# CHAPTER I

# INTRODUCTION

Citrus fruits are grown in tropical and sub-tropical regions throughout the world and rank probably third among the sub-tropical fruits of the world.

The origin and history of citrus fruits are not fully known. The most that can be said with confidence is that they are natives of Southern Asia (Bartholomew and Sinclair, 1951). Chinese literature written as early as 2200 B.C. refers to the cultivation of some species of citrus.

The lemon which belongs to the citrus species is commonly believed to be indigenous to India. Watt (1908) reported that species of 'lemons' such as C.medica Var-acida grow in India. These fruits are used for preparing beverages, pickles etc. The juice is also used for flavouring soups, curries, fish etc., since it imparts a pleasant taste and flavour. It is also used in domestic medicine.

The curative value of lemon juice in scurvy led to the identification of citrus fruits as important sources of ascorbic acid. However, citric acid is a major constituent in these fruits.

The origin of citric acid and other constituents present in the fruit has been a subject of great interest. The main question is whether they are formed in the fruit

tissue itself or whether they are formed in the leaf and transferred to the fruit. In the former case questions also arise regarding the mechanisms involved in their formation and accumulation in fruit tissue. The latter belief is referred to as the translocation hypothesis.

The translocation hypothesis received the support of Nitsch (1953) according to whom the acids are translocated from the leaves to the fruits which merely act as storage organs. Earlier, Gatet (1939 ) and Ballard, Magness and Hawkins (1922) found that the acidity in grapes and apples is higher at the centre than at the periphery of the fruit and that a reduction in leaf area decreases the acid content of the fruit. But the results must be considered equivocal as obviously such reduction would affect the supply of carbohydrate necessary for cellular synthesis and would affect organic acid content whether it is formed locally from carbohydrate or derived ready-made from the leaves. Similarly, regional differences in the concentration of enzymes could well account for the greater concentration of organic acid at the centre.

The alternate hypothesis that the presence of organic acids in fruits is due to conversion of carbohydrate into organic acids in the fruit vesicles and not due to translocation seems more plausible in the light of recent studies.

As early as 1933 Ricevuto suggested the formation in lemons of citric acid from reducing sugar and pentosans by enzymic action. Sinclair and Eny (1947) compared the total citric, malic and oxalic acids in mature leaves and in the juice and peel of mature Valencia orange fruits and found that the pattern of the acids in the three are different. They found the highest concentration of total malic and oxalic acids in leaves, whereas the juice contained the highest concentration of citric acid and the peel the lowest concentration of citric acid as well as total acids. These findings led them to suggest that the organic acids are synthesized in the vesicles from carbohydrates.

This hypothesis received substantial support from the ingenious studies carried out by Erickson (1957) who grafted a sweet lemon on a sour lemon plant and a sour lemon on a sweet lemon plant. Analyses of the fruits showed that the acidity of sweet lemons remained low (0.44%) and that of sour lemons remained high (5.2%) irrespective of the leaves by which they were nourished.

Aside from the characteristic of a lower acid concentration, the sweet lemon had a much higher reducing sugar concentration (5.17%) than the sour lemon (1.45%). The failure of sweet lemons to accumulate high concentrations of organic acids when grafted on sour lemon plants indicates

that the high concentration of organic acids in the lemons is more complex than would result merely from translocation of the acid from leaves to fruit and concentration in the latter site.

These studies suggest substantial differences between the composition of leaves and fruits and changes in the same with development. The suggestions deriving from these studies have been amply supported by enzyme studies which show that the fruit tissue possesses the enzyme machinery necessary for the synthesis of citric acid in the case of lemon (Ramakrishnan and Varma, 1959) and garcinia (Deshpande and Ramakrishnan, 1961).

Nada (1954) who analysed fruits of Vitis vinifera at different stages of development for their sucrose and total acid contents observed an increase in acidity and a decrease in sucrose and polysaccharides during the initial stages of growth and the reverse phenomenon during the later stages. A similar observation was made by Deshpande and Ramakrishnan (1961) in fruits of Garcinia (Xanthochymus guttiferae). Studies carried out by Ramakrishnan and Varma (1959) showed an association between increase in citric acid content and decrease in carbohydrate during the development of lemon fruit (Citrus acida). These authors (1959) also found that the contents of sugars, protein and acidity of young and

