METABOLIC STUDIES WITH BANANA FRUIT SLICES

II.* EFFECTS OF INHIBITORS ON RESPIRATION, ETHYLENE PRODUCTION, AND RIPENING

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Abstract

Transverse slices of green banana fruit were vacuum-infiltrated with aqueous solutions of 24 potential inhibitors of protein synthesis, respiration, or ethylene production. The effects of these compounds were examined in the absence or presence of 10 p.p.m. ethylene. Of the compounds which produced marked effects monofluoroacetate, 4-hydroxy-2-oxoglutarate (HKG), KCN, and cycloheximide were examined in more detail.

Monofluoroacetate (1 and 2×10^{-2}M) and cycloheximide (1 and 2 μg/ml) almost eliminated the peaks of induced respiration and ethylene production, almost eliminated the respiratory response to added ethylene, and prevented normal ripening. Ethylene production by slices treated with the inhibitors rose within a few hours after treatment to steady levels considerably above the preclimacteric rates of untreated slices, possibly as a result of cell damage. KCN at 5×10^{-2}M caused breakdown of the tissue. At 1 and $2 \cdot 5 \times 10^{-2}$M, KCN almost eliminated the peak of induced ethylene production, but only delayed the peak of induced respiration and retarded ripening in both ethylene-treated and ungassed slices. Before the resumption of a normal ripening pattern in slices treated with KCN ethylene production declined to normal preclimacteric rates. HKG at 3×10^{-2}M eliminated the induced rise in ethylene production, delayed the peak of induced respiration, and reduced respiration in ethylene-treated slices but did not affect ripening. There is evidence that banana tissue may metabolize HKG and KCN.

I. Introduction

Thick transverse slices of green banana fruit have proved useful in studies of ripening, and of the metabolic shifts ("aging") which are induced by cutting. Studies of ethylene production (McGlasson 1969), of carbon metabolism (Palmer and McGlasson 1969; Brady *et al.* 1970*a*; McGlasson *et al.* 1971), of protein biosynthesis (Brady *et al.* 1970*b*), and of the effects of plant growth substances (Vendrell 1969, 1970; Wade and Brady 1971) have been made using these slices.

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Certain of the changes induced by slicing fruit tissues are similar to those occurring during the ripening processes (Hulme *et al.* 1968) and the same regulatory functions may be involved. However, metabolic inhibitors may differentially influence components of the ripening and aging processes, and thus provide evidence as to which components are independent of others. An apparently normal respiratory climacteric in pear fruits in which ripening was inhibited by cycloheximide (Frenkel, Klein, and Dilley 1968) is an example of the dissociation of metabolic functions by an inhibitor.

This paper reports the effects of a number of metabolic inhibitors on the responses of banana fruit slices to cutting and to concentrations of ethylene which induce ripening.

II. Materials and Methods

(*a*) *Preparation and Handling of Slices*

Transverse slices (6 mm) were prepared from green fruit of the Williams cultivar of the intermediate Cavendish group grown at Avoca, N.S.W. and incubated at 20°C as described by Palmer and McGlasson (1969). Respiration (CO_2 production) was measured colorimetrically or with an infrared gas analyser (Palmer and McGlasson 1969) and ethylene was measured by gas chromatography (McGlasson 1969). Inhibitors were vacuum-infiltrated (1 min, 26 in. Hg) into the slices within 1 hr after cutting. The solution of the inhibitors added about 10% to the weight of the slices. Ethylene treatment (10 p.p.m.) of one-half of the slices (gassed slices) was begun immediately after vacuum infiltration (Vendrell 1969). Slices not treated with ethylene are referred to as "ungassed slices".

(*b*) *Analytical Methods*

Sugar levels in the pulp of slices were estimated by measuring total soluble solids with a refractometer (Palmer and McGlasson 1969). Organic acids in the pulp of slices treated with sodium monofluoroacetate were measured as described by McGlasson *et al.* (1971).

(*c*) *Inhibitors*

The following potential inhibitors were tested at the concentrations shown.

(i) *Inhibitors of Nucleic Acid and Protein Synthesis.*—Actinomycin D (Merck, Sharpe, and Dohme (Aust.) Pty. Ltd., Sydney, Australia), 2 and 4×10^{-5}M; carbonyl cyanide-*m*-chlorophenyldiazine (Calbiochem. Inc., Los Angeles, U.S.A.), 10^{-5}M in 4% (v/v) ethanol; D-*threo*-chloramphenicol (Sigma Chemical Co., St. Louis, U.S.A.), 5 mg/ml; chloroisopropylphenylcarbamate (Calbiochem. Inc., Los Angeles, U.S.A.), 0·1 mg/ml in 4% (v/v) ethanol; cycloheximide (Nutritional Biochemicals Corporation, Ohio, U.S.A.), 0·25–50 μg/ml; 5-fluorouracil (Roche Products Pty. Ltd., Sydney, Australia), 50 mg/ml; DL-*p*-fluorophenylalanine (Sigma Chemical Co., St. Louis, U.S.A.), 1 and 4 mg/ml; puromycin (Calbiochem. Inc., Los Angeles, U.S.A.), 1 and 5×10^{-4}M; tetracycline HCl (Cyanimid Corporation, U.S.A.), 0·25–20 mg/ml.

