Relationship between Cell Permeability and Respiration in Ripening Banana Fruit Tissue [1, 2, 3]

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Introduction

The hypothesis that changes in membrane permeability may be a causative factor in the climacteric rise in fruit respiration was suggested by Blackman (2) and also by Kidd in a lecture before the Royal Institute of Great Britain in 1934 (1). Support for this hypothesis has recently appeared in studies by Sacher (4) with banana and avocado fruits. He observed a close parallel during the respiratory climacteric between the respiratory increase and the rate of diffusion of substances from the tissue into distilled water. Young et al. (6) also observed that climacteric avocado tissue leaked P^{32} more readily than preclimacteric tissue.

Although differing in methodology, the studies reported here confirm the findings of Sacher (4) with banana fruit. Also, reported are the observations on ions leaking from the tissue, and the influence of 2, 4-dinitrophenol (DNP) on ion leakage and respiration of tissue slices taken from banana fruit ripening from green to fully ripe.

Materials and Methods

Experimental Material. The banana fruit (var. Valery and Fortuna) used in these experiments were grown in Honduras and were obtained from a local wholesale distributor. The hands of fruit were severed from the stem in Honduras and shipped in boxes. Each box contained 5 to 7 hands, the greenest of which were selected for the experiments. These fruits were all preclimacteric in respiration rate and just beginning to show a trace of yellow. All fruit were ripened in the absence of ethylene at 18° and 85 to 90 % relative humidity. At intervals of 24 or 48 hours, fruit samples were removed from the ripening room for experimentation. The rate of respiration of the intact fruit was determined and the fruit sectioned for permeability and tissue slice respiration measurements.

Respiration Measurements. The respiration rate of the intact fruit was measured immediately prior to sectioning with a Beckman Model 15A L/B infrared analyzer. The gas analyzer was calibrated between 0 and 625 ppm CO_2 and the background gas, commercially compressed air, ran approximately 300 to 325 ppm CO_2. This respiratory measurement established the position of the fruit on the climacteric curve.

Tissue slice respiration rates were determined in triplicate on cortical banana pulp tissue cut into slices 8 mm in diameter and 1 mm thick. The measurements were made at 25° on 0.75 g of tissue in 7-ml Warburg vessels.

To determine the influence of an uncoupler on tissue slice respiration, the tissue was soaked for 120 minutes in 10^{-4} M DNP. The control tissue was soaked for the same time in deionized water. After soaking, the slices were rinsed and immediately placed in the Warburg vessels and the rate of respiration determined over a 120 minute period.

Permeability Measurements. The changes in membrane permeability in pulp and peel tissue were determined by measuring the rate of ion leakage from tissue slices into deionized water. The slices used were from the same fruits used for tissue slice respiration measurement.

Since the number of pieces required for a given weight of tissue changes as the banana ripens, ion leakage measurements were made on a constant number of pieces rather than on a weight basis. At the time of the first measurement, when the fruit was quite green, the average number of slices in a 5-g sample was determined. At subsequent samplings as the fruit ripened, the same number of pieces were always used.

The slices, 10 mm in diameter and 3 mm thick, were placed loosely in bags constructed of Visking #28 No-Jax dialysis casings. These were suspended in 125 ml of deionized water which was being gently agitated with magnetic stirrers. The change in solution conductivity with time was measured at 20 ± 1° with a Model RC 16B2 Industrial Instruments conductivity bridge using a 1-ml pipette-type cell.

The effect of DNP on ion leakage was determined by soaking the pulp tissue slices in 10^{-4} M DNP for 120 minutes prior to measurement. Controls were held 120 minutes in deionized water.

Analytical Procedures. The inorganic ions leak-

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ing from pulp and peel tissue slices into the deionized water were determined on a Unicam SP 900 flame spectrophotometer. Analyses were made for Ca^{++}, Mg^{++}, and K^{+}.

