The Respiratory Pattern in the Tomato Fruit and its Alteration by Infiltration with Various Chemicals

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Whether the tomato fruit is left to ripen on or off the plant, a marked increase in its respiratory rate known as the climacteric is observed as it passes from the mature green to the ripe stage (4, 7, 15, 19). A number of hypotheses have been advanced to explain this climacteric rise in respiration (3, 10).

It has been suggested that the climacteric rise is caused by increased utilization of ATP or endogenous uncoupling of oxidative phosphorylation (16). At present there is no data indicative of uncoupling in situ, and tightly coupled mitochondria have been isolated from avocado fruits at the climacteric peak (20). Increased utilization of ATP could result from the onset of energy requiring processes such as protein synthesis (8,19). Increased turnover of ATP may be related to changes in mitochondrial permeability. Lyons and Pratt (12) reported that ethylene caused swelling in isolated mitochondria. They suggested that ethylene may increase the permeability of mitochondria within intact fruits, allowing more rapid movement of adenine nucleotides through mitochondrial membranes.

Most of the hypotheses about respiratory increases during the climacteric and the controlling mechanisms of respiration have been derived from studies on tissue slices and mitochondrial preparations rather than on whole fruit (17, 18, 20). Such inferences would hold true only if the conditions in the tissue slices and mitochondrial preparations are the same as those in the whole fruit.

The respiratory patterns of whole tomato, tissue slices, and mitochondrial preparations were compared over a wide range of fruit maturity. The climacteric rise in respiration as well as alterations of the normal respiratory pattern in the fruit brought about by various chemicals was investigated.

Materials and Methods

The Infrared Gas Analyzer. A Beckman infrared gas analyzer model 15a utilizing an Esterline-Angus direct current recorder was used in the respiratory studies on whole fruits and tissue slices. The analyzer was placed in a room with a constant temperature of 21° ± 0.5° and 50 % relative humidity (R.H.). An open system in which CO2 was removed from the air by passing it through a series of bottles containing 6 M KOH was used. CO2-free air was then passed through a saturated solution of Ca(NO3)2 to maintain approximately 53 % R. H. in the fruit chamber. This moisture was then removed by 2 columns of anhydrous CaSO4 (Drierite size 8 mesh) placed between the fruit chamber and the analyzer. The fruit chamber was made of plexiglas with an inner volume of 2.8 liters. Air was forced through the system by using a Dyna-Vac laboratory pump (3000 rpm). The rate of flow was controlled by 2 air-flow meters.

Infiltration of Fruits. Solutions were infiltrated into freshly harvested fruit (var. Kc 109) by a modification of the vacuum method described by Barbour et al. (2). The fruit was placed inside a bell jar with the stem scar up. The amount of solution required (0.5 % of fruit weight) was measured with a pipette and placed on the stem scar depression. The chamber was then evacuated to 8 inches of mercury until no air bubbles were seen coming out of the stem scar. When the vacuum was released, the solution was absorbed by the fruit through the vascular system. The control fruit was infiltrated with distilled water.

Preparation of Mitochondria. The procedure for preparing mitochondria, except for the preparation of the tissue for grinding, was that described by Kahn and Hanson (9). The fruits were washed with deionized water and cut cross-sectionally into slices approximately 1 inch thick. Only tissues from the outer pericarp were saved. Locular tissues were discarded due to their high titratable acidity in comparison with pericarp tissues (13). A 100 g sample was weighed for each treatment. The slices were washed again with deionized water then cut into thinner slices with a sharp knife. The thin slices were ground for 6 minutes in an ice-cold mortar with 100 ml of a precooled medium containing 0.5 m sucrose, 0.5 M KH2PO4, 0.5 M Tris, 0.01 M EDTA and adjusted to pH 7.65 with 14 M KOH.

Respiration was measured manometrically at 30° using the Warburg technique with air in the gaseous phase. The loss in orthophosphate was determined by the Fiske and Subbarow method (6). Protein was determined by the method of Lowry et al. (11). The fluid phase in each flask consisted of 0.2 ml of 20 % KOH in the center well and 2.5 ml of reaction medium pH 7.5. The reaction medium contained 30

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μmoles sucrose, 100 μmoles glucose, 2.5 μmoles MgSO₄, 50 μmoles KH₂PO₄, 20 μmoles potassium pyruvate, 40 μmoles l-malic acid, 0.2 mg TPP, 0.4 mg NAD, 1.5 mg ATP, 0.1 mg CoA, 20 K.M. units of hexokinase, and 0.5 ml of mitochondrial preparation.