old leaves and of stems bearing young fruits and those bearing mature fruits do not differ when considered in terms of percentage dry weight. But, in the case of fruits, the sugar and protein content decrease and the acidity increases with growth. The pattern of organic acids is the same in young and old leaves and stems bearing young fruits and those bearing mature fruits, citric, malic, succinic, fumaric and oxalic acids being present in both categories of leaves and stems. But in the case of fruits the pattern of organic acids varies with development. Young fruits are found to contain only malic, fumaric and oxalic acids. When the fruits attain a size of 1.5 cm diameter citric acid begins to accumulate, fumaric acid disappears, and oxalic acid is still present in small amounts. As they reach maturity, citric acid is the major constituent, malic acid being present in small amounts.

It was found that both the lemon and the garcinia fruit possess the enzymes necessary for the formation of citric acid and the mature fruit is characterised by a high activity of citrate synthase and the disappearance of aconitase (Ramakrishnan and Varma, 1959; Deshpande and Ramakrishnan, 1961).

The evidence cited above seems to have been ignored by Lioret and Moyse (1963) who favour the hypothesis of

translocation. They report the absence of citrate synthase in fruits accumulating citric acid. This is surprising since the enzyme has been found in lemon (Ramakrishnan and Varma, 1959) and garcinia (Deshpande and Ramakrishnan, 1961). It is possible that the enzyme preparation in their experiments was inactivated due to sudden release of excess acid during the preparation of the homogenate.

Recent studies suggest that even the carbohydrate necessary for organic acid synthesis may be derived from the outer skin of the fruit which is shown to have photosynthetic capacity. Bean and Todd (1960) while studying the $C^{14}O_2$ uptake by young oranges in light and dark found that the sugars are highly labeled in the photosynthesizing flavedo. Sucrose is found to be labeled to an appreciable extent in the albedo of the intact fruit but only a very small activity is found in sucrose in the illuminated isolated albedo. This suggested that the major activity in the albedo of the intact photosynthesizing fruit is due to translocation from flavedo. A very slight amount of activity is found in illuminated, isolated vesicles. On the other hand acid fractionation has shown that citric acid is low in concentration in the peel tissue whereas it is a major acid in the vesicles.

A number of factors indicate that products such as

citric acid formed during dark fixation must be formed within each tissue rather than by extensive translocation from one tissue to another. In the intact fruit photosynthesis results in the increase and redistribution of the activity in flavedo but has no effect on that found in the vesicles. Even in the albedo of the intact fruit the changes due to photosynthesis appear to be restricted to carbohydrate components. In contrast the vesicles accumulate a large amount of activity during dark fixation.

However the fixation of carbon dioxide also occurs in the dark and appears to be a general reaction for plants (Krotkov et al, 1958; Kunitake et al, 1959). In some succulent plants a net uptake of carbon dioxide may occur in the dark to give rise to an increase in organic acid (Gregory et al, 1954; Thomas, 1949; Thomas and Beevers, 1949; Thomas and Ranson, 1954).

The uptake of $CO_2$ during dark fixation appears to depend upon utilization of sugars to form acceptor units. Sugars are depleted while the acids increase. The reaction of phosphoenol pyruvate with $CO_2$ (Tchen and Vennesland, 1955) appears to be one of the reactions responsible for the dark fixation in succulents and other plants (Saltman et al, 1956; Walker, 1956, 1957). In citrus vesicles a high activity of phosphoenolpyruvate carboxylase has been demonstrated by Huffaker

and Wallace (1959). Davies (1956) has demonstrated the association of phosphoenol pyruvate carboxykinase with pea mitochondria and such systems have been more extensively studied in other tissues by Mazelis and Vennesland (1957) and Benedict and Beevers (1961). A number of enzymes which catalyze the fixation of carbon dioxide into acids in plants have been reported (Vennesland and Conn, 1952; Vennesland, 1960; Vishniac, Horecker and Ochoa, 1957; Walker, 1962).

Reports on the feasibility of cultivating the lemon fruit in vitro in a nutrient medium containing mineral salts and sucrose (Schroeder, 1960; 1961; Kordan, 1962; 1963a, b, c; 1964; 1965b) and the demonstration of starch synthesis in vitro in lemon fruit (Kordan, 1965a) indicate the independence of fruit tissue as a metabolic entity capable of synthesizing its own cellular constituents.

The mechanism by which glucose and other compounds are utilised for energy production has naturally been a subject of great interest since their oxidation in living tissues is quite different from their combustion outside the body.

