

CITRIC ACID METABOLISM IN THE FRUIT TISSUES OF *CITRUS ACIDA**

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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 of citric acid and other constituents present in the fruits 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, the question also arises 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 Nitsch¹ according to whom the acids are translocated from the leaves to the fruits which merely act as storage organs. Earlier Gatet² and Ballard, Magness and Hawkins³ found that the acidity in grapes and apples was higher at the centre than at the periphery of the fruit and that a reduction in leaf area decreased 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 supplied by 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, Ricevuto⁴ suggested the formation in lemons of citric acid from reducing sugar and pentosans by enzymic action. In their studies on valencia oranges, Sinclair and Eny⁵ found the distribution of citric, malic and oxalic acids in leaves, juice and peel to vary considerably. The highest concentration of total, malic and oxalic acids was found 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 Erickson⁶ 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. The sweet lemon also had a much higher reducing sugar concentration (5.17%) than the sour lemon (1.45%). These findings certainly did not support the translocation hypothesis and point to local synthesis. This suggestion has 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 lemon⁷ and garcinia.⁸

Nada⁹ who analysed fruits of *Vitis vinifera* at different stages of development for sucrose and total acid 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 Ramakrishnan⁸ in fruits of garcinia (*Xanthochymus guttifer*). Studies carried out by Ramakrishnan and Varma⁷ showed an association between increase in citric acid content and decrease in carbohydrate during the development of the lemon fruit (*Citrus acida*). These authors also found that the sugar and protein contents and total acidity of young and old leaves and of stems bearing young fruits and mature fruits do not differ when considered in terms of percentage dry weight. On the other hand, substantial differences are found between young and mature fruits in the case of which the sugar and protein contents decrease with growth whereas the acidity increases. Similarly, the pattern of organic acids is the same in young and old leaves and stems bearing young and mature fruits. Citric, malic, succinic, fumaric and oxalic acids are present in both categories of leaves and stems. But in the case of fruits the pattern of organic acids

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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.

Reports on the feasibility of cultivating the lemon fruit *in vitro* in a nutrient medium containing mineral salts and sucrose¹⁰⁻¹⁷ and the demonstration of starch synthesis *in vitro* in lemon fruit¹⁸ indicate the independence of fruit tissues as a metabolic entity capable of synthesizing its own cellular constituents.

It is found that both the lemon and the garcinia fruit contain the enzymes necessary for the formation of citric acid from glucose^{7,19} and that the mature fruit is characterised by a high activity of citrate synthase and the disappearance of aconitase.^{7,8}

The evidence cited above seems to have been ignored by Lioret and Moyse²⁰ 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 lemon⁷ and garcinia.⁸ It is possible that the enzyme preparation in their experiments was inactivated due to a 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 Todd¹² while studying the $C^{14}O_2$ uptake by young oranges with and without exposure to light found that the sugars are highly labelled in the photosynthesizing flavedo. Sucrose is found to be labelled to an appreciable extent in the albedo of the intact fruit exposed to sunlight but only to a limited extent if the same is isolated and exposed to light. This suggested that the major activity in the albedo of the intact photosynthesizing fruit is due to translocation from the flavedo. A very slight amount of activity is found in illuminated, isolated vesicles. However, they accumulate a large amount of citric acid during light deprivation although the peel has a low concentration of the same.

A number of factors indicate that products such as citric acid formed during light deprivation must be formed within each tissue rather than by extensive translocation from one tissue to another. In the intact fruit photosyn-

thesis results in an increase in the incorporation of labelled $C^{14}O_2$ and a redistribution in flavedo but has no effect on the vesicles. The changes in the albedo, however, appear to be restricted to carbohydrate components.

Recent studies carried out in this laboratory on carbohydrate metabolism in different areas of the citrus fruit show that the green skin is an active site for the photosynthesis of carbohydrate whereas the vesicle is the main site of citric acid formation followed by the septa. The white skin seems to be rather a relatively inert site of carbohydrate metabolism and its major function could be translocation of glucose from the green skin to the interior of the fruit. The juice seems to represent merely the spill-over products from the tissues. The vesicles and green skin are also found to be very active sites for the conversion of glucose to ascorbic acid.²²

These studies show that even different parts of the fruit are independent to a considerable extent.

