Ontogenetic Changes in Respiratory Pathways in Cortland Apple Skin During Storage

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Summary. Ontogenetic changes in respiratory pathways of skins of detached apple fruits were measured during a 4 month period. Results showed that an increase in pentose pathway occurred with increasing senescence in this tissue. Variation in temperature at any given age did not result in an alteration of the C\textsubscript{a}/C\textsubscript{i} ratio. CO\textsubscript{2} evolution from the pentose pathway in senescent fruit was directly related to O\textsubscript{2} uptake. Glucose uptake increased with increasing senescence indicating a change in cell permeability.

Metabolic pathways leading to respiratory CO\textsubscript{2} production in fruits have been of special interest to the physiologist for decades. While the literature on total respiration is quite voluminous, comparatively little is known concerning the qualitative aspects of CO\textsubscript{2} evolution and how it may change with tissue age. Attention was given recently to the occurrence of the pentose phosphate pathway (PPP) in fruits as a possible source of evolved CO\textsubscript{2}. The presence of PPP has been established in several fruits: apples (6, 10), pears (15), bananas (16), oranges (3), limes (3), and tomatoes (3, 9, 17). The CO\textsubscript{2} evolved through PPP has been variously estimated from 14% of total respiration in green tomatoes (3) to 40% in mature apples (6). Although calculations estimating the contribution of PPP to the overall respiratory CO\textsubscript{2} are not precise (13), the high proportion in such estimations point to the physiological importance of the PPP in fruits. In this investigation, the ontogenetic changes in PPP activity found during the storage of apples are described.

Materials and Methods

Preclimacteric Cortland apples were picked on September 20, about 2 weeks prior to commercial harvest in the orchard of the New York State Agricultural Experiment Station at Geneva, New York. The apples were placed in cold storage at 3.3\textdegree C immediately after picking. Samples for respiratory determinations were removed from storage and used 1 hour after warming to room temperature. Twice during storage a larger sample was removed and held for 1 week at 21\textdegree C. Respiratory determinations were made on these apples during this holding period.

Activity of the PPP was assessed by determining the C\textsubscript{a}/C\textsubscript{i} ratios by using glucose-6-\textsuperscript{14}C (G-6-\textsuperscript{14}C) and glucose-1-\textsuperscript{14}C (G-1-\textsuperscript{14}C). This method was originally designed by Bloom, Stetten and Stetten (4) and was reviewed by Gibbs (11). For our determination, 1 mm thick by 8 mm diameter discs of apple skin were cut with a cork borer and razor blade. One g samples of disks (ca 30 pieces) were placed into each of 8 respiratory flasks and 10 ml of 0.03 m phosphate buffer at pH 5.2 and 2 \mu c of labelled glucose added. Specific activities of the sugars were used 29.6 mc/mole for G-1-\textsuperscript{14}C and 4.9 mc/mole for G-6-\textsuperscript{14}C. The flasks were attached to the respirometer and shaken gently by means of a mechanical incubator shaker. CO\textsubscript{2} free air was passed through the system at the rate of 55 ml/minute. The reaction was allowed to proceed at 25\textdegree C for 4 hours. Respiratory CO\textsubscript{2} was trapped in 0.5N NaOH and sampled hourly. The carbonate was precipitated from the alkali with BaCl\textsubscript{2} and was counted with a Nuclear Chicago Auto-scaler with a Model 447 Gas Flow Detector.

Immediately following incubation, the tissue was extracted with 10 ml of 80 % (v/v) ethanol, filtered, and both the ethanol soluble and insoluble residue counted. Activity obtained by counting BaCO\textsubscript{3}, ethanol extract, and residue were added and the total used as the total uptake by the tissue. The respiratory CO\textsubscript{2} was then expressed as percent of total uptake. C\textsubscript{a}/C\textsubscript{i} ratios were determined by dividing the percent of activity recovered for G-1-\textsuperscript{14}C. Measurements were made in duplicate in at least 2 separate runs throughout, and the standard deviations were calculated.

The total respiration was determined separately

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Figure 1

![Graphs showing changes in respiration of Cortland apple skin during storage.](image)

**Fig. 1.** Changes in respiration of Cortland apple skin during storage. A) Changes in total respiration expressed as O₂ uptake. B) Changes in recovery of CO₂ from G-1-¹⁴C and G-6-¹⁴C. C) Changes in the glucose absorption during storage. Standard deviation did not exceed for any point in A ± 15; in B for G-1-¹⁴C ± 1.05; for G-6-¹⁴C ± 0.39; in C ± 5300.

as O₂ uptake by the Warburg method at 25° by using 0.75 g samples of disks per flask. Results were calculated as μl O₂/hour/g fresh weight.

**Results**

The pattern of respiratory O₂ uptake of skin disks taken from these apples was typical of that of whole fruit under cold storage conditions (fig 1A). Respiration decreased for a few days after harvest, then rose to a slight but significant climactic before following a downward trend throughout the storage.