(ii) *Inhibitors of Respiration.*—Acetaldehyde (A.R.), 10^{-2}M; sodium arsenite (A.R.), 10^{-2} and 10^{-3}M; carbon tetrachloride (A.R.), saturated aqueous solution; chloral hydrate (A.R.), 10^{-2}M; potassium cyanide (A.R.), 10^{-4}–10^{-1}M; 2,4-dinitrophenol (A.R.), 10^{-4}M; sodium fluoride (A.R.), 10^{-2}M; glyceraldehyde (A.R.), 10^{-2}M; 4-hydroxy-2-oxoglutaric acid (HKG), 10^{-3}–3×10^{-2}M, pH 5·0 [prepared as described by Payes and Laties (1963) and Laties (1967), except that the compound was eluted from the silicic acid column with a gradient of methanol in chloroform instead of with chloroform–butanol 65 : 35 (v/v)]; lithium chloride (A.R.), 10^{-2}M; malonate (A.R.), 2×10^{-2}M, pH 5·0; sodium mercaptobenzothiazole, 10^{-3}M [purified immediately before use as described by Palmer and Roberts (1967)]; sodium monofluoroacetate (A.R.), 1 and 2×10^{-2}M; *trans*-2-hexanal (Compagnie Parento Inc., New York, U.S.A.), 10^{-2}M.

(iii) *Inhibitors of Ethylene Production.*—Sodium-*cis*-3-chloroacrylate (Union Carbide Aust. Ltd., Sydney, Australia), 10^{-4}–10^{-2}M.

III. Results

(*a*) *Screening Experiments*

Each of the compounds listed in Section II(*c*) was infiltrated into groups of slices which were then incubated with and without added ethylene in the air stream. Treated slices were compared to controls (infiltrated with water) for respiration rate, sugar content, peel colour, and pulp softness. Ethylene production by ungassed slices was measured only in slices treated with HKG and *cis*-3-chloroacrylate.

Of the inhibitors of nucleic acid and protein synthesis, only cycloheximide, tetracycline, and *p*-fluorophenylalanine had marked effects. At concentrations above 5 μg/ml cycloheximide caused severe tissue damage, and at lower concentrations damage was sometimes apparent after about 4 days. Cycloheximide inhibited both increases in respiration and ripening, at concentrations less than 1 μg/ml these inhibitions were not consistently observed.

Tetracycline reduced respiration and delayed ripening in gassed slices only at very high concentration (10 and 20 mg/ml). *p*-Fluorophenylalanine increased the peak of induced respiration in ungassed slices and did not inhibit ripening in gassed slices.

Sodium fluoride (10^{-3}M) increased the respiration of preclimacteric slices, but did not retard the development of the climacteric; 10^{-2}M fluoride severely damaged the slices. Sodium arsenite (10^{-3}M) and sodium monofluoroacetate (10^{-2}M) inhibited respiration in both gassed and ungassed slices. Ripening was inhibited, and there was some surface browning of treated slices after 1 or 2 days. Severe damage occurred when the concentration was 10-fold higher. HKG (10^{-2}M) reduced the peak of induced ethylene production but had little effect on induced respiration. Respiration during ripening was lower than in control slices, although in other respects ripening appeared normal.

2,4-Dinitrophenol (10^{-4}M) caused a 25–50% increase in CO_2 production by both gassed and ungassed slices. The pattern of the respiratory climacteric, and other ripening changes were normal. KCN inhibited respiratory responses only at concentrations of 10^{-2}M or above, while severe damage resulted at 5×10^{-2}M. It was shown by titration of unabsorbed KCN with $AgNO_3$ that samples of green tissue homogenized in 3×10^{-2}M KCN absorbed about 5×10^{-7} mole of KCN per g fresh weight. Infiltration of slices with 10^{-2}M KCN adds about 10^{-6} mole of KCN per gram, half of which would be absorbed by the tissue. This effect explains the need to infiltrate such high concentrations of cyanide to inhibit induced or climacteric respiration.

Other inhibitors listed in Section II(*c*) did not markedly inhibit respiratory responses or ripening. Sodium *cis*-3-chloroacrylate did not affect ethylene production. This compound has been reported to inhibit the production of labelled ethylene from [1-^{14}C]acetate in *Penicillium digitatum* (Jacobsen and Wang 1968). More detailed studies were made with cycloheximide, sodium monofluoroacetate, HKG, and cyanide. Cycloheximide, sodium monofluoroacetate, and cyanide were chosen because of their marked effects in the screening studies and because of their widely different sites of action; HKG was included because of its apparent differential effects on induced ethylene and respiration.

(*b*) *Detailed Experiments*

(i) *Cycloheximide.*—Cycloheximide at concentrations of 1 and 2 μg/ml largely prevented the rise in induced respiration and eliminated the peak of induced ethylene production, but caused ethylene production to rise to a level which was several times higher than the preclimacteric rate of control slices (Fig. 1). There were climacteric-like rises in respiration and ethylene production in ungassed cycloheximide-treated slices (Fig. 1). There was some sugar accumulation in these slices, but the peel remained green, and the pulp did not soften (Table 1). Cycloheximide-treated slices which were exposed to ethylene did not develop a climacteric (Fig. 1), and did not ripen (Table 1).