In the case of animal tissues and micro-organisms studies carried out by Hopkins, Meyerhof, Parnas, Lohmann, Embden, Harden, Neuberg, Warburg, Cori and Cori and others helped to postulate the glycolytic pathway for the initial breakdown of carbohydrate (Dickens, 1951; Nord and Weiss,

1951; Green, 1954). The widespread operation of this pathway in other animal tissues (Novikoff et al, 1948; DuBois et al, 1948; Wu and Chang, 1948; de Vincenti, 1947), plants (James et al, 1941; Giri and Ramasarma, 1956), bacteria (Utter and Werkman, 1941; LePage and Umbreit, 1943) and molds (Bernhauer and Iglauer, 1936a; b; Clutterbuck, 1936; Butkevich and Gaevskaya, 1935; Johnson et al, 1937; Bentley and Thiessen, 1957; Butkevich and Fedorov, 1929a; b; 1930a; b; Foster and Waksman, 1939a; b; Foster et al, 1949; Foster and Carson, 1950) suggest that this is a major pathway for the conversion of carbohydrate to pyruvate in most living tissues, although evidence is now available that glucose may also be metabolized through reactions that bypass anaerobic glycolysis down to glyceraldehyde-3-phosphate by enzyme systems present in yeast, bone marrow and other sources (Warburg et al, 1935; Dickens, 1938a; b; Seegmiller and Horecker, 1952) through a sequence known as the hexose monophosphate shunt.

Several studies were carried out by Thunberg (1920), Knoop (1923), Szent - Gyorgyi (1935, 1936), Stare and Baumann (1936), and Krebs and Johnson (1937) to understand the formation of organic acids during the oxidation of carbohydrates and finally Krebs and Johnson proposed the citric acid cycle, otherwise known as the tricarboxylic acid cycle or the Krebs cycle, to explain the formation of

organic acids as intermediates in the oxidation of pyruvate. The operation of the tricarboxylic acid cycle has been demonstrated in animal tissues (Green et al, 1948; Elliott and Kalnitsky, 1950), bacteria (Gilvarg and Davis, 1956; King et al, 1956; Atkinson, 1956; Tourtellotte et al, 1955; Stone and Wilson, 1952; Beck and Lindstrom, 1955; Wiame and Bourgeois,1953; Altenbern and Housewright, 1952; Swim and Krampitz, 1954; Saz and Kramptiz, 1955; Blakley, 1952; Englesberg and Levy, 1955; Delwiche and Carson, 1953; Campbell and Stokes, 1951; Barrett and Kallio, 1953; Kogut and Podoski, 1953; Stoppani et al, 1955; Crook and Lindstrom, 1956; Stedman and Kravitz, 1955) and molds (Mickelson and Schuler, 1953; Ramakrishnan, 1954; Ramakrishnan et al, 1955; Lewis, 1948; Strauss, 1955; Goldschmidt et al, 1956; Hockenhull et al, 1954; Schatz et al, 1955; Moses, 1955).

These studies show that carbohydrates can be utilized in animal tissues and micro-organisms by a major pathway shown in Fig. 1.

