Respiratory Contribution of the Alternate Path during Various Stages of Ripening in Avocado and Banana Fruits[1]

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ABSTRACT

The respiration of fresh slices of preclimacteric avocado (*Persea americana* Mill. var. Hass) and banana (*Musa cavendishii* var. Valery) fruits is stimulated by cyanide and antimycin. The respiration is sensitive to *m*-chlorobenzhydroxamic acid in the presence of cyanide but much less so in the presence of antimycin. In the absence of cyanide the contribution of the cyanide-resistant pathway to the coupled preclimacteric respiration is zero. In uncoupled slices, by contrast, the alternate path is engaged and utilized fully in avocado, and extensively in banana. Midclimacteric and peak climacteric slices are also cyanide-resistant and, in the presence of cyanide, sensitive to *m*-chlorobenzhydroxamic acid. In the absence of uncoupler there is no contribution by the alternate path in either tissue. In uncoupled midclimacteric avocado slices the alternate path is fully engaged. Midclimacteric banana slices, however, do not respond to uncouplers, and the alternate path is not engaged. Avocado and banana slices at the climacteric peak neither respond to uncouplers nor utilize the alternate path in the presence or absence of uncoupler.

The maximal capacities of the cytochrome and alternate paths, V_{cyt} and V_{alt}, respectively, have been estimated in slices from preclimacteric and climacteric avocado fruit and found to remain unchanged. The total respiratory capacity in preclimacteric and climacteric slices exceeds the respiratory rise which attends fruit ripening. In banana V_{alt} decreases slightly with ripening.

The aging of thin preclimacteric avocado slices in moist air results in ripening with an accompanying climacteric rise. In this case the alternate path is fully engaged at the climacteric peak, and the respiration represents the total potential respiratory capacity present in preclimacteric tissue. The respiratory climacteric in intact avocado and banana fruits is cytochrome path-mediated, whereas the respiratory climacteric of ripened thin avocado slices comprises the alternate as well as the cytochrome path. The ripening of intact fruits is seemingly independent of the nature of the electron transport path.

Uncouplers are thought to stimulate glycolysis to the point where the glycolytic flux exceeds the oxidative capacity of the cytochrome path, with the result that the alternate path is engaged.

The ripening of many fruits is attended by a burst of respiratory activity, the well known climacteric (5). Ethylene, the quintessential ripening hormone, not only initiates the climacteric, but is also a major metabolic product of the ripening process (19). The earliest theory put forward to explain the respiratory burst invoked the breakdown of "organization resistance," a concept implying a marked increase of membrane permeability, with accompanying cellular decompartmentation and metabolic deregulation (26). A second major theory centered on the evocation of protein synthesis, which on the one hand was thought to provide one or more enzymes particular to the ripening process (5, 8, 21), and on the other, to enhance ATP turnover with its attendant respiratory stimulation (5). While permeability changes assuredly characterize the ripening process (3), it is an open question whether such changes are causative or consequential. With respect to the second theory, it is to be noted that the respiratory capacity of mitochondria from preclimacteric and climacteric tissue is the same (6), that the respiration rate of uncoupled preclimacteric slices is more than enough to support the climacteric rise of intact fruits (17), and that anaerobiosis stimulates glycolysis in intact avocado fruit, indicating a considerable latent glycolytic capacity (27). Thus, we have focused our attention on regulatory mechanisms of respiration rather than on *de novo* enzyme synthesis.

Recently we discovered that CN as well as ethylene engenders the climacteric and ripening in a variety of fruits, and that ethylene-responsive fruits are invariably CN-resistant (25, 27–29). For this reason we came to the view that the CN-resistant, or alternate, path is central to the climacteric and to ripening, and that CN as well as ethylene positively modulates, and thereby favors, the alternate path. This view was reinforced by Burg's suggestion that ethylene (and therefore CN [24]) acts by complexation with a transition metal (10), by Beyer's observation that Ag^+ ion inhibits physiological responses to ethylene (4), by Sisler's evidence that π-acceptor compounds like CO, methyl isocyanide, and vinyl olefins exert an ethylene-like effect on tobacco leaf respiration (24), by the reported role of H_2O_2 in fruit ripening (9), and by the suggestion that the alternate path leads to peroxide formation (11, 20).

Despite these provocative observations, direct evidence is lacking for the participation of the CN-resistant electron transport path in the respiratory climacteric and fruit ripening. Herein we examine the contribution of the alternate path to the uninhibited respiration of preclimacteric and climacteric avocado and banana fruits. We come to the view that the alternate path is not required to sustain the elevated respiration rates which characterize the climacteric.

MATERIALS AND METHODS

PLANT MATERIAL

Avocado fruits, *Persea americana* Mill. var. Hass, were collected from a private orchard in Los Angeles. Banana fruits, *Musa cavendishii* Lambert, var. Valery, were obtained from Central America by refrigerated ships 11 to 14 days after picking. They were always dark green and preclimacteric.

