synthetase from Staphylococcus aureus. The authors partially purified the enzyme and studied its several properties.

Maxwell et al. (1975) isolated microbodies from different groups of fungi, both higher and lower. Microbodies were found to be concentrated at the hyphal tips. Their morphology varied among the fungi and their number varied with the variation of carbon sources in the growth medium. Isocitrate lyase and malate synthetase activities were obtained in hyphal extracts of all 13 fungi tested. Wagner and Levitch (1975) obtained a mutant of Pseudomonas MS which was unable to grow on 1-Carbon methyamine.
and also on acetate. Growth on methionine caused an induction of isocitrate lyase, a key enzyme of glyoxylate cycle. This mutant lacked isocitrate lyase and an enzyme of serine pathway malate lyase. It was suggested that utilisation of 1-Carbon unit by Pseudomonas MS resulted in the net accumulation of acetate which was then assimilated via the glyoxylate pathway. Chell and Sundaram (1975a) prepared identical and homogeneous isocitrate lyase and malate synthase from wild and a mutant PC2NG35 of Bacillus stearothermophilus. Chell and Sundaram (1975b) also found that Bacillus stearothermophilus mutants PC2NG35, PC2E2 and PC2S1 produced more isocitrate lyase and malate synthase than wild type. According to the authors these might be the cause why the mutants grew more slowly than the wild on acetic acid as sole carbon source. Varimo and Oura (1975) purified isocitrate lyase from Saccharomyces cerevisiae. They found that oxalacetate could not act as feedback inhibitor of isocitrate lyase in bakers yeast. Sariaslani et al. (1975) found that Nocardia salmonicolor showed higher activities of isocitrate lyase when grown on acetate, commercial DL-lactate or hydrocarbon substrate compared with those resulting from growth on other carbon sources. This presumably reflected the anaplerotic role of glyoxylate cycle during growth on the former substrates. When acetate was added to the cultures growing on glucose, there followed de novo synthesis of isocitrate lyase and isocitrate dehydrogenase with increase in growth rate and glucose utilisation and both glucose and acetate were metabolised simultaneously. Only succinate and fumarate prevented an increase in enzyme activity in the presence of acetate. The authors also found that even a minute amount of acetate (40μM) when added to glucose limited continuous culture caused a 3 fold increase in isocitrate lyase activity within 3 min of addition. These results were consistent with the induction of isocitrate lyase synthesis by acetate or closely related metabolites and catabolites.
repression by a 4-Carbon acid of the bicarboxylic acid cycle, possibly fumarate.

Takahashi (1975) showed that Escherichia coli cells grown in presence of 2% glucose had low respiratory activity with respect to acetate and succinate. The author also found that succinate dehydrogenase and isocitrate lyase were synthesised during the recovery from glucose repressed respiration.

Bellion and Woodson (1975b) reported that isocitrate lyase, produced by Pseudomonas MA when grown on acetate differed from that produced when grown on methylamine as carbon source. Kim and Wee (1975) reported that isocitrate lyase and malate synthase were active in Brevibacterium ammoniagenes grown on acetate media indicating that the glyoxylate cycle was operational. The specific activities of isocitrate lyase from the acetate media was about 70 times greater than that from glucose media.

Holten (1976) found that both acetate and pyruvate were oxidised via tricarboxylic acid cycle in species of Neisseria; N. elongata and false neisserias (N. catarrhalis, N. ovalis and N. caviae) oxidised acetate in absence of other substrates indicating operative glyoxylate cycle in these species. In the true neisserias other than N. elongata acetate was oxidised only in the presence of glutamate indicating that these species did not possess a glyoxylic acid cycle. Lozinov et al. (1976) showed that the yeast strain not metabolising n-alkanes but growing on acetate as the only carbon source, the activity of isocitrate lyase were comparable to those of the alkane metabolising strain. The authors concluded that this enzyme was apparently not responsible for the lack of growth of yeast on n-alkanes.

Armit et al. (1976) isolated and genetically analysed 119 acetate non-utilising mutants of Aspergillus niger and revealed 10 new loci affecting acetate metabolism. The enzyme lesion associated with mutation at 7 of the acu loci were: acu A, acetyl-CoA synthase (EC6.2.1.1) acu D isocitrate
lyase (EC 4.1.3.1); acu E, malate synthase (EC 4.1.3.2), acu F, phosphoenol pyruvate carboxykinase (EC 4.1.3.2), acu G, fructose 1,6-diphosphatase (EC 3.1.3.11), acu K and acu M, malic enzyme (EC 1.1.1.40). The acu loci were widely distributed over the A. nidulans genome, close linkage was observed only between acu A and acu D (<1% recombination). Poor induction of isocitrate lyase and malate synthase in mutants lacking acetyl-CoA synthase and also in two classes of fluoroacetate resistant mutants indicated that acetate might be metabolised to a trace metabolic inducer, possibly acetyl-CoA to induce the enzyme. The acu D and acu E genes seemed to be subject to negative control.