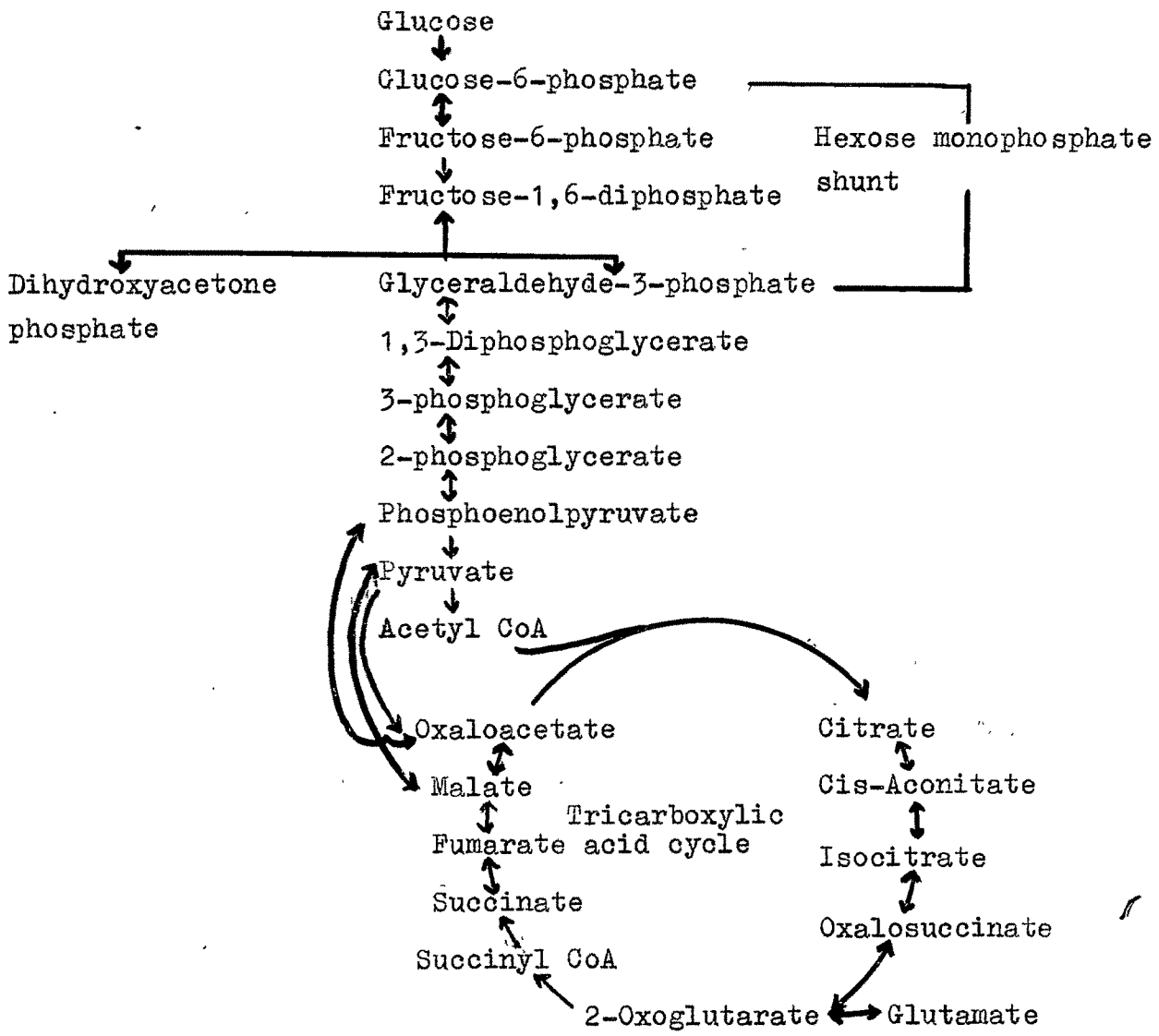



Fig. 1.- Carbohydrate metabolism in Living cells.

In the case of plant tissues, studies of Tanko (1936) and Hanes (1940a) on the transformation of starch into phosphorylated sugars by pea meal extract and those of James et al (1941) on the conversion of sugars into pyruvic acid by barley sap have led to the belief that the mechanism for the conversion of carbohydrates into pyruvate in plants may be similar to those in animals and yeast. A considerable amount of evidence has accumulated during the past few years for the presence of a fermentative system in higher plants similar to the glycolytic cycle. Further, individual enzymes of the glycolytic cycle have been detected in several plant tissues (Tanko, 1936; Hanes, 1940a; b; Bliss and Nylor, 1946; Kursanov and Pavlinova, 1948; Stumpf, 1948; Tewfik and Stumpf, 1949; Stumpf, 1950; Porter, 1950; Cardini, 1951; Saltman, 1953; Axelrod and Bandurski, 1953; Ramasarma et al, 1954; Edelman et al, 1955; Gibbs, 1955; Hageman and Arnon, 1955a; b; Giri and Ramasarma, 1956). The demonstration of all the enzymes of the glycolytic cycle in green gram (Phaseolus radiatus) by Giri and Ramasarma (1956) added substantially to the evidence for the operation of this cycle in plant tissues as well.