The above studies show that carbohydrates are utilized for the formation of citric acid in the fruit tissues due to the operation of glycolytic pathway and the tricarboxylic acid cycle and citric acid accumulates in the mature fruit due to absence of aconitase. It is found that while 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 able to oxidise all other TCA cycle intermediates except citric acid. This raises the question regarding the mode of respiration of the mature fruit tissue.

The accumulation of citric acid due to absence of aconitase raises the question regarding the operation of respiratory mechanisms in the mature fruit. The breakdown of protein and the utilization of amino-acids for respiration suggests itself as a possibility on the basis of studies carried out during starvation,²³ senescence²³ and water stress.²⁴

Several techniques have been used for inducing starvation in plants by restricting or preventing photosynthesis. These include light deprivation, water deprivation and CO_2 deprivation.

Studies have been made of the effects of light deprivation on nitrogen metabolism in etiolated seedlings.²⁵⁻²⁷ Barley seeds were allowed to germinate in the dark and determinations made on different days of the respiratory rate and the contents of carbohydrate

and nitrogen both in the endosperm and embryo. During the first six days of germination the reserves of the embryo, namely, sucrose, raffinose and small amount of fat were rapidly broken down. The respiratory quotient during this time was maintained at a value close to unity indicating that carbohydrates furnished most of the respiratory substrate. Thereafter the stores were found to be exhausted and the respiratory quotient began to fall to a value of about 0.8 suggesting that non-carbohydrate sources, presumably proteins, were used for respiration. Similarly, tobacco leaves detached from the plant and immersed for 72 hours in water showed an initial depletion of starch. Subsequently, a breakdown of protein was evident. After 70 hours hexose and sucrose along with free amino-acids especially glutamic acid and aspartic acid were used for respiration.²⁸

Studies carried out by Thompson *et al.*²⁴ on the effect of water stress in turnip leaves showed that protein content decreases at a rapid rate in the wilted leaves and this is associated with a rise in ammonia suggesting that deamination of amino-acids provides oxidisable substrates for respiration.

In the case of fruits such as apples and bananas, ripening is accompanied by a sharp rise in respiration and this rise is associated with a net initial protein synthesis followed by a steady fall in protein content and an increase in amino nitrogen and asparagine.²⁹ These studies suggest that protein is utilized for respiration during ripening. Studies were therefore carried out in this laboratory to find out whether a similar phenomenon occurs in the mature fruit tissues of *Citrus acida*. Respiratory studies carried out on tissue slices of young and old fruits showed that the R.Q. was unity in the former case whereas it was 0.8 in the latter case suggesting that non-carbohydrate sources, presumably proteins, are used for respiration in the latter case (Sakariah and Ramakrishnan, unpublished data). In other studies a decrease in protein nitrogen associated with increases in amino and amide nitrogen as well as glutamic acid and asparagine was found in the mature fruit.³⁰ In studies on respiration using different amino-acids as substrates glutamic acid was found to be used for respiration by the mature fruit tissue but not by the young tissue. While the addition of glutamate had no effect on oxygen uptake in the young fruits it increased the same by 50% in the mature fruit. Addition of glutamic acid resulted in the increased formation of ammo-

nia but this was prevented by the simultaneous addition of aspartic acid which resulted in an increase in asparagine.³⁰ The specific activities of enzymes concerned with glutamate utilization were high in the mature fruit.³⁰ The results of these studies suggest that the mode of respiration in the mature fruit is by the conversion of glutamate to 2-oxoglutarate which gets further oxidised by the Krebs cycle. The ammonia formed appears to be removed and detoxified by the formation of asparagine.

In conclusion, studies carried over the last several years in this laboratory and elsewhere suggest that the citrus fruit tissue is metabolic entity in its own right capable of synthesizing many of its major constituents. The fruits of *Citrus acida* possess the metabolic machinery for photosynthesis as well as the operation of the glycolytic and TCA cycles. A block in the operation of the TCA cycle in the mature fruit at citrate level would account for the accumulation of citrate in the mature fruit. A failure in respiration as a result of this block is apparently prevented by the utilization for respiration of glutamate derived from the breakdown of proteins. The accumulation of ammonia during the deamination of glutamate appears to be prevented by its incorporation into aspartate to form asparagine. The different parts of the fruit tissue seem to have quite distinct metabolic characteristics and differ with regard to their photosynthetic capacity and organic acid metabolism.

