PERMEABILITY, SUGAR ACCUMULATION, AND RESPIRATION RATE IN RIPENING BANANA FRUITS

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Summary

The permeability of pulp tissue of banana fruits and aseptically prepared transverse slices of bananas increases before the respiratory climacteric begins. The permeability change may be measured as leakage of amino acids from pulp tissue, and is not dependent on the soluble carbohydrate content of pulp cells. During the climacteric, amino acid leakage into water increases further. This increase in part results from the increase in the soluble carbohydrate content of the pulp during ripening, but leakage of amino acids into sugar solutions also increases.

Application of 1–100 p.p.m. of ethylene results in a respiratory response within 8 hr, and the respiration rate is half the peak climacteric rate after 24 hr. No increase in amino acid leakage, nor in "apparent free space" to mannitol can be measured before 32 hr. The respiratory increase in banana fruit during ripening is thus not dependent on changes in tissue permeability.

Preclimacteric banana fruit has a respiratory quotient of 1·0. With or without added ethylene, when the climacteric begins, the respiratory quotient drops rapidly to 0·8 before rising gradually to 1·0 at about the time of the climacteric peak.

I. Introduction

During the ripening of many fruits, a rise in respiration rate occurs. The relation between this respiratory climacteric and other ripening events involving changes in texture, colour, and sweetness has interested physiologists for many years.

One theory of ripening links the respiratory climacteric to a decreased isolation of cell compartments (Blackman and Parija 1928; Sacher 1967). A similar explanation has been advanced for the respiratory rises which occur in response to fungal infection (Wheeler and Hanchey 1968), and in senescent tissues (Sacher 1967). Of particular interest is evidence of increased tissue permeability in banana (Sacher 1966, 1967) and avocado fruits (Ben-Yehoshua 1964) prior to the commencement of the climacteric. Sacher (1966, 1967) has noted that "apparent free space" (Briggs and Robertson 1957) increased through the climacteric and concluded that pulp tissue of bananas at the climacteric peak was all freely permeable to low molecular weight solutes. Burg, Burg, and Marks (1964) emphasized that the increasing sugar concentrations in pulp cells may influence estimates of apparent free space, and Burg (1968) measured an apparent free space of about 60% in climacteric pulp tissue.

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In the work reported here we have examined further the connection between permeability changes, soluble sugar content of tissue, and respiration rate in banana fruits. We have observed the sequence of changes occurring in banana fruits and banana fruit slices in which the time to ripen was varied by controlling the ethylene concentration in the atmosphere. By this means we sought to distinguish those changes which are linked during natural ripening only by coincidence in time from those which may be causally related.

II. Materials and Methods

(*a*) *Fruit*

Green fruits of the Williams Hybrid strain of the Dwarf Cavendish variety were obtained from Avoca, N.S.W. Experiments were performed using either intact fruit, or 6-mm thick transverse slices prepared as described by Palmer and McGlasson (1969). Before treatment with ethylene, slices were kept for 4 days in air.

Respiration measurements were made on individual whole fruit, or bulked samples of 4 or 10 slices. Flow rates of the ventilating airstreams were adjusted to maintain the oxygen content inside respiration jars above 20%(v/v).

(*b*) *Ethylene Application*

Fruit was ventilated continuously with air containing ethylene (0·1–100 p.p.m.), following the procedures of Vendrell (1969), or Pratt *et al.* (1960).

(*c*) *Respiration Measurements*

Carbon dioxide production was measured with an Infra-red Gas Analyser, model SB2, Grubb Parsons and Co. Ltd., England, and oxygen uptake was measured with a D.C.L. Servomex Analyser, type 83, Servomex Controls Ltd., Crowborough, England.

To determine the respiratory quotient (R.Q.) (Thomas 1960) carbon dioxide and oxygen measurements were made sequentially on individual intact bananas within a period of 10 min. No increment in respiration rate could be detected during this time interval, even when the slope of the climacteric was maximal.

(*d*) *Soluble Carbohydrate Analysis*

Extracts were made from fresh pulp tissue, or from pulp frozen in liquid nitrogen and stored as a powder at −15°C.

Samples of pulp tissues were extracted three times by boiling under reflux in 200 volumes of 80%(v/v) ethanol. Ethanol was removed from the bulked extracts *in vacuo* at 40°C. Total carbohydrate in the extracts was assayed by the anthrone reaction with glucose as standard (Pinnegar 1965). Results are expressed as percentages of dry matter, estimated on samples dried for 16 hr at 80°C in a forced-air draught.