Results

Ion Leakage—Respiration Relationship. A total of 8 separate hands were studied during ripening and the relationship between ion leakage and intact fruit respiration was similar in each (fig 1). The rate of ion leakage from pulp tissue was correlated with the intact fruit respiration rate in each of the eight hands. The correlation coefficients ranged from +0.896 to +0.990 and were significant at the 0.05 level. The results of only one experiment are shown.

There was a marked increase in the rate of ion leakage from pulp tissue at the onset of the climacteric rise. The rate of ion leakage continued to increase until the seventh day of ripening at 18° and then remained nearly constant. The climacteric peak was attained on the ninth day.

Loss of ions from the peel tissue was less dramatic. However, a slight increase in leakage rate did occur during the first 8 days of ripening at 18° and a fairly sharp increase in the rate of ion loss occurred after the ninth day. Possibly this lag in the rate of ion loss is due to the fact that ripening is initiated in the placental region of the pulp and progresses outward through the cortical region to the peel (3).

Ions Present in the Diffusate Solution. Potassium was the predominant cation present in the bathing medium. Calcium and magnesium ions were not detected. A comparison was made between K^{+} content and the maximum conductivity attained in

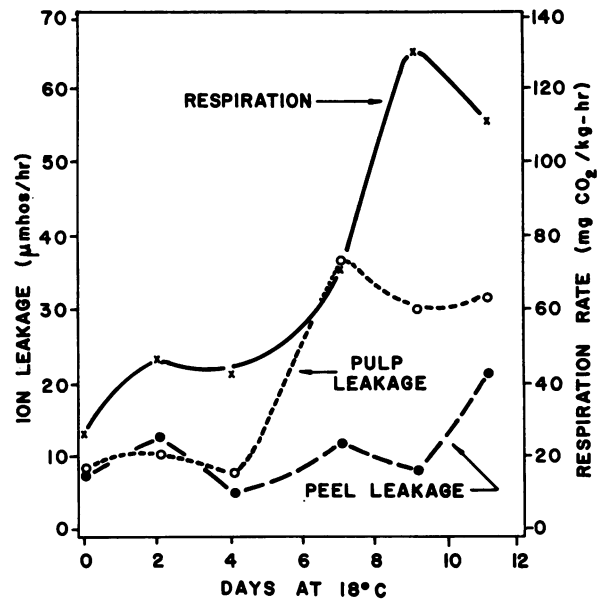

FIG. 1 (*upper left*). Relationship between the rate of ion leakage from pulp and peel tissue and the intact fruit respiration rate of the ripening banana. Fruit was ripened at 18° and sampled at the times indicated. The correlation coefficient between pulp leakage and respiration rate is + 0.942, which is significant at the 0.05 level.

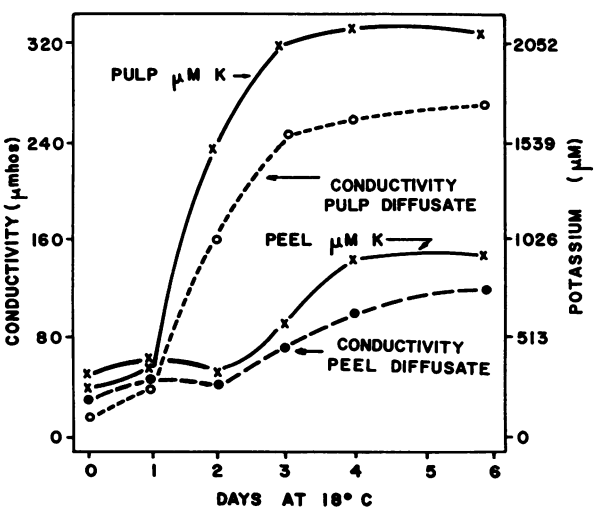

FIG. 2 (*lower left*). Relationship between the potassium content and conductivity of the diffusate solutions. Measurements were made after a leakage period of 360 minutes. Fruit was ripened at 18° and sampled at the times indicated.