TABLE 1

CONDITION, APPEARANCE, AND SUGAR LEVELS IN SLICES TREATED WITH CYCLOHEXIMIDE

The data refer to the same experimental material used in Figure 1. The assessment of ethylene-treated slices was made 7 days after cutting; ungassed slices (ethylene concn. 0) were assessed on day 15

Inhibitor Concn. (μg/ml)	Ethylene Concn. (p.p.m.)	Appearance and Condition of Slices	Sugar Concn. (%)
0 (control)	0	Peel yellow, pulp soft, strong ripe fruit aroma	17·8±0·2*
	10	As above	18·5±0·2
1	0	Peel green, pulp hard, slight ripe fruit aroma	10·9±0·2
	10	Peel green, pulp hard, no ripe fruit aroma	5·3±0·1
2	0	Peel green, pulp hard slight ripe fruit aroma	11·9±0·2
	10	Peel green, pulp hard no ripe fruit aroma	4·6±0·2

* In all tables the statistical limits are estimates of the standard deviation of the population.

(ii) *Monofluoroacetate.*—Figure 2 shows that 10^{-2}M sodium monofluoroacetate reduced the response to cutting in terms of respiration and of ethylene production, while 2×10^{-2}M almost eliminated both responses. However, subsequent to the induced peak, ethylene production remained higher in slices treated with the inhibitor than in the control slices.

Respiration rate and ethylene production in slices treated with monofluoroacetate increased about 2 days after the control slices commenced to ripen. However, treated slices did not ripen normally. Table 2 shows that inhibitor treatment interfered with colouring of the skin, softening of the pulp, production of aroma, and conversion of starch to sugar.

Monofluoroacetate-treated slices did not respond normally to exposure to ethylene. The respiratory response was delayed and 7 days after treatment peel