Satyanarayana and Klein (1976) purified acetyl-CoA synthetase from aerobic cells of Saccharomyces cerevisiae. They determined the molecular weight of the enzyme which was 250,000 ± 500. The molecular weight of a single poly peptide chain was 83000 ± 500. These studies indicated that acetyl-CoA synthetase of aerobic S. cerevisiae was composed of three sub-units of identical or nearly identical size.

Sundaram et al. (1976) purified isocitrate lyase, malate synthase and pyruvate carboxylase of Bacillus stearothermophilus var nondiastaticus. Coleman and Bhattacharjee (1976) showed that acetyl-CoA synthetase level was repressed when the cells of S. cerevisiae were grown in a complex nutrient medium as opposed to the minimal medium. However, the authors found that a glutamic acid auxotroph when grown on excess glutamic acid demonstrated a 5 fold increase of acetyl-CoA synthetase. Krulwich et al. (1976) found that Arthrobacter pyridinophilis and A. crystallopoetis both possessed glyoxylate pathways that were induced by acetate but not by hexoses. Isocitrate lyase deficient mutant of A. pyridinophilis failed to grow on rhamnose and fructose as well as acetate.
Taylor and Anthony (1976) concluded from the growth pattern of a mutant (ICT54) of Pseudomonas AMI which lacked inducible acetyl-CoA synthetase (EC 6.2.1.1) and that this enzyme was essential for growth on ethanol, malonate and acetate plus glyoxylate. The properties of other mutants suggested that 'malate synthetase' activity was normally essential for growth of AMI strain on ethanol, malonate and 3-hydroxy-butyrate. The growth properties of a revertant (ICT51R) and of a mutant lacking maryl-CoA lyase (PCT57) indicated that an alternative route must existed for assimilation of compounds metabolised exclusively by way of acetyl-CoA. Hanozet et al. (1976) showed that the enzymes of alcohol metabolism, alcohol dehydrogenase, aldehyde dehydrogenase and acetyl-CoA synthetase of Rhodotorula gracilis were repressed by glucose but induced by 2-Carbon metabolic fuels such as ethanol, acetic acid and acetyl phosphate. Albers and Gottschalk (1976) studied acetate metabolism in Rhodopseudomonas gelatinosa and several other Rhodospirillaceae. R. gelatinosa produced both enzymes of glyoxylate cycle, isocitrate lyase and malate synthase when grown on acetate aerobically in the dark. However, under anaerobic conditions in the light only isocitrate lyase could be detected. Other Rhodospirillaceae were tested for isocitrate lyase and malate synthase activities after growth on acetate and according to this they could be divided into three groups: (1) organisms possessing both enzymes, (2) organisms containing malate synthase only and (3) R. gelatinosa containing only isocitrate lyase when grown anaerobically in the light.

Coleman et al. (1976) worked with Neurospora lineolate. Enzymes of glyoxylate cycle and (or) Krebs cycle were detected in mycelial extract when the organism was grown on minimal medium with 0.044 M acetate or sucrose. These findings led the authors to indicate the involvement of
these cycles in the metabolism of *N. lingo lata*. Westwood and Higgins (1976) found, cyclic AMP (3 or 30 mM) supplied exogenously to cultures of *Nocardia salmonicolor* growing on acetate, incompletely released catabolite repression of isocitrate lyase (EC 4.1.3.1) by Na-fumarate (0.1-0.5%). Failure to achieve total release of the repression might be due to low permeability of the bacterial membrane to cyclic AMP. Stupnikova and Tel’nova (1975) purified acetyl-CoA synthetase 70-fold from extracts of *Staphylococcus aureus*. The molecular weight of the enzyme was 50,000 as detected by the authors by gel filtration on sephadex G-200. Assis and Amaral (1976) demonstrated the glyoxylic acid cycle in wood rotting basidiomycete *Piceporus sinnabarinus*. The uptake of the intact cells grown on glucose, acetate or ethanol indicated the presence of the cycle. Both the enzymes of the cycle, isocitrate lyase and malate synthase were induced when the mold was grown on acetate or ethanol.

Nabeshima et al. (1977a) performed comparative studies on the activities of isocitrate lyase and malate synthase in *Candida* yeast grown on media containing glucose, n-alkanes or acetate as sole carbon source. In general the activities of both enzymes were higher in the cells growing on n-alkanes or acetate than in those growing on glucose. When cultivated on glucose, the level of isocitrate lyase activity in *C. tropicalis* was enhanced after the middle phase of growth, while the activity in *C. lipolytica* was maintained at a low level. The synthesis of isocitrate lyase in *C. tropicalis* growing on n-alkanes or acetate was repressed by the addition of glucose to the medium and derepressed when the cells grown on glucose were transferred to n-alkanes or acetate media indicating that the glyoxylate cycle had an important role in the metabolism of n-alkanes and acetate. Cycloheximide
inhibited the synthesis of isocitrate lyase in *C. tropicalis* growing on n-alkanes or acetate. Nabeshima et al. (1977b) found that the synthesis of isocitrate lyase in *Candida tropicalis*, the growth of which was stimulated by exogenously added biotin, was released from repression by glucose under biotin deficient conditions. Acetyl-CoA donating compounds such as pyruvate, acetate and alkanes on the other hand stimulated the synthesis of isocitrate lyase regardless of the presence or absence of biotin. The stimulated synthesis of isocitrate lyase under biotin deficient conditions was also observed in *C. albicans* and *C. guilliermondii*.