Several investigations have similarly been made on the operation of the hexose monophosphate shunt in plant tissues. Conn and Vennesland (1951) demonstrated the

presence of glucose-6-phosphate dehydrogenase in several plant extracts. Benson (1951) and Axelrod et al (1953) were similarly able to identify ribulose and sedoheptulose 7-phosphate respectively in spinach leaf preparations fed with ribose-5-phosphate. Ginsburg and Hassid (1956) introduced $C^{14}$ labeled pentoses into canna plant and wheat seedlings and succeeded in isolating labeled sucrose. The detection in plant tissues of the individual enzymes of the hexosemonophosphate shunt such as glucose-6-phosphate dehydrogenase (Gibbs, 1952),6-phosphogluconic dehydrogenase (Axelrod and Bandurski, 1952), phosphoriboisomerase (Axelrod and Jang;1954) and transketolase (Horecker et al, 1953) and the work of Clayton (1959) on the pentose cycle activity in cell-free extracts of tobacco leaves and seedlings have added substantially to the evidence for the operation of pathways other than glycolysis in plant tissues.

It is thus evident that plant tissues possess the enzymes required for the operation of both the Embden-Meyerhof pathway and the hexose monophosphate shunt. To determine the relative strengths of these two pathways in plant tissues Beevers and Gibbs (1954) carried out isotopic studies on a variety of plant tissues. They introduced equal amounts of glucose-1-$C^{14}$ and glucose-6-$C^{14}$ into several plant tissues and compared the initial yields of $C^{14}O_2$. They found that in most of these tissues some of

the glucose was broken down through a reaction sequence in which C-1 was split off at an earlier stage than C-6. They therefore concluded the Embden-Meyerhof pathway to be the more dominant.

Regarding the further oxidation of pyruvate in plant tissues, the hypothesis that a cycle similar to the tricarboxylic acid cycle in animal tissues might be operating in plant tissues was first advanced by Chibnall (1939).

A number of *in vitro* experiments on a variety of plant materials such as segments of *Avena* coleoptiles (Bonner, 1948) and barley root (Laties, 1949), spinach leaves (Bonner and Wildman, 1946) and slices of potato tubers (Barron *et al*, 1950) demonstrate the utilization of various di and tricarboxylic acids like succinic, fumaric and $\alpha$-ketoglutaric acid by respiring plant tissue cells and an increased rate of oxidation in these cells on addition of these acids, thus showing the similarity of plant tissues to animal tissues regarding their ability to oxidize these acids.

The oxidation of the intermediates of the tricarboxylic acid cycle have also been shown in a large number of other plant tissues like *Avena* (Tager, 1954), castor bean endosperm (Beevers and Walker, 1956; Walker and Beevers, 1956), pea internodes (Price and Thimann, 1954), cauliflower (Laties, 1953a; b), sweet potato (Akazawa and Uritani, 1954;

Lieberman and Biale, 1956), spinach leaves (Ohmura, 1955), soybean (Switzer and Smith, 1957), developing pepper (Howard and Yamaguchi, 1957) and double beans (Kalimi, 1968).

Bhagvat and Hill (1951) separated particulate fraction from plant homogenate and showed that the addition of succinate led to increase in the rates of oxygen uptake in the presence of cytochrome C. Millerd *et al* (1951) isolated active mitochondria from plant materials from mung bean (*Phaseolus aureus*) hypocotyls and showed its ability to oxidize some of the tricarboxylic acid cycle intermediates. Similarly active mitochondrial fractions were isolated from cauliflower (*Brassica oleracea* Var *botrytis*) by Laties (1953a, b, c) and from peas (*Pisum sativum*) by Davies (1953). Davies (1953) demonstrated the occurrence of the essential steps of the tricarboxylic acid cycle in the pea mitochondria. Similarly mitochondrial particles have been isolated from roots, seeds, hypocotyls, floral parts, fruits, petioles and tubers of different plants (Freebairn and Remmert, 1956; Hackett, 1955). These suggested the ability of the plant tissues to use tricarboxylic acid cycle intermediates for respiration. Regarding the utilization of pyruvate formed from glucose by glycolytic pathway, Brummond and Burris (1953) showed the net production of citrate from pyruvate and malate in lupine mitochondria. They incubated lupine mitochondria with pyruvate labeled in the carbonyl carbon using malate as a sparking acid. In addition other individual acids of the cycle were added as

traps for any labeled acid of the same type which might be produced. After one hour the reaction was stopped and the acids were separated chromatographically. The carbonyl-C of pyruvate appeared in each of the acids of the tricarboxylic acid cycle. Similarly Freebairn and Remmert (1957) showed that carbon from succinate-2-$C^{14}$ was incorporated into malate, pyruvate, citrate and glutamate when it was oxidized by a preparation from cabbage.