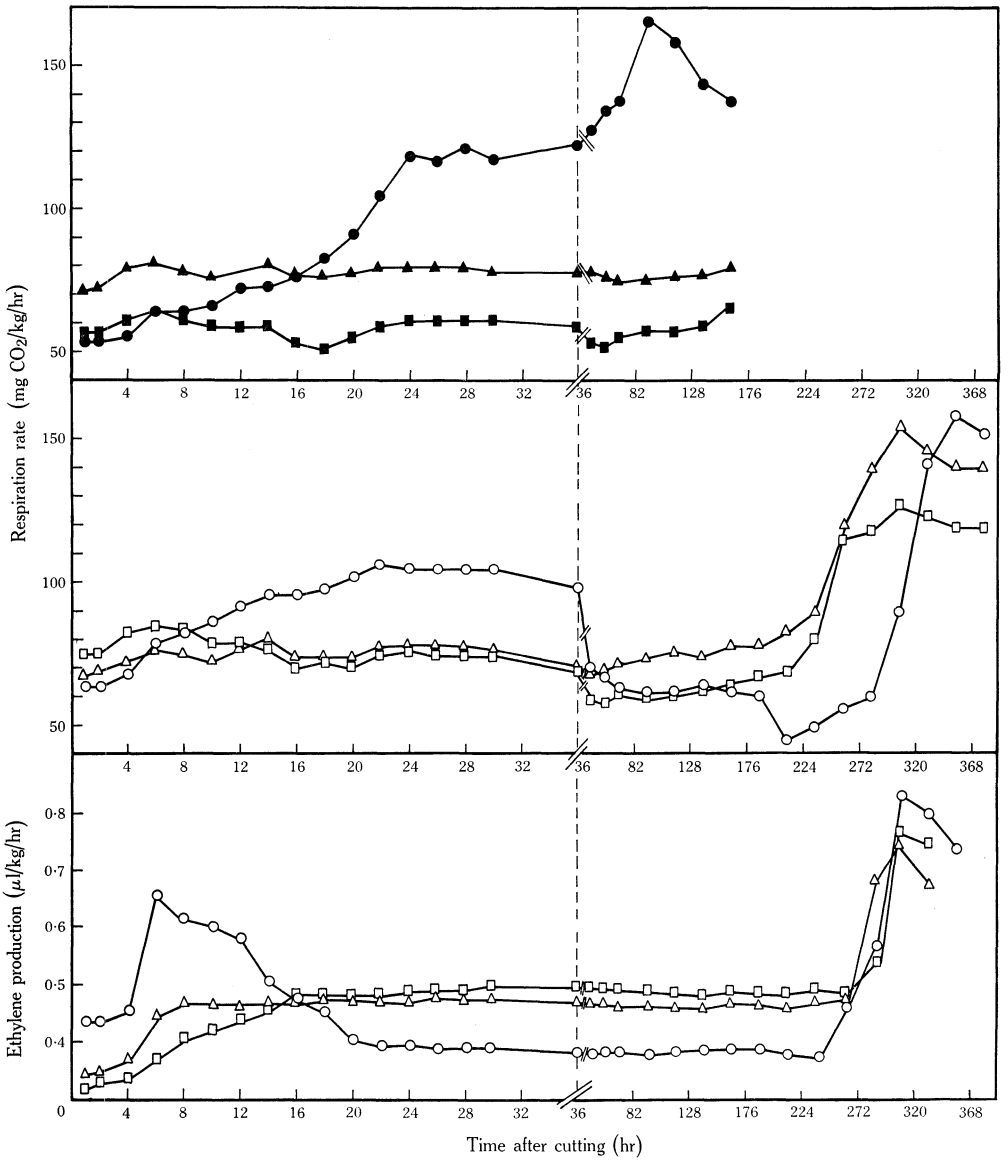

Fig. 1.—Effects of cycloheximide on respiration and ethylene production. Each point is the average of data from three composite samples each of four slices 6 mm thick. The inhibitor treatments are indicated as follows: water control (○, ●), 1 μg/ml (△, ▲), and 2 μg/ml (□, ■). Open symbols indicate ungassed slices, closed symbols indicate slices treated continuously with 10 p.p.m. ethylene. S.E. of means ($n' = 3$): *Respiration*: control gassed ±8·93 (46 d.f.); 1 μg/ml gassed ±4·87 (46); 2 μg/ml gassed ±4·40 (46); control ungassed ±6·67 (66); 1 μg/ml ungassed, 0–284 hr, ±4·09 (48), 308–370 hr, ±13·25 (8); 2 μg/ml ungassed, 0–284 hr, ±5·37 (50), 308–370 hr, ±10·24 (8). *Ethylene production:* control ungassed ±0·045 (64); 1 μg/ml ungassed ±0·032 (62); 2 μg/ml ungassed ±0·040 (62).