While the overall O₂ uptake declined during storage, the evolution of CO₂ from labeled glucose increased (fig 1B). This was particularly true with glucose labelled in C₁ position. The increase of C₁ in evolved CO₂ increased from less than 2% to above 7% of label taken up by the tissue. Although evolution of CO₂ from the C₆ of glucose also increased, this increase was only slight. The change in evolution of CO₂ from C₁ and C₆ was reflected in the C₆/C₁ ratio, which decreased with time from 0.7 at harvest to 0.32 following the 4 months of storage.

Radiorespirometric measurements were made twice during the storage period to determine the type of respiration in senescent fruit resulting from 1 week holding periods at 21°. Results obtained in November are shown in figure 2, and in January in figure 3. The C₆/C₁ ratio increased during the first 2 hours of the experiment while the fruit was physiologically relatively young (fig 2A, 2B, 2C). This change did not take place in very senescent fruit, either after 8 days at high temperature in November or after 4 months of cool (3.3°) storage in January. The increase in C₆/C₁ ratio is normally expected and the significance of its absence will be discussed later.

The fact that no major change in the respiratory quotient was observed throughout storage suggested that the metabolism of glucose through the PPP may be directly related to O₂ uptake. This theory was tested by conducting experiments under the reduced O₂ conditions produced by pretreating apples with N₂ for 12 hours at 3.3° and again measuring the C₆/C₁ ratio. While there was only a slight decrease in glucose uptake in N₂, the CO₂ evolution from both the C₆ and the C₁ labels decreased significantly. The evolution of both the C₆ and the C₁ labels was decreased equally indicating a proportional decrease in both pathways.

**Discussion**

It was found that the PPP plays an increasingly important role in the respiratory mechanism during storage of apples to senescence. A shift that occurred towards the PPP during storage was observed by a progressive lowering of the C₆/C₁ ratios. To circumvent calculation artifacts due to known differences in glucose absorption during storage, the C₆/C₁ ratios were calculated as percent of actual glucose uptake. Although we recognize that the C₆/C₁ ratio cannot be used for accurate calculation of the precise contribution of the PPP to the total respiratory CO₂, the ratio being significantly less than unity indicates a strong PPP. A decreasing C₆/C₁ ratio therefore indicates an increasing glucose metabolism through the PPP. That the total respiration as indicated by O₂ uptake did not increase at the time when the activity through the PPP increased points to a true change in metabolic pathways rather than an additional CO₂ evolution through the PPP.

Results of other workers also suggest that such
a change may take place. Dilley et al. (8) postulated a change in metabolism of senescent fruit by measuring CO₂ production in nitrogen and in air. He found that after 5 months of storage, CO₂ production of Red Delicious apples in nitrogen was much lower than in air. His results can be explained by the presence of PPP. In nitrogen, CO₂ production decreased in both pathways (PPP and citric acid cycle) and was replaced only by the anaerobic CO₂ production which is less than the total of the 2 pathways. That such an interpretation is valid was shown by Daly et al. (7).

The increased rate of glucose uptake during senescence of fruit may indicate a change in permeability of cells. It also can reflect, however, the internal metabolism. Laties (12) postulated that glucose absorption depends on the internal cytoplasmic glucose concentration and the latter in turn depends upon the type and intensity of respiratory metabolism. This would necessitate a very rapid use of glucose at times when glucose uptake is high. The rate of CO₂ evolution from glucose did not reflect such a rapid internal glucose utilization. Labelled CO₂ production was slightly less in fruit kept at 21°C in January than in fruit kept in similar conditions in November, yet the glucose uptake greatly increased with time. The important difference in the 2 groups of fruit was that in November the C₆/C₁ ratio changed during the course of experiment, while in January it did not. It started very low and increased significantly after the first hour (fig 2A, 2B, 2C). In a tissue which is meta-

**Fig. 2.** Radiorespiratory patterns after 6 weeks in cold storage and various time at 21°C. A) Immediately after removal from storage. B) After 3 days at 21°C. C) After 5 days at 21°C. D) After 8 days at 21°C. Standard deviation did not exceed for any point in A ± 0.10; in B ± 0.09; in C ± 0.16; in D ± 0.12.
bolizing glucose simultaneously by the PPP and citric acid cycle the C₆/C₁ ratio would be expected to increase with time. ApRees, Blanch and Davies (2) suggested that the rate at which this increase occurs would be affected by the time taken for the tissue to metabolize the added glucose. The shorter this time the more rapid the increase in the C₆/C₁ ratio. Since there was only a slight decrease in the rate of metabolism at the time when glucose uptake increased, the absence of change in the C₆/C₁ ratio in senescent fruit (fig 2D,3A,3B,3C) probably indicates that glucose moved into the cell so rapidly that it could not be utilized fast enough to alter the C₆/C₁ ratio. Such a supposition would require a drastic change in cell permeability in senescent tissue and a partially nonactive glucose uptake. Direct evidence for this is presently being sought.

**Literature Cited**