SLICE PREPARATION

Avocado. Preclimacteric or climacteric avocado fruits were halved, the seed removed, and the mesocarp pierced with a 9-mm-diameter stainless steel borer, the cores being left in the fruit.

Slices 1 mm thick were cut into ice water from the pierced fruit halves with a microtome blade fixed in an appropriate bed. Slices were rinsed with distilled H_2O and used promptly as fresh slices.

Banana. The same procedure was followed to prepare preclimacteric banana slices from banana fruit. Because of the softness of ripe banana fruit, climacteric banana dics were made by piercing 2-mm-thick slices of the fruit with a 9-mm-diameter stainless steel borer.

RESPIRATORY MEASUREMENTS

Intact Fruits. Single fruits were put into 1-liter respiratory jars through which a stream of air was passed at a rate of 50 ml/min at 20 C. CO_2 output was recorded with a Beckman IR CO_2 analyzer.

Slices. Respiration was measured by conventional Warburg manometry at 25 C. Warburg flasks contained 14 slices (about 1 g fresh wt for 1-mm discs) in 4 ml of 0.1 mM $CaSO_4$ with the appropriate respiratory inhibitor (pH 5.5). Two-tenths ml of 10% NaOH and a fluted filter paper were in the center well. With climacteric banana slices; six to seven slices (about 1 g fresh wt, 2 mm thick) were placed in each flask in 0.3 M sucrose (to limit swelling). When used, KCN was added directly to the experimental solution and the pH was adjusted to 5.5 with 0.4 N H_2SO_4. Six-tenths ml of a suitable mixture of $Ca(CN)_2$-$Ca(OH)_2$ (22) was added to the center well. $CCCP^3$ was provided in 0.01 M K-phosphate (pH 7.3) in 0.1 mM $CaSO_4$. Respiratory rates were obtained over a 2-hr period. Stock solutions of CCCP and antimycin A were made in absolute ethanol. One hundred μl of the stock solution were diluted with 100 ml of the appropriate external solution to give the maximum inhibitor or uncoupler concentration used.

RIPENING OF AVOCADO SLICES IN MOIST AIR

An intact preclimacteric avocado fruit was surface-sterilized with a 1:3 dilution of Clorox for 3 min, then rinsed. Slices 1 mm thick and 9 mm in diameter were prepared as described above, and seven slices (about 0.5–0.6 g fresh wt) placed in a Warburg flask in which a moist Whatman 3MM filter paper layered the bottom. Two-tenths ml of 10% NaOH was placed in the center well. The course of respiration was followed at 25 C. When slices reached the climacteric peak respiration was measured in the conventional way in the presence and absence of inhibitors as described in the previous section. The effect of CLAM on aging was determined by vacuum-infiltrating a 3 mM CLAM solution for 1 min at 500 mm of Hg before discs were incubated in moist air. All glassware and reagents were autoclaved, and tissue slicing was done in a transfer hood under aseptic conditions.

ANALYSIS OF TITRATION DATA

Titration of preclimacteric and climacteric avocado and banana slice respiration with CLAM and CN were carried out as described by Bahr and Bonner (1). The total respiration is described by equation 1, while the respiration with the residual component subtracted is defined by equation 2.

$$V_T = \rho \cdot g(i) + V_{cyt} + V_{res} \qquad (1)$$

$$V_T' = \rho \cdot g(i) + V_{cyt} \qquad (2)$$

The meaning of the terms in the equations, and their estimation, have been fully described (31). V_{alt} represents the maximal capacity of the alternate path, the upper limit of $\rho \cdot g(i)$.

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[3] Abbreviations: CLAM: *m*-chlorobenzhydroxamic acid; CCCP: carbonyl-cyanide *m*-chlorophenyl hydrazone.

BIOCHEMICALS

CCCP and antimycin A were obtained from Sigma. Methyl-*m*-chlorobenzoate was obtained from Eastman. CLAM was synthesized as previously indicated (31).

RESULTS

EFFECT OF KCN, ANTIMYCIN A, CLAM, AND CCCP IN PRECLIMACTERIC AND CLIMACTERIC AVOCADO AND BANANA SLICES

Table I summarizes the effect of various inhibitors on the respiration of intact preclimacteric and climacteric fruits, as well as on slices prepared from preclimacteric and midclimacteric avocado and banana fruits.

Intact Fruits. The ripening of intact preclimacteric avocado fruit results in a 5-fold increase in respiration, the well known climacteric. Treatment of preclimacteric fruit with 400 μl/l of HCN stimulates the respiration 4-fold in a period of 2 days, and causes ripening akin to natural or ethylene-induced ripening (27). The respiration of intact climacteric peak banana fruits is 7 to 10 times that of preclimacteric fruits. CN gas elevates the respiration of green bananas and causes ripening much as in natural or ethylene-ripened fruit (Goldmann and Laties, unpublished).