**Experimental Results**

Respiration, as CO₂ output, was determined on 5 uniform fruits harvested at the mature green stage. Measurements were made daily until the fifth day after incipient coloring and thereafter on the seventh, tenth, fourteenth, and eighteenth day. CO₂ production for each fruit was recorded during a 20-minute period for each test.

The data (fig 1) show a uniform rise in respiration as the fruits advance from mature green to the second day after incipient coloring. At the climacteric the respiratory rate was approximately 30% above that of the mature green stage. Following the climacteric, respiration declined uniformly until the fruit became soft ripe.

![Respiratory rates of whole tomato fruits and slices of outer pericarp at 21°C. MG, IC, and DA refer to mature green, initial coloring, and days after IC, respectively. Data represent means of 5 replicates for whole fruits and 4 replicates for tissue slices.](image)

**Fig. 1.** Respiratory rates of whole tomato fruits and slices of outer pericarp at 21°C. MG, IC, and DA refer to mature green, initial coloring, and days after IC, respectively. Data represent means of 5 replicates for whole fruits and 4 replicates for tissue slices.

Fruits to be used in determining the respiratory rates of tissue slices were harvested at incipient coloring (except for the samples representing the mature green fruits) and stored at 21°C and 50% R. H. until they were used. Four replicates of 3 fruits each were used at each of the 5 stages of maturity consisting of (1) mature green, (2) incipient coloring, (3) 2 days after, (4) 6 days after, and (5) 10 days after. Two large slices about 25 g each were prepared from the outer pericarp of each fruit. The slices were gently washed twice with deionized water and blotted for several minutes with paper toweling to absorb the excess moisture before placing them on petri dishes with the outer surfaces in contact with the dish. An average of 6 slices weighing about 150 g was used per sample. It was then placed in the respiratory chamber and left until respiration reached a steady state in about 2 hours, after which the rate was recorded.

The tissue slices (fig 1) exhibit no climacteric. Their respiratory rates which are highest at the mature green stage decline with subsequent ripening. Though CO₂ evolution is approximately double that of whole fruits at the preclimacteric stage, they show the same rate as whole fruits at the climacteric stage and 8 days after.

The oxidative-phosphorylative activity of the mitochondria was measured over a period extending from mature green to 21 days after incipient coloring. All fruits, except for the mature green, were harvested at incipient coloring and stored at 21°C until they were used.

Mitochondrial preparations (fig 2), like tissue slices, show no climacteric. Their ability to oxidize malate and pyruvate was highest at the mature green stage, and very low at the stage where whole fruits show maximum CO₂ production. The addition of bovine serum albumin (2 mg/flask) to the reaction mixture did not have any stimulating effect. The decline in the rate of oxidation of the tricarboxylic acids with ripening was not accompanied by a change in the P/O ratio.

Since the results with tissue slices and mitochondrial preparations revealed that only whole fruits exhibit a climacteric, and that it cannot be explained on the basis of uncoupling of oxidative phosphoryla-
tion, the possible limitation of adenylate was investigated. This was done indirectly by infiltrating fruits with DNP neutralized to pH 7.0 with 1 N KOH. Fruits at incipient coloring and others 7 days after were supplied with 2 concentrations, $10^{-4} \text{ M}$ and $10^{-3} \text{ M}$. No stimulation of CO$_2$ production resulted from concentrations lower than $10^{-4} \text{ M}$, and injury resulted from $5 \times 10^{-3} \text{ M}$ concentration.

An increase of 29% in the respiratory rate of fruits at the preclimacteric stage resulted from the higher but none from the lower concentration of DNP (fig 3). DNP caused a smaller but consistent increase in respiration of postclimacteric fruits (fig 4). In all cases fruits reached maximum respiratory rates 6 hours after the treatments were started, and at the end of the second day, the rates had dropped to the levels shown by untreated fruits. Fruits treated with DNP at the preclimacteric stage showed no climacteric whereas the control fruits infiltrated only with water showed the same respiratory pattern as normal fruits.