Gonzalez (1977) found that when glucose grown cells of *Saccharomyces cerevisiae* were transferred to fresh medium containing 2-and 3-Carbon substrate, the assimilative capacity for 2-Carbon substrates developed more slowly and followed the induction of isocitrate lyase. Washed yeasts transferred to basic medium containing no added substrate possessed only low levels of isocitrate lyase after 6 hour adaptation. After 6 hours, isocitrate lyase was present at high levels in cells transferred to a range of ethanol concentration but was present in only low amounts in cells transferred to acetate. Choinski and Mullins (1977) identified glyoxysome type microbodies in the fungus *Achlya ambisexualis*. The microbodies contained many enzymes including isocitrate and malate synthase. Al-SSum and White (1977) obtained high activity of isocitrate lyase in *Bacillus megaterium* when grown in presence of acetate but in absence of biotin; acetate allowed normal growth probably by induction of the glyoxylate cycle. Taniguchi et al. (1977) demonstrated about 3 fold increase of isocitrate lyase in *Rhizopus japonicus* in the presence of both sclerin and Na-acetate.
Brown et al. (1977) isolated mutants of *Escherichia coli* K12 which were devoid of acetate kinase (EC 2.7.2.1) or phosphotransacetylase (EC 3.2.1.8). These mutants incorporated labeled acetate in the presence of glycerol but not glucose and uptake was affected by an inducible acetyl-CoA synthetase (EC 6.2.1.1). Kelly and Hynes (1977) found that *cre* A 204, *cre* B 15 and *cre* C 27 mutations caused carbon catabolite derepression of acetyl-CoA synthase and isocitrate lyase in *Aspergillus nidulans*. The authors investigated the acitamidase levels of strains containing *cre* mutations and supported the hypothesis that an acetate metabolite rather than acetamide induced this enzyme. King and Casselton (1977) identified 13 chromosomal loci which affected acetate metabolism in *Coprinus cinereus*. Mutants at only 2 loci, *acu*-1 and *acu*-7 were deficient in isocitrate lyase (EC 4.1.3.1) activity, *acu*-1 was unable to induce isocitrate lyase because they lacked acetyl-CoA synthetase which was required to convert acetate to the metabolic inducer of isocitrate lyase. The authors showed this by selecting temperature sensitive *acu* revertants resulting from a second mutation within the *acu*-7 gene.

Stovall and Cole (1978) found that *Rhizobium japonicum* bacteroids isolated from soybean nodule oxidised $^{14}$C labelled succinate, pyruvate and acetate in a manner consistent with operation of the tricarboxylic acid cycle and partial glyoxylate cycle. Substrate carbon was incorporated into all major cellular components such as cell wall plus membrane, nucleic acids and proteins.
Bellione and Kim (1978) obtained 1-Carbon and 2-Carbon type of isocitrate lyase in the cells of Pseudomonas MA grown on acetate with methylamine as sole nitrogen source. Sealey et al. (1978) showed that acu-1 locus in Coprinus cinereus was the structural gene for acetyl-CoA synthetase. They induced five suppressor gene mutations which suppress the acu-1.34 missense allele by mutagenic treatment. Extracts of unsuppressed acu-1.34 mutant contained less than 2% of wild type acetyl-CoA synthetase activity, whereas extracts of 4 of the 5 suppressors strain showed activities ranging 28-37% of wild type. Only a slight increase in activity was detected in the fifth suppressor strain, but this was associated with a temperature sensitive sup* phenotype. All five sup* mutations restored the ability of the acu-1.34 mutant to induce isocitrate lyase, an enzyme which under the conditions of growth used, could only be induced when acetyl-CoA synthetase activity was present. Thus all five suppressors acted to restore normal acu-1 protein function.

Kleber and Wiss (1978) showed the activities of malic enzymes, eight enzymes of citric acid cycle and glyoxylate bypass in the cells of Acinetobacter calcoaceticus grown on acetate, C13-18 n-alkanes, L-malate and succinate. Isocitrate lyase activity was increased about 10 fold and malate dehydrogenase about 4 fold in the cells grown on acetate or n-alkanes. Inhibitor studies showed the increased activities to be due to induction of the enzyme synthesis. The importance of glyoxylate cycle in the utilisation of alkanes was concluded by the authors. Chell et al. (1978) isolated isocitrate lyase in a homogeneous state from a thermophilic Bacillus. This enzyme resembled...
in size with the cognate enzyme from the mesophilic Pseudomonas indigofera but was smaller than the enzyme from Neurospora crassa.

Chaudhuri and Sen (1979) isolated acetate non-utilising (acu) mutants of Aspergillus terreus IRRL 16043 by mutagenic treatment of the conidia with NTG.