CHANGES IN RATE OF LEAKAGE OF POTASSIUM FROM EXCISED DISKS OF APPLE FRUITS HELD AT 20°C AFTER HARVEST*

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As early as 1928, Blackman and Parija suggested that the climacteric rise in respiration which is associated with ripening in most fruits might be due to changes in cell permeability. This view has been supported by recent observations on some tropical and subtropical fruits, viz. the avocado (Sacher 1962; Ben-Yehoshua 1964; Young and Biale 1967), the banana (Baur and Workman 1964; Sacher 1962, 1966), and the nutmeg melon (Von Abrams and Pratt 1967).

Information is lacking regarding permeability changes in temperate fruits during ripening and during the subsequent senescent phase which is much more prolonged than in the case of the fruits that have so far been studied. This paper reports an investigation of the course of permeability in mature apple fruits held at 20°C from the time of harvest, through ripening and senescence until just before the death of the fruit.

Materials and Methods

Four to five bushels of apples, harvested at commercial maturity from each of two Jonathan and two Sturmer trees (Jonathan: March 16; Sturmer: May 4), were divided into random samples of 20 fruits and stored at 20°C. Samples were removed from storage successively, initially every 1–3 days, and subsequently at increasingly longer intervals, until a marked reduction in resistance to infection by decay organisms indicated that the fruit was approaching the end of its life. After removal of a sample, 10 disks of mid-cortical tissue, 6 mm in diameter and 2 mm thick, were cut from each fruit. The juice was extracted from a representative sample of the remaining tissue, and its refractive index was determined with a refractometer.

The pooled disks from the 20 fruits were used for the measurement of rate of leakage of potassium into an aqueous bathing medium. If such disks are suspended in distilled water osmotic entry of water causes rupture of some cells. It was therefore necessary to use an approximately isotonic solution as the bathing medium and also for washing the freshly cut disks. The soluble solids content of the juice, expressed as percentage sucrose, was derived from the observed refractive index with the aid of conversion tables, and a sucrose solution of this concentration was prepared. Depending on duration of storage, the observed concentration ranged between 12·5 and 14·5% for Jonathan, and between 15·0 and 17·0% for Sturmer. Trials showed that for a given sample of disks, potassium leakage rate was independent of sucrose concentration in a range from 1% below to 1% above the isotonic concentration.

The freshly cut disks were washed rapidly in several changes of isotonic sucrose to remove sap from injured cells, and then blotted dry. Four 4-g samples of disks were weighed out and two samples were placed in conical flasks each containing 100 ml isotonic sucrose. The inclusion of some calcium (1 mM CaCl₂) in this solution was essential to maintain the disks in a fully turgid condition. After the flasks had been gently shaken for 5 hr the liquid contents were filtered through Whatman No. 541 filter paper. Each of the other two disk samples was blended with

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300 ml isotonic sucrose. Cell debris was removed by filtering as before, and potassium was determined in all filtrates using an EEL flame-photometer, with a blank consisting of isotonic sucrose solution which had been filtered through Whatman No. 541 paper. The rate of leakage of potassium ions was calculated as that percentage of the potassium present after washing which diffused out of the disks in 5 hr. The calculated rate was thus independent of any variation in potassium content between samples of fruit.

**Results and Discussion**

The limited number of fruits available from each tree, together with the relatively large number of sampling times, allowed only one sample per tree to be removed from storage at each sampling time. Therefore the two trees of each variety were treated as duplicates for the statistical analysis, which was carried out on logarithm transformation of the data in order to reduce the heterogeneity of variance.

Figure 1 shows, for Jonathan and Sturmer varieties, the means of the transformed data plotted against time, the calculated trend, and the least significant differences for the comparison of any two means. In both varieties there was a significant upward trend in potassium leakage rate during the first three-fourths of the post-harvest life of the fruit. This was followed by a significant downward trend which continued at least until just before the end of storage life. Although the Jonathan fruit had about twice the storage life of the Sturmer fruit, the trend during the major part of storage life was similar in the two varieties. In Sturmer fruit the leakage rate increased steadily during the relatively brief ripening period immediately after harvest, and continued to increase thereafter. In Jonathan fruit the data do not afford a clear picture of the course of potassium leakage rate during the ripening period.

Changes in the rate of potassium leakage out of tissue disks have been regarded by a number of workers as an indication of changes in the permeability of cell membranes (e.g. Lieberman et al. 1958; Baur and Workman 1964). In studies of
permeability, the measurement of potassium leakage rate is preferable to the measurement of leakage of other important cell sap solutes (e.g. sucrose) because potassium determinations are simple and reliable and because the concentration of potassium in the tissue of detached organs is not affected by any metabolic changes which may occur with time.

However, the potassium concentration gradient and cell permeability may not be the only factors determining the rate of diffusion of potassium out of tissue disks into an aqueous bathing medium. As potassium ions are the main cations providing electrical balance for the anions present in the cell, the rate of outward diffusion of potassium may depend in addition upon the number of anions present which are being balanced by potassium ions, and upon the number of anions which are sufficiently mobile to diffuse out along with potassium ions.

Supplementary experiments showed the rate of leakage of potassium from apple fruit disks to be independent of the concentration of calcium ions in the bathing medium over the range 0.01–1 mm. The number of equivalents of calcium absorbed by the disks was greater or less than the number of equivalents of potassium lost, depending on the variety and on the concentration of calcium in the bathing medium.

In the main experiments reported here, while calcium chloride was initially present in the bathing medium at a concentration of 1 mm, the concentration of leached potassium was always less than 0.4 mm. Hence there was never any shortage of chloride to balance the potassium leaking out, or of calcium to replace this potassium in balancing intracellular anions. The fact that a hundredfold reduction in the concentration of these ions in the bathing medium had no effect on potassium leakage rate suggests that they were not indispensable in this role. While calcium ions may have functioned in the maintenance of ion pumps possibly operating within the cells (Morrill, Kaback, and Robbins 1964), potassium leakage rate was apparently not limited by requirements for the maintenance of electrical balance.

Since the apple fruit cell consists of a large vacuole bounded by an extremely thin cytoplasmic layer and a thin cellulose wall, by far the major proportion of intracellular potassium is likely to be in free solution in the sap. Therefore observed changes in rate of potassium leakage would be more likely to result from changes in cell membrane permeability than from changes in the degree to which potassium ions are bound in the cell wall, in intracellular membranes, or in organelles such as mitochondria or microsomes.

The increase reported by Sacher (1966) in the permeability of banana fruit tissue during ripening resulted from a progressive increase in the proportion of cells which were completely permeable to solutes. If an increase in cell permeability was responsible for the observed increase in potassium leakage rate from disks of senescent apple fruits, it would appear to have been due rather to a progressive increase in the permeability of all the cells simultaneously. Two observations lead to this conclusion. Firstly, apple disks at all stages of ripening and senescence retained normal turgor, whereas banana tissue lost turgor during the climacteric (Sacher 1966). Secondly, the rate of leakage from apple disks declined late in senescence. It seems unlikely that an increase in the proportion of completely permeable cells would be a reversible effect.

It is suggested that the observed increase in permeability of apple fruit cell membranes may cause alteration of intracellular compartmentation, as proposed by
Sacher (1962) for banana, resulting in the promotion of enzyme–substrate interactions deleterious to the maintenance of normal cell organization. On the other hand, the observed decline in permeability late in senescence may slow down reactions necessary at this stage for such maintenance. It seems possible, therefore, that the observed changes in cell permeability may exert an influence on the rate of loss of cell organization during senescence, and hence on the duration of fruit storage life.

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References