When fruits were infiltrated with water, there was an immediate drop in respiration. After about 2 hours recovery was 100% in preclimacteric but only 86% in postclimacteric fruit.

Factors other than adenylate which might be limiting respiration in tomato fruit were tested. These included an organic substrate (malate) and Pi. Three levels of l-malic acid (0.1 M, 0.25 M, and 0.5 M) and 2 levels of Pi (0.1 M and 0.25 M) were infiltrated into preclimacteric and postclimacteric fruits. Neither substance stimulated respiration. The higher concentration of malate did cause a slight reduction in the respiratory rate during the first several hours following the treatment.

**Discussion**

The respiratory data on whole tomato fruits, tissue slices, and mitochondrial preparations (fig 1, 2) indicate that the climacteric is a characteristic of whole fruit only, since it was not shown by tissue slices and mitochondrial preparations. The low respiratory rate of preclimacteric fruit in comparison to the high rate in tissue slices may be attributable to an internal controlling mechanism, which becomes less effective as the fruit reaches the climacteric. The control of respiration also seems to be minimized by the preparation of tissue slices. This may be due in part to better gas exchange in the slices than in the whole fruit. Injury may also be a factor but climacteric and postclimacteric slices respired at rates similar to that for whole fruit.

Although the respiratory rate of mitochondria dropped sharply during ripening, their efficiency for phosphorylation was rather constant. These data are in agreement with those of Dickinson and Hanson (5) on tomato. The results are also interesting in view of a recent observation by Bain and Mercer (1). They show that as the climacteric progresses in the pear, there is extensive disorganization in the cell but no appreciable breakdown of the mitochondria.

The stimulation of respiration by infiltration with DNP indicates that respiration of intact fruits may be limited by ADP or its turnover (8,17). The fact that greater stimulation occurred in preclimacteric than in postclimacteric fruits indicates that the climacteric may result from the utilization of high energy phosphate from ATP in synthetic reactions, thus increasing the availability of ADP. This suggestion is in agreement with the work of Rowan et al. (19) who showed that the ratio of ADP to ATP in postclimacteric tomato was higher than in preclimacteric fruit. Another possibility for the greater stimulative effect of DNP on preclimacteric than on postclimacteric fruit may be due to an increase in membrane permeability of the latter. Since membrane permeability increases with ripeness (10), less response would be expected from treating postclimacteric fruit with DNP. Although the magnitude of response to DNP in preclimacteric and postclimacteric fruit treated at incipient coloring. Data represent means of 5 fruits.

**Fig. 3.** Short-term effect of DNP on the respiration of tomato fruits treated at incipient coloring. Data represent means of 5 fruits.

**Fig. 4.** Short-term effect of DNP on the respiration of tomato fruits treated 7 days after incipient coloring. Data represent means of 5 fruits.
macteric fruit differed, the time required to reach the maxima (6 hr) did not. This lag may be due in part to the time required for DNP to reach the active sites in mitochondria.

In contrast to the stimulation of respiration by DNP, neither malate nor Pi had any effect. Whether the lack of stimulation by malate was due to the availability of endogenous malate in adequate amounts or to resistance of cell membranes to movement of exogenous malate into the cells is a matter of conjecture. On the other hand, Pi has been shown to move freely into tomato fruit (14), thus a lack of stimulation by Pi may indicate that this element is not limiting oxidative phosphorylation and respiration.

Summary

Respiratory patterns during ripening were determined on intact tomato fruits, tissue slices, and mitochondrial preparations. The climacteric was found to be a characteristic of intact fruits only. Respiration of tissue slices was highest at the mature green (preclimacteric) stage and showed a continual decline as ripening progressed. The capacity of mitochondrial preparations to oxidize malate and pyruvate decreased sharply during ripening in a pattern similar to respiration of tissue slices. However, P/O ratios remained rather constant.

The effect of various chemicals on respiration of intact fruits was determined by vacuum infiltration of solutions through the stem scars. Respiration of pre- and postclimacteric fruits was not stimulated by inorganic phosphate or 1-malic acid. Respiration was stimulated by 2,4-dinitrophenol at both pre- and postclimacteric stages, indicating that availability of ADP limits respiration of intact fruits.

Literature Cited