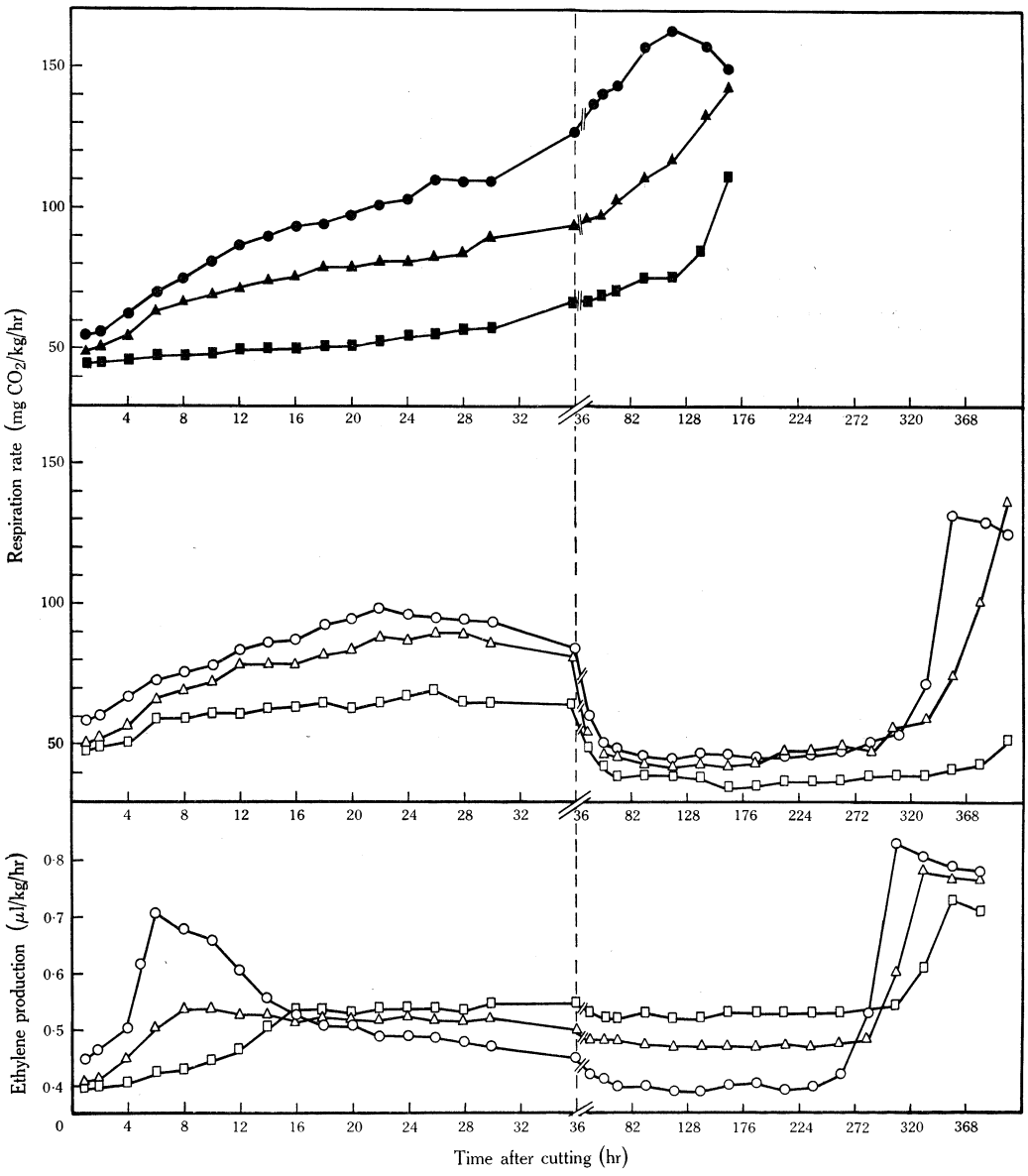

Fig. 2.—Effects of sodium monofluoroacetate on respiration and ethylene production. Each point is the average of data from three composite samples each of four slices 6 mm thick. The inhibitor treatments are indicated as follows: water control (○, ●), 10^{-2}M (△, ▲), and 2×10^{-2}M (□, ■). Open symbols indicate ungassed slices, closed symbols indicate slices treated continuously with 10 p.p.m. ethylene. S.E. of means ($n' = 3$): *Respiration*: control gassed $\pm$9·30 (48 d.f.); 10^{-2}M gassed $\pm$ 4·50 (48); 2×10^{-2}M gassed $\pm$4·50 (48); control ungassed $\pm$5·77 (68); 10^{-2}M ungassed, 0–320 hr, $\pm$4·50 (62), 336–380 hr, $\pm$13·35 (6); 2×10^{-2}M ungassed $\pm$3·74 (68). *Ethylene production*: control ungassed $\pm$ 0·051 (66); 10^{-2}M ungassed $\pm$0·035 (66); 2×10^{-2}M ungassed $\pm$0·044 (66).

colouring, softening of the pulp, production of aroma and sugar accumulation were limited (Table 2).

TABLE 2

CONDITION APPEARANCE, AND SUGAR LEVELS IN SLICES TREATED WITH SODIUM MONOFLUOROACETATE

The data refer to the same experimental material used in Figure 1. The assessment of ethylene-treated slices was made 7 days after cutting; ungassed slices were assessed on day 16

Inhibitor Concn. (M)	Ethylene Concn. (p.p.m.)	Appearance and Condition of Slices	Sugar Concn. (%)
0 (control)	0	Peel yellow, pulp soft, strong ripe fruit aroma	18·2±0·2
	10	As above	18·5±0·2
1×10^{-2}	0	Peel discoloured, some browning of pulp, pulp hard, slight ripe fruit aroma	10·1±0·2
	10	Traces of yellow in peel, pulp hard, no aroma	6·4±0·2
2×10^{-2}	0	Peel discoloured, some browning of pulp, pulp hard, no aroma	9·4±0·2
	10	Peel green, pulp hard, slight ripe fruit aroma	6·1±0·3

In slices 24 hr after infiltration with monofluoracetate, the concentration of citrate was higher than in controls but the concentrations of glutamate, aspartate, succinate, and malate were unchanged (Table 3).

TABLE 3

ETHANOL-SOLUBLE ACIDIC COMPOUNDS IN SLICES 24 HR AFTER INFILTRATION WITH SODIUM MONOFLUOROACETATE

Values are means of duplicate samples each of four slices 6 mm thick

Inhibitor Concn. (M)	Ethylene Concn. (p.p.m.)	Acid Concentrations (m-equiv/100 g)				
		Glutamic	Aspartic	Succinic	Malic	Citric
0	0	0·224	0·080	0·260	4·48	2·07
(control)		±0·032	±0·012	±0·028	±0·08	±0·04
	10	0·192	0·096	0·244	4·51	2·33
		±0·020	±0·016	±0·028	±0·09	±0·05
2×10^{-2}	0	0·200	0·084	0·248	4·39	2·96
		±0·024	±0·008	±0·018	±0·08	±0·06
	10	0·240	0·096	0·248	4·36	3·17
		±0·020	±0·004	±0·016	±0·07	±0·06

Infiltration of slices with 2×10^{-2}M sodium acetate did not affect respiration, ethylene production, or ripening.