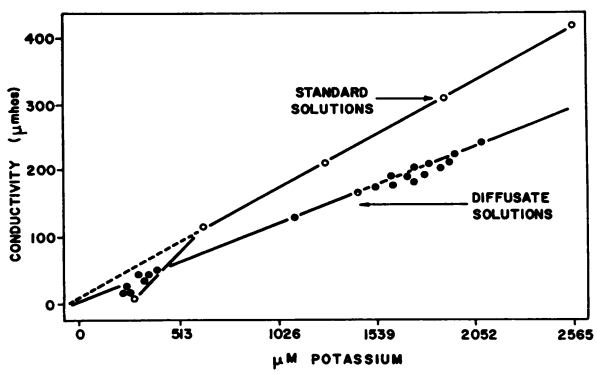

FIG. 3 (*upper right*). Comparison of the conductivity of standard potassium solutions and the conductivity of pulp diffusate solutions containing equivalent amounts of potassium ion.

the diffusate solution. This was done at regular intervals as the fruit ripened (fig. 2). It is evident that the increase in conductivity resulting from ion leakage from peel or pulp tissue closely paralleled the increase in K^+ ion in the diffusate solution.

Another comparison was made between the conductivity of standard K^+ solutions (KCl) and the conductivity of diffusate solutions containing equivalent amounts of K^+ (fig 3). The diffusate solutions always showed a lower conductivity. This may be due to immobilization of the K^+ or perhaps to the nature of the anion. Possibly the attendant anions in the diffusate solution were organic in nature and less mobile than Cl^- in pure solution.

Effect of DNP on Tissue Slice Respiration and Rate of Ion Leakage. Several concentrations of DNP (10^{-3} M to 10^{-6} M) were tested in order to determine the concentration giving the maximum stimulation of respiration. Based on these observations, 10^{-4} M which stimulated O_2 uptake 53 % was used in all experiments.

The influence of DNP on tissue slice respiration and the rate of ion leakage from pulp tissue slices is shown in table I. The intact fruit respiration determined just prior to sectioning is also shown. The DNP induced respiratory stimulation ran between 42 and 55 % as the fruit ripened during the climacteric rise in respiration. However, at the climacteric maximum DNP inhibited tissue slice respiration.

Table I. *Influence of 10^{-4} M DNP on Tissue Slice Respiration and Rate of Ion Leakage*

Respiration			Ion leakage	
Intact Fruit*	Tissue slice**		Rate***	
	Control	DNP	Control	DNP
21.0	51.0	75.0	4.0	5.0
35.0	58.0	90.0	5.0	6.2
58.0	31.0	44.0	12.8	10.0
113.0	18.5	11.8	8.0	6.8

* Respiration rate of whole fruit immediately before sectioning, mg CO_2/kg-hr.

** Slices soaked in deionized water or 10^{-4} M DNP prior to respiration measurements, μl O_2/g-hr.

*** Conductivity of bathing solution in μmhos/hr. Slices soaked in deionized water or 10^{-4} M DNP prior to ion leakage measurements. Values are averages of 4 replications.

The respiration rate of the tissue slice controls did not increase parallel with the increase in the intact fruit respiration rate during the climacteric rise. Rather an inverse relationship was shown. This may reflect the greater sensitivity of the riper tissue to sectioning.

Although not large, DNP also increased the rate of ion leakage from preclimacteric pulp tissue (table I). This stimulation of ion leakage continued for a short time as the fruit ripened but ceased before the climacteric maximum was attained.

Discussion

A dramatic increase in the rate of ion leakage from tissue slices occurred as the banana fruit ripened from the green to the ripe condition. This increase closely paralleled the climacteric rise in respiration. However, neither these observations nor those of Sacher (4) establishes the increase in membrane permeability as a causative factor in the climacteric rise. In our work, the increase in membrane permeability seemed to occur simultaneously with the increase in respiration. Hence, it could be one of many changes initiated in fruit during the climacteric rise in respiration.

Initially, we advanced the hypothesis that this increase in membrane permeability during fruit ripening was due to insufficient energy to both maintain the cellular membranes and still permit synthesis of enzymic proteins, volatiles, and other constituents during fruit ripening. To test this hypothesis, the influence of an uncoupling agent on the rate of ion leakage from pulp slices taken from green through ripe bananas was determined. Preclimacteric fruit, treated with DNP, demonstrated a small but persistent increase in ion leakage. As the fruit ripened, the effect diminished and eventually ion leakage was inhibited.