(*e*) *Leakage of Amino Acids*

To measure the leakage of amino acids from pulp tissue, lots of 4·0 g of disks, 1 mm thick and 1 cm in diameter, were immersed in 80 ml of solution and agitated by an air stream. After 1 hr at 0°C, the solution (diffusate) was decanted, centrifuged, and sampled. The tissue and the solution remaining with it were extracted by grinding in 5%(w/v) trichloracetic acid. Amino nitrogen in this extract and in the diffusate was estimated by the colorimetric ninhydrin method (Cadavid and Paladini 1964). Assuming a tissue density of 1·0, leakage of amino nitrogen was calculated as

$$\frac{\text{amino nitrogen in diffusate } (\mu\text{mole/ml}) \times 84 \text{ (ml)}}{\text{amino nitrogen in diffusate + extract } (\mu\text{mole})} \times 100.$$

To measure leakage from the pulp of fruit slices, the outer 1·5 mm was removed from each cut

surface, and disks prepared from the central pulp tissue. Leakage estimates were made on duplicate pulp samples.

When the time course of leakage was measured, bulked samples of pulp disks were prepared from groups of 12 fruit, and 8-g lots bathed in 200 ml of solution at 0°C. Samples (2 ml) were taken at appropriate times, filtered through glass wool to remove tissue pieces, and analysed. Each treatment was replicated four times.

Total tissue leakage of amino acids includes components moving from damaged cells and from the "free space", as well as components transversing the plasmalemma. Leakage from both preclimacteric and climacteric tissue slices is initially very rapid (Fig. 1). Amino nitrogen from damaged cells and from the free space will be the major component of this rapid phase which has a half-time of about 1·5 min. Since preclimacteric and climacteric fruit slices lost similar amounts of amino nitrogen in the first 2·5 min of washing, differences in cell damage in slicing, or in amino nitrogen in the free space, were not important to the difference in total leakage. The greatest difference between preclimacteric and climacteric tissue was in the slope of the graph after about 10 min.

Most measurements of leakage reported in this paper involve a balance of amino nitrogen between solution and tissue at the completion of a 60-min washing period. This will include amino nitrogen lost during the rapid phase of leakage. From slices of a range of maturities, leakage within 2·5 min has accounted for 8·4±0·8% of the amino nitrogen of the tissue.

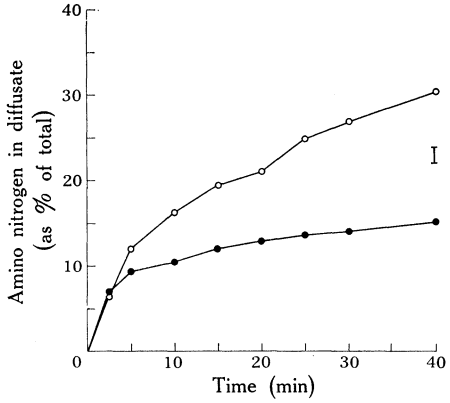

Fig. 1.—Leakage of amino nitrogen into water from slices of the pulp of preclimacteric (●) and climacteric (○) fruit. Bar shows least significant difference ($P = 0·05$).

(*f*) *Apparent Free Space to Mannitol*

From groups of six bananas, pulp disks 1 mm thick and 1 cm in diameter were cut. Lots of disks weighing 2·00±0·10 g were incubated in 4·00 ml of [1-^{14}C]mannitol (400 μmoles and 0·033 μCi) solution for 30 min at 25°C. The solution was then decanted and centrifuged, and the radioactivity in 0·10 ml lots of the supernatant measured in a Packard 300 Tri-Carb liquid scintillation spectrometer after mixing with 0·9 ml of water and 10 ml of the scintillation mixture 1 of Patterson and Greene (1965).

Apparent free space was calculated (Sacher 1966) as

$$\left[\left\{\frac{\text{disintegrations/min in 4·00 ml solution}}{\text{disintegrations/min/ml after incubation}} - 4·00\text{ ml}\right\} \times \frac{1}{\text{tissue weight (g)}} \times 100\right].$$

(*g*) *Amino Acid Analyses*

The methods of tissue extraction and amino acid analyses are described in Brady *et al.* (1970).