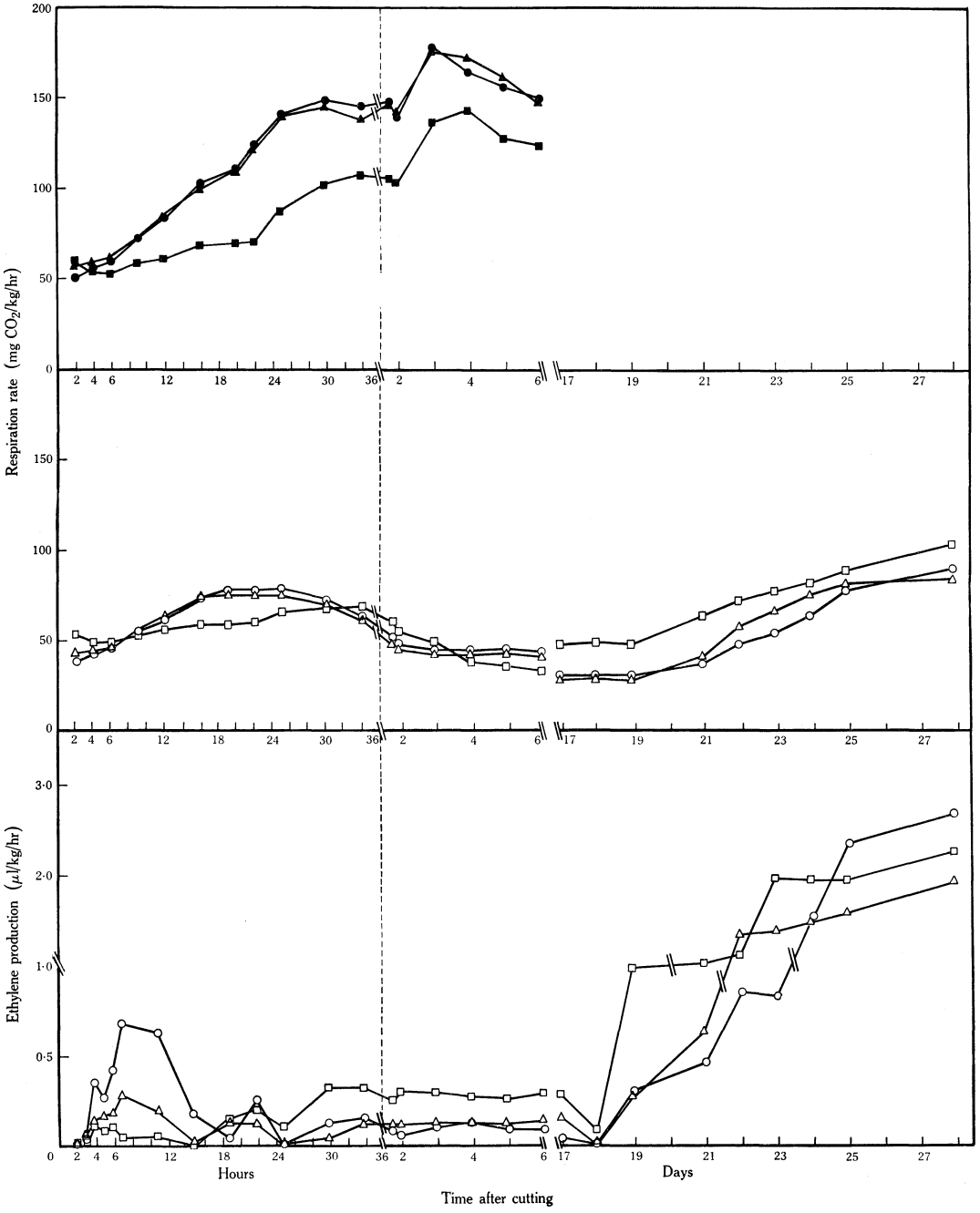

Fig. 3.—Effects of 4-hydroxy-2-oxoglutarate on respiration and ethylene production. Each point is the average of data from three composite samples each of four slices 6 mm thick. The inhibitor treatments are indicated as follows: control (○, ●), 3×10^{-3}M (△, ▲), and 3×10^{-2}M (□, ■). Open symbols indicate ungassed slices, closed symbols indicate slices treated continuously with 10 p.p.m. ethylene. S.E. of means ($n' = 3$): *Respiration:* control gassed $\pm2\cdot64$ (34 d.f.),