The operation of the tricarboxylic acid cycle in plant tissues was further strengthened by the study on the individual enzymes involved. Price and Thimann (1954) studied malic, succinic and oxoglutarate dehydrogenases from pea mitochondria. Davies (1953, 1954) showed the presence of NAD and NADP specific isocitrate dehydrogenases, fumarate hydratase and aconitate hydratase in the supernatant obtained by treating washed pea mitochondria in a Mickle shaker.

Several attempts have been made to show similar oxidation in fruit tissues. Pearson and Robertson (1954) tried to isolate mitochondria from apples which can oxidise the tricarboxylic acid cycle intermediates. But their preparations showed only a limited activity with succinate, malate and citrate. Later, Neal and Hulme (1958) demonstrated the oxidation of two intermediates, viz., succinate and malate by the particles of apples in presence of added

protocatechuate. Biale and his associates could isolate metabolically active cytoplasmic particles from avocado (Abramsky and Biale, 1957a; b; Biale et al, 1957) which were found to carry on the oxidation of all the intermediates of the cycle as well as phosphorylation. From these and other related studies Abramsky and Biale (1957a) concluded that the tricarboxylic acid cycle is a major pathway of oxidation of carbohydrates in a variety of tissues in higher plants.

Ramakrishnan and Varma (1959) studied the oxidation of the intermediates of tricarboxylic acid cycle by the homogenates of young and old fruit tissues of Citrus acida. The young fruit tissue in which no citric acid accumulates is able to effect the oxidation of all the intermediates of the tricarboxylic acid cycle. The mature fruit tissue in which citric acid accumulates is not able to oxidise citric acid suggesting a partial block of the tricarboxylic acid cycle at this level. This supports the view expressed by Bonner (1950), namely, that the accumulation of a particular cycle acid in a plant tissue might mean that one of the reactions of the cycle was blocked or especially slow. While this may account for the accumulation of citric acid the question arises regarding the respiration in these tissues. Studies carried out recently in this laboratory show that there is a very high protein break-down in the

mature fruit tissues and glutamic acid formed is utilised for respiration. Glutamic acid gets converted to 2-oxoglutarate which is used for respiration and the ammonia formed combines with aspartic acid to form asparagine which accumulates in large quantities in mature fruits (Sakariah, Parekh, and Ramakrishnan, unpublished). The enzymes concerned with the breakdown of protein into amino acids, utilization of glutamic acid and formation of asparagine have been isolated from fruit tissues confirming the possible utilization of glutamic acid for respiration in mature fruit tissues (Sakariah, Parekh and Ramakrishnan, unpublished).

Ramakrishnan and Varma (1959) also examined the cell-free extracts of fruit tissues of Citrus acida at different stages of development for the presence of tricarboxylic acid cycle enzymes. While young fruit tissue extract shows the presence of all the enzymes of the tricarboxylic acid cycle mature fruit tissues show a total absence of aconitate hydratase activity and an increase in the activities of citrate synthase, pyruvate oxidase and $C_4$ forming enzymes. The block in the operation of the tricarboxylic acid cycle at the citrate level can be explained in terms of the total absence of the aconitase activity whereas the increase in the activity of citrate synthase, pyruvate oxidase and $C_4$ enzymes suggests an accelerated formation of citrate.

The above studies show that carbohydrates are utilized for the formation of citric acid in fruit tissues due to the operation of the glycolytic pathway and the tricarboxylic acid cycle although the operation of the former has yet to be demonstrated.

The other acid which accumulates in citrus fruits is ascorbic acid. Regarding its presence, Smith and Gillies (1940) suggested its translocation from leaves to other parts of plants. Bacharach et al (1934) who studied the levels of ascorbic acid in oranges, tangerines and lemons showed that ascorbic acid is present in gradually diminishing amounts in the flavedo, albedo etc., and it may be synthesized in them, the greatest synthesis being in the skin. Studies carried out in tissues of other plants such as cress seedlings (Isherwood et al, 1954; Mapson et al, 1954; Mapson and Isherwood, 1956) and in the detached ripening strawberry and in the germinating cress seedling (Loewus et al, 1956; Loewus and Jang, 1957; Loewus, 1961) also suggested the potency of different plant tissues to convert sugar into ascorbic acid.