The increase in ion leakage caused by DNP was relatively small compared to the increase which occurs during the climacteric rise. Most likely energy relations are only one of several factors responsible for the large increase in ion leakage.

The tremendous changes in the osmotic concentration due to the starch-sugar shift may have an influence. This increase in osmotic concentration may be primarily responsible for the movement of water from the peel to the pulp resulting in increased hydration of the cellular membranes. Possibly this increased hydration could render the membranes more permeable to ions (5).

Summary

The rate of ion leakage from tissue slices taken from banana fruit in several stages of ripeness was determined. At the onset of the climacteric rise, the rate of ion leakage from pulp tissue began to increase. The rate of loss continued to increase as the fruit ripened and reached a constant rate about 2 days before the climacteric peak in respiration. Increases in the rate of ion leakage from peel tissue did not occur until the climacteric peak in respiration had been reached. The major cation leaking from the tissue was K^+.

Preclimacteric banana pulp tissue when treated with 10^{-4} M 2, 4-dinitrophenol shows a marked increase in respiration and a moderate increase in ion leakage. As the fruit ripened the stimulatory effect

diminished and when ripe both respiration and ion leakage were inhibited.

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Proteins and Plant Cell Walls. Proline to Hydroxyproline in Tobacco Suspension Cultures [1, 2, 3]

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Hydroxyproline occurs in higher plants in peptides and proteins, which are found in the plant cell walls (6, 9, 18, 19). Considerably more hydroxyproline is found in the protein of rapidly proliferating tissue than in the protein of slowly proliferating tissue (2, 7, 19). Hydroxyproline-rich plant protein and collagen are similar in the large amounts of proline and hydroxyproline they contain. The function of hydroxyproline-rich plant protein may thus be structural, similar to that of collagen in animals, or perhaps structural and regulatory as suggested by Lamport (8). In this connection Lamport has proposed that such hydroxyproline-rich plant proteins be called "extensin" (8).

The conversion of proline to hydroxyproline is very similar in animal and plant systems. In both systems free proline, but not free hydroxyproline, is a precursor of protein bound hydroxyproline (19). In general hydroxyproline-containing proteins are metabolically inert (2, 11). Hydroxyproline hydroxyl O_2 of both animal and plant protein is derived by the fixation of atmospheric O_2 (3, 4, 8, 17).

Cultured plant cells are ideal for study of the location, function, and biochemistry of hydroxyproline-containing plant protein because of the large amounts of the protein present. This paper presents results of an investigation of the hydroxyproline-containing protein of the cell walls of suspension cultures of tobacco cells grown on a completely defined medium. As reported by others, these cells rapidly incorporate proline-C^{14} into all protein fractions as proline-C^{14} or hydroxyproline-C^{14}. Data are presented which suggest that in this system proline may be hydroxylated after incorporation into a protein or large peptide.

Materials and Methods

Tobacco Suspension Cultures. Suspension cultures of tobacco cells were grown in a completely defined liquid medium developed by Mr. Philip Filner and described in table I (private communication). The cells were provided by Mr. Filner who originally isolated them from tobacco stem callus (*Nicotiana tabacum* var. Xanthi).

Cultures were grown from 5 to 10 days at 27 to 28° in liter flasks on a platform-type reciprocal shaker operating at 100 cpm with a horizontal excursion of 4.5 cm.

Collection of the Cells and Their Fractionation. Cells were collected by filtering suspensions through Miracloth[5], a porous paper, and squeezing them free

[1] Received Nov. 12, 1963.

[2] Work conducted under a cooperative agreement with the California Institute of Technology, at the Division of Biology, Pasadena, Calif.

[3] Reported in part at the Western Section meeting of the American Society of Plant Physiologists, June 17 to 21, 1963, Stanford University.

[4] A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

[5] Reference to a company name does not imply approval or recommendation of the product by the Department of Agriculture to the exclusion of others that may also be suitable.