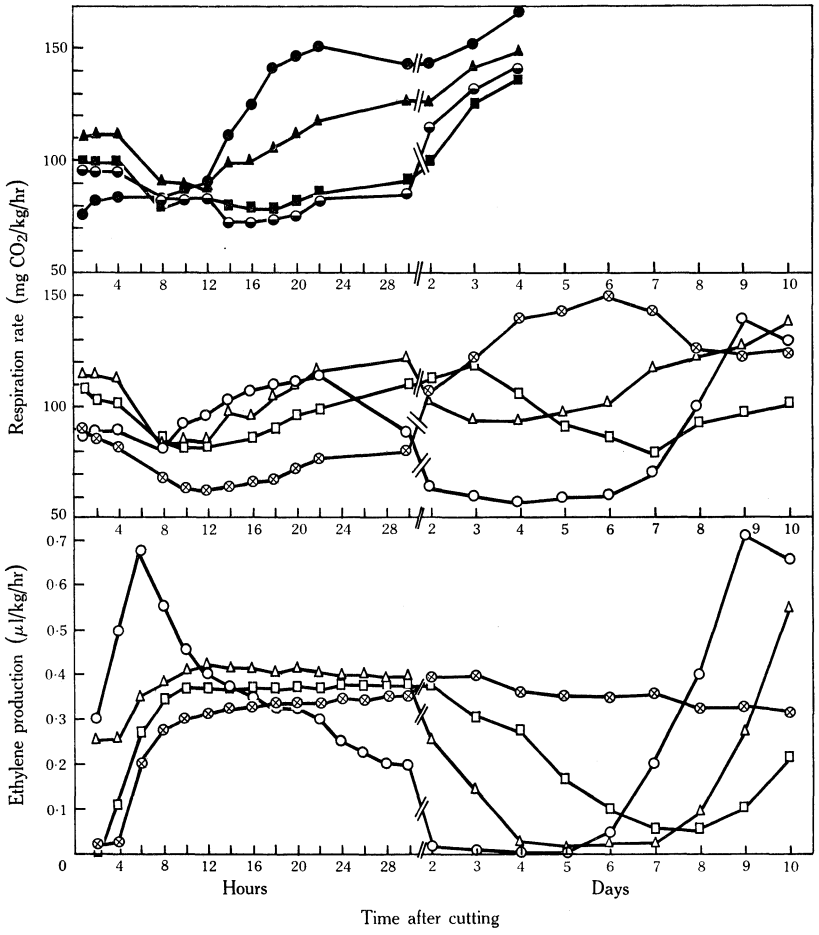

Fig. 4.—Effects of potassium cyanide on respiration and ethylene production. Each point is the average of data from two composite samples each of four slices 6 mm thick except for the ungassed controls where data for four samples were averaged. The inhibitor treatments are indicated as follows: water control (,○ ●), 10^{-2}M (△, ▲), $2{\cdot}5\times10^{-2}$M (□, ■), and 5×10^{-2}M (⊗, ◒). Open symbols indicate ungassed slices, closed symbols indicate slices treated continuously with 10 p.p.m. ethylene. S.E. of means ($n' = 4$ for ungassed controls, $n = 2$ for other treatments): *Respiration*: control gassed $\pm5{\cdot}22$ (15 d.f.); 10^{-2}M gassed $\pm6{\cdot}03$ (15); $2{\cdot}5\times10^{-2}$M gassed $\pm5{\cdot}15$ (15); 5×10^{-2}M gassed $\pm4{\cdot}4$ (15); control ungassed $\pm4{\cdot}33$ (63), 10^{-2}M ungassed $\pm4{\cdot}54$ (20); $2{\cdot}5\times10^{-2}$M ungassed $\pm7{\cdot}00$ (15); 5×10^{-2}M ungassed $\pm7{\cdot}60$ (21). *Ethylene production*: control ungassed $\pm0{\cdot}041$ (72); 10^{-2}M ungassed $\pm0{\cdot}049$ (24); $2{\cdot}5\times10^{-2}$M ungassed $\pm0{\cdot}046$ (23); 5×10^{-2}M ungassed $\pm0{\cdot}034$ (24).

3×10^{-3}M gassed $\pm2{\cdot}98$ (34); 3×10^{-2}M gassed $\pm8{\cdot}21$ (34); control ungassed, 0–6 days, $\pm1{\cdot}64$ (33), 17–28 days, $\pm8{\cdot}58$ (22); 3×10^{-3}M ungassed $\pm7{\cdot}90$ (56); 3×10^{-2}M ungassed $\pm5{\cdot}33$ (56). *Ethylene production*: control ungassed, 0–6 days, $\pm0{\cdot}099$ (38), 17–28 days, $\pm0{\cdot}59$ (18); 3×10^{-3}M ungassed, 0–36 hr, $\pm0{\cdot}050$ (26), 2–6 days, $\pm0{\cdot}113$ (12), 17–28 days, $\pm0{\cdot}75$ (18); 3×10^{-2}M ungassed, 0–6 days, $\pm0{\cdot}119$ (38), 17–28 days, $\pm0{\cdot}52$ (18).

(iii) *4-Hydroxy-2-oxoglutarate (HKG).*—HKG at 3×10^{-2}M eliminated the induced rise in ethylene production and delayed the peak of induced respiration by about 8 hr (Fig. 3), but had no effect on the time to ripen or the rate of respiration and ethylene production during ripening. Respiration in ethylene-treated slices was reduced about 25%, but the peak was not delayed, and the associated ripening changes were not affected.

The only effect observed following treatment with 3×10^{-3}M HKG was a 50% reduction in the size of the peak of induced ethylene production.

(iv) *Cyanide.*—Cyanide at 5×10^{-2}M obviously damaged the tissue. Peaks of induced ethylene production and respiration did not occur, nor did the tissue ripen. The rate of ethylene production was high throughout, and the high rate of CO_2 production was accompanied by a high respiratory quotient.

Cyanide at 1 and $2{\cdot}5\times10^{-2}$M did not result in irreversible damage, and the respiratory quotient was 1·0 throughout. These treatments eliminated the peak of induced ethylene production, and delayed the attainment of the peak of induced respiration (Fig. 4). Treatment caused ethylene production to be high for some days, but it returned to a low level before the beginning of the climacteric which was slightly delayed. Other ripening changes were also delayed slightly (Table 4).

In gassed slices, cyanide (1 and $2{\cdot}5\times10^{-2}$M) delayed both the onset of the climacteric, and other ripening changes (Fig. 4; Table 4).