III. Results

(*a*) *Effect of Added Ethylene on the Respiratory Climacteric*

A minimal concentration of 0·1–1·0 p.p.m. ethylene was required to induce the climacteric of both whole fruit and slices within 6 hr (Fig. 2). The climacteric was not induced immediately by 0·1 p.p.m. ethylene, but its occurrence was advanced

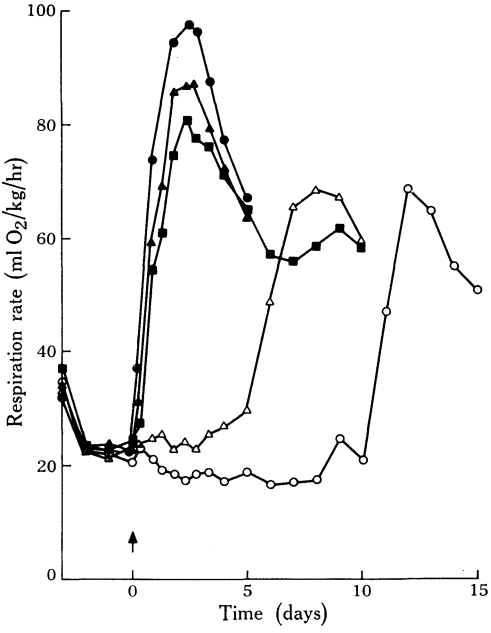

Fig. 2.—Effect of treatment with ethylene at concentrations (p.p.m.) of 0 (○), 0·1 (△), 1·0 (■), 10 (▲), and 100 (●) on oxygen uptake by banana slices. Six replicates, each of four slices, were taken in each treatment; each point is the mean of the replicates. Least significant difference ($P = 0·05$) is 4·1 ml O_2/kg/hr.

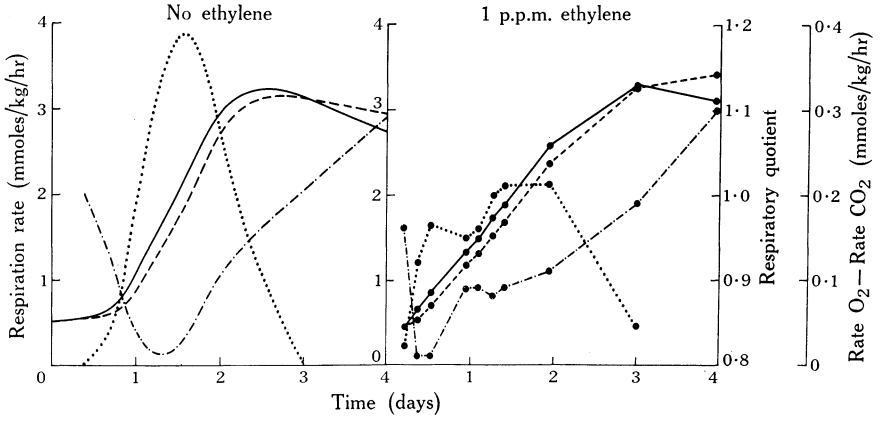

Fig. 3.—Effect of 1 p.p.m. ethylene on carbon dioxide output (– – –) and oxygen uptake (——) of individual bananas. Six fruits were included in each treatment, and the mean responses are shown. Fruits in air varied by several days in the time taken to enter the climacteric. For presentation, a common time was assigned to all samples in air, and the respiration reading shifted appropriately along the time axis. The respiratory quotient (-·-·-) and the difference between oxygen uptake rate and carbon dioxide output rate (···) were calculated for individual fruits, and mean values are presented.

relative to slices not treated with ethylene, and was preceded by a slow upward drift in respiration rate. Similar respiratory responses of banana fruit to ethylene have been described by Biale (1960).

Preclimacteric fruit had an R.Q. of about 1·0. Within 8 hr of adding 1 or 100 p.p.m. ethylene, the R.Q. fell to about 0·8, then rose gradually towards 1·0 as the climacteric developed (Fig. 3). A similar fall followed by a rise in the R.Q. occurred during the climacteric in fruit ripening without added ethylene (Fig. 3).

(*b*) *Amino Nitrogen Leakage and Respiration Change*

In banana fruit slices not treated with ethylene, leakage of amino nitrogen increased 3–4 days before the climacteric began [Fig. 4(*a*)]. Leakage increased progressively throughout the climacteric so that when the respiration rate was half-maximal, the leakage of amino nitrogen was about 50% of the total pool.