Studies carried out in recent years have shown the mechanism of utilization of glucose for synthesis of L-ascorbic acid in animal tissues (Jackel, et al, 1950; Horowitz et al, 1952; Horowitz and King, 1953). Isherwood

and co-workers (1954) reported that the administration of D-glucuronolactone and L-gulonolactone to rats produced an increase in urinary excretion of L-ascorbic acid. Hassan and Lehninger (1956) tested the enzymes in rat liver which could convert D-glucuronic acid and L-gulonic acid to L-ascorbic acid. The detection of enzymes involved in the conversion of glucose to glucuronic acid (Strominger et al, 1954; and Storey and Dutton, 1955), reduction of D-glucuronic acid to L-gulonic acid (Hassan and Lehninger, 1956; Ashwell et al, 1961; Mano et al, 1961) and the interconversion of L-gulonic acid and L-gulonolactone (Winkelman and Lehninger, 1958; Yamada et al, 1959) showed that glucose can be converted to L-ascorbic acid in rat liver. The enzyme responsible for the conversion of L-gulonolactone to ascorbic acid was found to be localized in microsomes (Hassan and Lehninger, 1956; Burns et al, 1956b) and 2-keto-L-gulonolactone is suspected to be the most likely intermediate (Kanfer et al, 1959). Evans et al (1960) found that galactose-1-$^{14}$C was a better precursor of L-ascorbic acid in rats than glucose-1-$^{14}$C as it had about 92% of its total activity in C-6 compared to 60% for that from glucose-1-$^{14}$C indicating that galactose is converted to L-ascorbic acid without intermediate formation of glucose. In addition, rat liver was shown to have the enzymes required for conversion of D-galactose

to L-ascorbic acid through D-glucuronic acid. Based upon currently available evidence, the most likely overall scheme for biosynthesis of L-ascorbic acid in animal tissues from glucose or galactose is shown in Fig. 2.

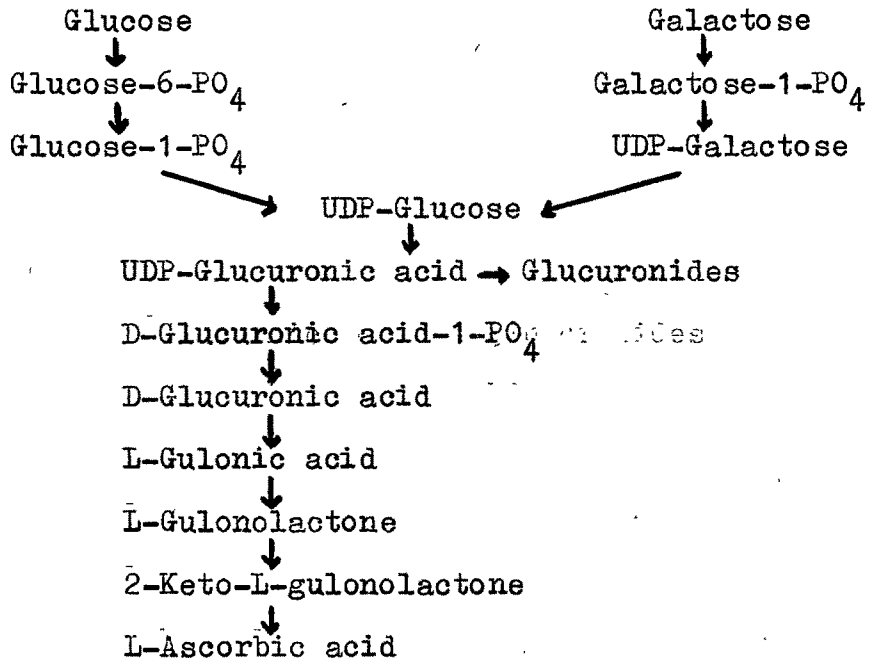



Fig. 2.- Biosynthesis of L-ascorbic acid in animal tissues

Regarding the biosynthesis of ascorbic acid in plants, Isherwood and co-workers (Isherwood et al, 1954; Mapson et al, 1954; Mapson and Isherwood, 1956) demonstrated that L-gulonolactone, L-galactonolactone, D-glucuronolactone and methyl-D-galacturonate were converted to L-ascorbic acid in cress seedlings. From these they postulated two pathways, one similar to that postulated for animals and another different from this (Figures 3 and 4).