TABLE 4

SUGAR LEVELS IN SLICES TREATED WITH POTASSIUM CYANIDE

The data refer to the same experimental material used in Figure 2. The measurements on ethylene-treated slices were made 4 days after cutting, and on ungassed slices 10 days after cutting

Inhibitor Concn. (M)	Ethylene Concn. (p.p.m.)	Sugar Concn. (%)	Inhibitor Concn. (M)	Ethylene Concn. (p.p.m.)	Sugar Concn. (%)
0	0	19·2±1·0	$2{\cdot}5\times10^{-2}$	0	9·7±0·4
(control)	10	15·2±0·4		10	10·9±0·6
10^{-2}	0	15·0±0·5	5×10^{-2}	0	6·3±0·1
	10	12·9±0·4		10	5·5±0·4

IV. DISCUSSION

An aim in this study was to compare the effects of various potential inhibitors on the increases in ethylene production and respiration found after cutting with those occurring during ripening. In assessing our observations, some characteristics of the slice system need consideration. A limited volume of solution is introduced when the slice is infiltrated so that, following uptake, the concentration of the inhibitor within the cell will be less than that in the infiltrated solution. This situation contrasts with that operating when thin slices are exposed to a large volume of solution. In the latter case, the inhibitor concentration within the cell may well exceed that in the solution. The limited amount, as opposed to concentration, of inhibitor introduced means that the system may be particularly influenced by non-specific adsorption, decomposition, or metabolism of the inhibitor. This was demonstrated with cyanide [Section III(*a*)]. Consequently higher concentrations must be used than when larger

volumes of solution are available, and the difference between a specifically inhibitory and a toxic concentration may not be large.

Two patterns of the inhibition of ripening were observed after treatment of slices with cyanide, cycloheximide, and monofluoroacetate. Cyanide inhibited the respiratory climacteric and other ripening changes to about the same extent. In contrast, there was evidence that cycloheximide and monofluoroacetate dissociated the normal relationship between the respiratory climacteric and other ripening changes.

Cycloheximide treatment inhibited the respiratory climacteric and the onset of ripening when ethylene was applied immediately after the inhibitor. A similar response was reported by Brady *et al.* (1970*b*). However, when the slices were left to ripen without added ethylene, ripening was clearly inhibited relative to the climacteric peaks of ethylene and respiration (Fig. 1; Table 1). This is comparable to the effect of cycloheximide infiltrated into whole pear fruits (Frenkel, Klein, and Dilley 1968), when ripening was prevented although the climacteric was extended. These differential responses to low concentrations of cycloheximide may indicate effects on cellular metabolism other than inhibition of protein synthesis as shown by Ellis and MacDonald (1970).

When slices infiltrated with monofluoroacetate were treated with ethylene, there was no correlation between the progress of the climacteric and the changes in sugar content, although this content normally rises in parallel with the respiration rate (Brady *et al.* 1970*a*). This may indicate that modification of the tricarboxylic acid (TCA) cycle by monofluoroacetate limits the activation of starch hydrolysis.

Inhibitor treatments tended to abolish the initial peak of ethylene production induced by cutting although the subsequent output of ethylene was higher than in untreated tissue. Increased ethylene output continued for a long time and may indicate cell damage. Only with cyanide was the normal level of ethylene output regained, and it was only in this case that ripening appeared normal. The inhibition of the induced ethylene peak would appear to be a further indication of the special role ethylene may play in regulating metabolism within fruit tissues (McGlasson 1969). Large increases in ethylene production by citrus fruits have been measured following treatment with cycloheximide (Cooper, Rasmussen, and Hutchison 1969).

There is evidence that respiration in bananas proceeds through the Embden–Meyerhoff–Parnas pathway and the TCA cycle, and through at least the initial steps of the pentose phosphate shunt (Tager 1956; McGlasson *et al.* 1971). Inhibition of induced respiration by arsenite and monofluoroacetate point to the involvement of the TCA cycle in this response to cutting. Over long periods, CO_2 production by banana tissue is not inhibited by malonate (McGlasson *et al.* 1971), but inhibition of respiration immediately after malonate infiltration has not been investigated. Specific inhibition of respiration by fluoride was not observed. Concentrations of about 10^{-2}M fluoride are required for substantial inhibition of enolase (Miller 1958), and this concentration, introduced by infiltration, damaged banana slices.

HKG, chloral hydrate, acetaldehyde, glyceraldehyde, and lithium chloride all maintain glycolysis and the TCA cycle in an inhibited state after potato tuber tissue is sliced (Laties 1963). In banana tissue these compounds were without effect on the development of induced respiration. This may be due to the fact that the TCA cycle is already operative in unsliced bananas.

V. Conclusions

Glycolysis and the TCA cycle are the principal pathways of respiration following slicing and at least during the early stages of ripening. Inhibitors which retard respiration cause an initial suppression of ethylene production. However, a longer-term indicator of disturbed metabolism is an increase in ethylene production which begins within a few hours after treatment and reaches steady levels several times those of untreated tissue. Induced ethylene production may be inhibited without an inhibition of induced respiration so the one does not appear to be dependent on the other.

Ripening could be inhibited when a respiratory climacteric did occur and thus the respiratory rise is not dependent upon and integrated with other ripening changes. On the other hand compounds which inhibited the respiratory climacteric invariably inhibited ripening indicating that some essential non-respiratory components of ripening are dependent on the respiration rise or on the factors which lead to the respiration rise. This dependence could involve synthesis of proteins. However, such experiments cannot of themselves establish such a dependence, and information on the degree of respiratory control and the efficiency of phosphorylation during the respiratory climacteric which occur after cycloheximide and monofluoroacetate treatment would aid the interpretation of these results.

VI. Acknowledgments

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