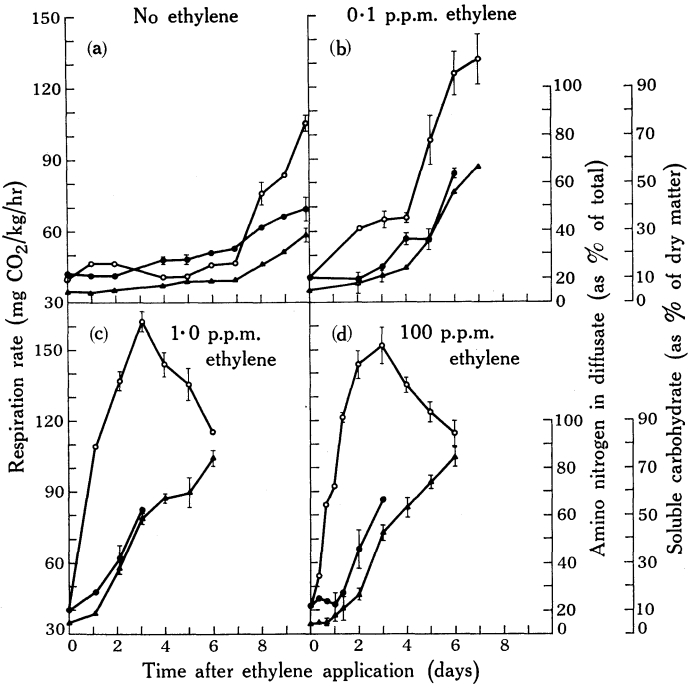

Fig. 4.—Effect of ethylene on respiration rate (CO_2 production) (○), amino acid leakage (●), and ethanol-soluble carbohydrate content (▲) of banana slices. Each treatment consisted of groups of 10 slices, and at each time interval two groups were removed for analysis. Duplicate samples of pulp tissue were selected from each group for each analysis. The respiration graphs are of mean readings of all groups still held at any time. Standard errors which exceed the size of the symbols are shown as bars.

In contrast, slices treated with 1 or 100 p.p.m. ethylene [Figs. 4(*c*), 4(*d*)] showed no increase in leakage until 32 hr after ethylene was applied. A half-maximal respiration rate was attained within 24 hr of ethylene application. Slices treated with 0·1 p.p.m. ethylene [Fig. 4(*b*)] showed an increase in respiration but not in leakage by the second day after ethylene was applied. Subsequently both respiration rate and leakage increased, slowly at first and then rapidly.

The relationship between changes in respiration rate and amino nitrogen leakage was also measured in whole fruit which were ventilated with ethylene-free air, or air containing 1 or 100 p.p.m. ethylene. In fruit treated with ethylene no increase in leakage occurred within 24 hr, although respiration rate was half-maximal

by this time (Table 1). After 48 hr, when the respiratory peak was approached, an increase in leakage was measured.

TABLE 1

RESPIRATION RATE AND AMINO ACID LEAKAGE FROM PULP OF WHOLE BANANA FRUIT TREATED WITH ETHYLENE

Twelve fruits were assigned at random to each ethylene treatment. Respiration readings were made on individual fruit and, at daily intervals, two fruits per treatment were examined for amino acid leakage into water from pulp disks. Duplicate samples of disks were used from each fruit. The values presented are means together with their standard errors

Time (hr)	Ethylene Concn. (p.p.m.)	Respiration Rate (μl CO_2/kg/hr)	Amino Acid Leakage (%)
0		18·56±1·07	22·0±1·4
24	0·0	19·12±0·80	22·5±1·8
	1·0	48·79±0·45	19·5±1·0
	100	67·68±1·57	21·1±1·2
48	0·0	18·70±1·18	22·9±1·1
	1·0	114·14±2·24	38·6±3·5
	100	140·26±4·22	46·1±5·6

In fruit not treated with ethylene, the respiration rate was more or less constant for about 10 days. However, amino nitrogen leakage varied between the samples taken at daily intervals during this time (Fig. 5). The frequency of occurrence of