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A NEW CLASS OF ELECTROMAGNETIC FIELDS

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RECENTLY, several authors¹⁻⁴ have derived theorems which may be used to generate electromagnetic solutions of the field equations from given vacuum solutions. These results are generalisations of an earlier work by Weyl⁵ who investigated axially symmetric gravitational fields. Such electromagnetic fields are, therefore, known as Weyl class of electromagnetic fields. In this note we discuss a procedure by which one can generate a new class of electromagnetic fields, which are not of Weyl type, from a given axially symmetric, vacuum gravitational field.

Consider the following set of field equations :

$$R_{ij} - (1/2) g_{ij} R \equiv G_{ij} = -8\pi E_{ij}, \quad (1)$$

$$F^i{}_{j;j} = 0, \quad (2)$$

$$F_{[ij;k]} = 0, \quad (3)$$

where

$$E_{ij} = (1/4\pi) [g^{kl} F_{ik} F_{jl} - (1/4) g_{ij} F_{kl} F^{kl}]. \quad (4)$$

The symbols used here have their usual meaning. We choose the axially symmetric static metric in the form⁶

$$ds^2 = e^{2u} dt^2 - e^{2k-2u} [(dx^1)^2 + (dx^2)^2] - h^2 e^{-2u} d\phi^2, \quad (5)$$

where u , k and h are functions of x^1 and x^2 only. The vacuum field equations for the metric (5) are easily obtained as :

$$u_{,11} + u_{,22} + (1/h) (u_{,1} h_{,1} + u_{,2} h_{,2}) = 0, \quad (6)$$

$$2(u_{,2}^2 - u_{,1}^2) + (2/h) (k_{,1} h_{,1} - k_{,2} h_{,2}) + (1/h) (h_{,22} - h_{,11}) = 0, \quad (7)$$

$$2u_{,1} u_{,2} - (1/h) (k_{,2} h_{,1} + k_{,1} h_{,2}) + \frac{h_{,12}}{h} = 0, \quad (8)$$

$$h_{,11} + h_{,22} = 0. \quad (9)$$

However, if one takes the stress-tensor of an electromagnetic field as the source of the gravitational field equations, i.e., Eqn. (1), one can obtain the field equations with the help of only two components of the four potential. If one introduces the potentials A and B in the following manner^{3,7,8}

$$\left. \begin{aligned} F^{\alpha\beta} &= (-g)^{-1/2} \epsilon^{\alpha\beta\gamma} A_{,\gamma} \\ F_{0\alpha} &= B_{,\alpha} \end{aligned} \right\}, \quad \alpha, \beta, \dots = (1, 2, 3) \quad (10)$$

where $A = A(x^1, x^2)$ and $B = B(x^1, x^2)$, one obtains the stress-tensor E_{ij} in a symmetrical form with respect to A and B . Further, there are only two non-trivial Maxwell equations amongst (2) and (3) which are identical with regard to A and B . This situation allows one to introduce a single potential C such that :

$$\left. \begin{aligned} A &= C \sin \alpha \\ B &= C \cos \alpha \end{aligned} \right\}, \quad (11)$$

where α is a constant. Equations (11) are equivalent to a 'duality rotation' of Misner and Wheeler.⁹ These considerations lead to the following field equations :

$$u_{,11} + u_{,22} + (1/h) (u_{,1} h_{,1} + u_{,2} h_{,2}) = -e^{-2u} [C_{,1}^2 + C_{,2}^2], \quad (12)$$

$$u_{,2}^2 - u_{,1}^2 + (1/h) (k_{,1} h_{,1} - k_{,2} h_{,2}) + (1/2h) (h_{,22} - h_{,11}) = e^{-2u} [C_{,1}^2 - C_{,2}^2], \quad (13)$$

$$2u_{,1} u_{,2} - (1/h) (k_{,2} h_{,1} + k_{,1} h_{,2}) + (h_{,12}/h) = -2e^{-2u} C_{,1} C_{,2}. \quad (14)$$

Further, the only non-vanishing Maxwell equation is obtained as :

$$C_{,11} + C_{,22} + (1/h) (C_{,1} h_{,1} + C_{,2} h_{,2}) - 2(C_{,1} u_{,1} + C_{,2} u_{,2}) = 0. \quad (15)$$