D-Glucose → D-Glucuronic acid → L-Gulonic acid → L-Ascorbic acid

Fig. 3.- Postulated pathway for ascorbic acid synthesis in plants.

D-Galactose → Methyl-D-galacturonate → L-Galactono-lactone → L-Ascorbic acid

Fig. 4.- Postulated pathway for ascorbic acid synthesis in plants.

Studies by Loewus et al (1956) and Loewus (1961) in the detached ripening strawberry and in the germinating cress seedling do not support these mechanisms as major pathways for the biosynthesis of the vitamin in plants since no evidence for an inversion between D-glucose and L-ascorbic acid was found.

D-glucose-1-$^{14}$C yielded L-ascorbic acid containing 65-70% of its activity in C-1 and 14-19% in C-6. With D-glucose-6-$^{14}$C, 73% of the total activity in L-ascorbic acid was present in C-6 and 24% in C-1. When D-galactose-1-$^{14}$C was used as the substrate, L-ascorbic acid had about the same labeling in C-1 and C-6. These findings are in marked contrast to those on rats in which both C-1 of glucose and C-1 of galactose are converted to C-6 of L-ascorbic acid. These data suggest that different pathways may exist in plants and animals for biosynthesis of L-ascorbic acid. Loewus and coworkers have postulated the following mechanism for conversion of glucose to L-ascorbic acid :

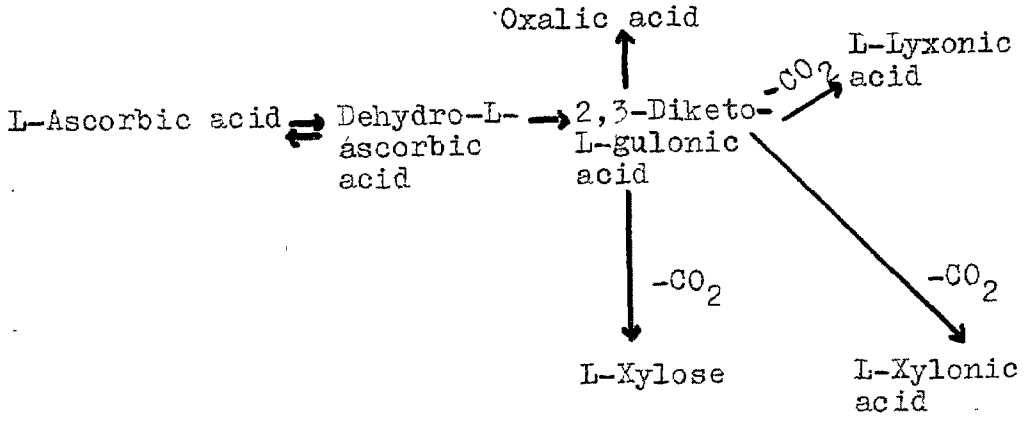
D-Glucose → D-Glucose-6-phosphate → D-Gluconate-6-phosphate → 3-Keto-D-gluconate-6-phosphate → L-Ascorbic acid

This hypothesis is yet to be confirmed by the identification of specific enzyme systems.

The formation of dehydroascorbic acid and 2,3-diketogulonic acid from L-ascorbic acid was postulated initially by Penn and Zilva (1943).

Regarding the catabolism of L-ascorbic acid, recent studies in animals have shown that it is oxidized to respiratory $CO_2$ to some extent in rats and guinea pigs (Burns *et al*, 1951; Burns *et al*, 1956a; Dayton *et al*, 1959; Rudolff *et al*, 1956; Curtin and King, 1955).

On the basis of available evidence it appears that the catabolism of the vitamin in animals is taking place as given below :




It is possible that the accumulation of ascorbic acid in fruits such as strawberry is due to its not being able to

metabolize the vitamin formed. It seems plausible that a similar mechanism is operating in citrus fruits.

The presence of tricarboxylic acid cycle enzymes in fruit tissues such as lemon suggest the operation of the glycolytic cycle as well in these tissues but this has not been empirically demonstrated. The present studies were therefore undertaken on fruit tissues of Citrus acida to investigate the presence in them of (a) glycolytic enzymes and glycolytic intermediates and (b) different forms of ascorbic acid and ascorbic acid synthesizing enzyme system and ascorbic acid oxidase. Further studies were undertaken on the distribution of these enzymes and metabolites in different parts of the fruit at different stages of development. These studies are detailed in this thesis.