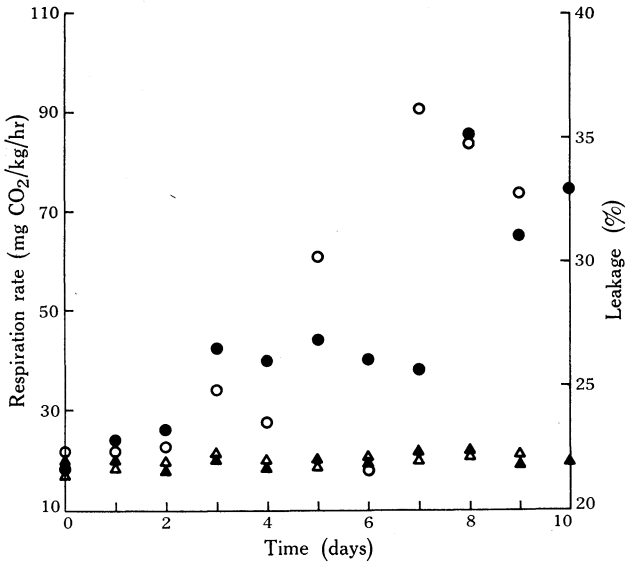

Fig. 5.—Leakage of amino acids from pulp tissue, and the respiration rate (CO_2 production) of individual banana fruits ventilated with ethylene-free air, on successive days after harvest. On each day after harvest, two fruits were selected randomly, and amino acid leakage from pulp tissue measured. The graph presents the respiration rate (△, ▲) of the fruit immediately before analysis, and the corresponding leakage rate (○, ●) for each individual fruit.

fruits with a high leakage value increased with time after harvest, strongly suggesting that an increase in leakage precedes the onset of the climacteric.

(c) *Influence of Sugar Accumulation on Leakage Rate*

In slices ripening with or without added ethylene the content of soluble carbohydrate in pulp cells increased at about the same time as the leakage rate (Fig. 4). A similar coincidence in time between increased leakage rate and soluble carbohydrate accumulation occurred in whole fruit.

TABLE 2

AMINO ACID LEAKAGE AND SOLUBLE CARBOHYDRATE CONTENT OF THE PULP OF PRECLIMACTERIC BANANA FRUITS

Respiration rate (CO_2 production), leakage of amino acids, and ethanol-soluble carbohydrate content of the pulp of individual whole fruits were measured on successive days after harvest. Two fruits were examined on each day, and duplicate pulp samples were used for both leakage and carbohydrate estimates on each fruit. The values presented are means together with their standard errors over the time intervals shown

Time Post-harvest (days)	Respiration Rate (μl CO_2/kg/hr)	Amino Acid Leakage (%)	Ethanol-soluble Carbohydrate (% dry wt.)
5–7	19·3±1·2	21·9±0·4	1·39±0·45
8–11	19·5±0·8	26·3±0·4	1·76±0·37
12–15	20·8±1·0	34·6±1·3	1·95±0·32

These results suggested that the leakage characteristics may be influenced by an increase in turgor in cells as the osmotic pressure increased. However, the results in Table 2 clearly show that the leakage rate can increase in preclimacteric fruit without a corresponding increment in soluble carbohydrate content.

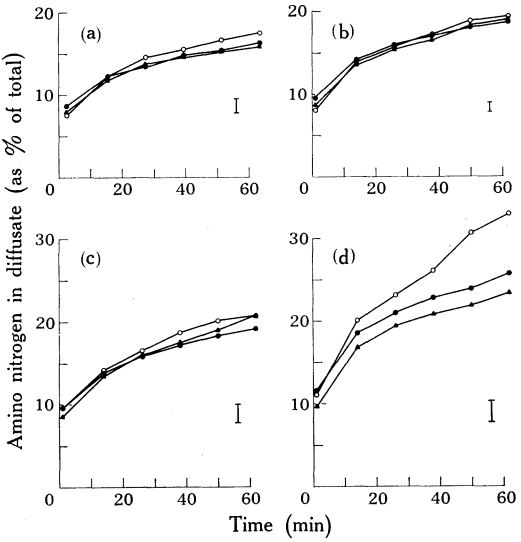

Fig. 6.—Effect of solution tonicity on amino acid leakage from pulp tissue disks prepared from whole bananas treated with 10 p.p.m. ethylene for 0 (*a*), 24 (*b*), 48 (*c*), and 72 hr (*d*). Leakage into water (○), 0·4M mannitol (●), and 0·6M mannitol (▲) was measured. The ethanol-soluble carbohydrate contents of the pulp were: (*a*) 3·08±0·12, (*b*) 3·48±0·08, (*c*) 10·72±1·48, and (*d*) 21·96±3·04% of dry matter. Bars show least significant differences ($P = 0·05$).

The kinetics of amino nitrogen leakage from preclimacteric and climacteric pulp tissue into water, 0·4M, and 0·6M mannitol solution are shown in Figure 6. Two

components of leakage may be recognized. The rapid initial phase was independent of solution tonicity in all samples. The second slower phase was dependent upon solution tonicity only in tissue exposed to ethylene for 72 hr [Fig. 6(*d*)].

(*d*) *Amino Acids Leaking from Pulp Tissue*

The molar ratios of amino acids in diffusates and tissue extracts were identical, and similar to those described for preclimacteric and climacteric samples by Brady *et al.* (1970). Since the amino acids in the diffusates were representative of the total free amino acid pool, a change in the Donnan system between the osmotic volume and the free space did not contribute significantly to total leakage.

(*e*) *Change in Apparent Free Space*

No increment in the apparent free space to mannitol was found in disks from ethylene-treated whole fruit until late in the climacteric period (Table 3). The apparent free space to inulin, measured by isotopic dilution, also increased late in the climacteric.

TABLE 3

APPARENT FREE SPACE TO MANNITOL IN RIPENING BANANAS

A bulked sample of fruit was ventilated with 10 p.p.m. ethylene. At each time, apparent free space to mannitol was measured on eight lots of 2·0 g of a bulked sample of pulp slices prepared from 12 bananas. Apparent free space was determined by isotope dilution as detailed in Section II. Values presented are means together with their standard error

Time (hr):	0	16	24	36	48	72	72*
Apparent free space (%)	18·4 ±2·5	16·9 ±1·7	21·4 ±4·2	20·3 ±5·8	27·3 ±6·4	38·1 ±5·1	19·5 ±2·2

* Ventilated with ethylene-free air.

IV. DISCUSSION

Before the onset of the climacteric a change in the permeability characteristics of fruit tissue occurs during ripening in the absence of added ethylene. This permeability change may be measured as leakage of cell contents [Figs. 4(*a*), 5], or as the apparent free space to various ions or organic molecules (Sacher 1966). The change in permeability before the onset of the climacteric, and the increase in respiration rate as the tissue becomes more permeable [Fig. 4(*a*)] have led to the postulate that the respiration rises because cellular compartmentation decreases (Sacher 1966, 1967; Young and Biale 1967).

However, fruit treated with ethylene at a concentration above 1 p.p.m. develops a typical respiratory climacteric without change in permeability as measured by leakage [Figs. 4(*c*), 4(*d*)] or as apparent free space to mannitol (Table 3). Thus, a change in total tissue permeability cannot be a prerequisite for the development of the climacteric. More subtle changes in intracellular compartments may be involved.

Leakage from some fruit tissues bathed in aqueous solutions can be reduced by increasing the tonicity of the bathing medium (Burg, Burg, and Marks 1964). Leakage from preclimacteric and early climacteric banana fruit tissue is, however, essentially

independent of solution tonicity (Fig. 6). Leakage from late climacteric tissue into mannitol solution is markedly lower than into water [Fig. 6(*d*)]. However, leakage into 0·6M mannitol solution, from late climacteric tissue (48 and 72 hr after applying ethylene) in the 2–62-min interval of bathing, is significantly greater than that from preclimacteric tissue ($P = 0{\cdot}05$, calculated from results summarized in Fig. 6). This component of leakage from tissue exposed to ethylene for 72 hr also significantly exceeds that from tissue from fruit sampled 24 hr after exposure to ethylene. There is then an increment in the late climacteric in a component of leakage which is independent of solution tonicity. The observed increases in leakage are thus not dependent only on the accumulation of soluble carbohydrate and attendant increases in tissue osmotic pressure during the climacteric.

Bananas ripening in air are reported to have an R.Q. of unity (Langworthy and Milner 1912; Olney 1926; Nelson 1939; Palmer and McGlasson 1969). Although a quotient of less than unity has been reported in unripe (Wardlaw 1940) and ripening bananas (Gane *et al.* 1953), a change in R.Q. has not previously been associated with climacteric respiration.

An apparent change in R.Q. may arise from purely physical factors associated with the diffusion coefficients and solubilities of carbon dioxide and oxygen. These factors may have a marked effect when respiration rate is changing rapidly (James 1953). Studies of the movement of gases through fruit tissues suggest, however, that these factors are not important in the ripening banana (Burg and Burg 1965).

The observed trend in R.Q. is similar to that which occurs in potato tuber slices developing an "induced" respiratory response. In this case, Laties and Hoelle (1967) have presented evidence that the initially low R.Q. reflects the oxidation of fatty acids. In contrast to the potato tuber, an active tricarboxylic acid cycle functions in the banana (Palmer and McGlasson 1969). A low R.Q. may also reflect an accumulation of carboxylic acids by the tissues. Malic and citric acids accumulate in bananas during ripening (Wyman and Palmer 1964).

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