Avocado Slices. Slicing an intact preclimacteric avocado fruit results in an immediate 5-fold increase of respiration, which is reminiscent of the "wound-respiration" observed with potato tubers (15). Moreover, slicing *per se* elevates the respiration to the level of natural or ethylene ripened fruit. When a midclimacteric fruit is sliced the rate rises to the same maximum typical of fresh slices and intact climacteric fruit (Table I).

CN stimulates preclimacteric slice respiration 20% as previously observed by Lips and Biale (17). CLAM, a selective inhibitor of the alternate path (23), is in itself without effect on the respiration, but exerts marked inhibition in the presence of CN. The synergistic effect of CLAM and KCN indicates the presence of the alternate path in preclimacteric avocado tissue. Whereas antimycin also stimulates respiration, it shows little inhibitory effect in the presence of CLAM, in marked contrast to CN (32). CCCP markedly stimulates fresh preclimacteric slice respiration. The uncoupled respiration rate of fresh slices is eight times that of the intact

Table I. Effect of KCN, Antimycin A, CLAM, and CCCP on the Respiration of Preclimacteric and Mid-climacteric Avocado and Banana Slices.

	Avocado			
Inhibitor	Intact fruit[1]		Slices 1 mm thick	
	Preclimacteric	Climacteric peak	Preclimacteric	Climacteric rise[2]
	μl CO_2/g fresh wt·hr		μl O_2/g fresh wt·hr	
Control	35	180	171	170
KCN 0.3 mM	140	–	200	180
CLAM 2 mM	–	–	165	173
KCN + CLAM	–	–	80	88
Antimycin 10 μM	–	–	213	192
Antimycin + CLAM	–	–	171	154
CCCP 10 uM	–	–	275	258

	Banana			
Inhibitor	Intact fruit[1]		Slices 2 mm thick	
	Preclimacteric	Climacteric peak	Preclimacteric	Climacteric rise[3]
	μl CO_2/g fresh wt·hr		μl O_2/g fresh wt·hr	
Control	7-10	65	57	54
KCN 0.1 mM	60	–	70	46
CLAM 1 mM	–	–	64	55
KCN + CLAM	–	–	20	11
Antimycin 10 μM	–	–	69	42
Antimycin + CLAM	–	–	52	16
CCCP 10 μM	–	–	152	60

(1) Cyanide treated intact preclimacteric fruits exposed to 400 μl/l HCN.
(2) Slices were prepared from a single avocado fruit which was on the climacteric rise. Its respiratory rate was 100 μl O_2/g fresh wt·hr.
(3) Climacteric slices were made from a single banana fruit which was on the climacteric rise. Its respiratory rate was 39 μl O_2/g fresh wt·hr.

preclimacteric fruit, reflecting the unexpressed respiratory capacity in intact avocado fruit.

Slices from fruit in midclimacteric rise are slightly stimulated by CN but markedly inhibited by CN and CLAM together, indicating the presence of the CN-resistant pathway in midclimacteric fruit. CCCP stimulates the respiration of slices from midclimacteric fruit, while slices from the climacteric peak do not respond to uncoupler (17).

Banana Slices. The respiration of a fresh preclimacteric banana slice is six to eight times that of the intact organ (18) and quantitatively similar to that of the climacteric fruit. Slicing an intact banana in midclimacteric results in a 38% increase of respiration (Table I), bringing the respiration to that of the peak climacteric fruit and preclimacteric slice.

CN and antimycin stimulate preclimacteric slice respiration while CLAM alone is without effect. When CLAM and CN are presented together the respiration is strongly inhibited, indicating the presence of the alternate path in banana tissue. Whereas antimycin affects the Cyt path (stimulation of respiration), antimycin and CLAM together are barely inhibitory. Thus, antimycin and CN are not equivalent in evoking alternate path respiration (32). Uncouplers stimulate preclimacteric slice respiration more than 2-fold, the uncoupled respiration rate being 15 times that of the intact preclimacteric fruit. Thus, a huge unexpressed respiratory capacity exists in the intact banana fruit.

Banana slices prepared in midclimacteric rise are CN-resistant, and sharply inhibited by CLAM in the presence of CN. In contrast to avocado, the respiration of midclimacteric banana slices is inhibited synergistically by antimycin and CLAM.

CONTRIBUTION OF ALTERNATE PATH IN COUPLED AND UNCOUPLED SLICES PREPARED FROM PRECLIMACTERIC FRUITS: QUANTITATIVE RELATIONS OF V_{cyt}, V_{alt} AND V_{res}

Values of ρ in Avocado. The results of titrating coupled and uncoupled preclimacteric avocado slices with CLAM in the presence and absence of CN are shown in Figure 1, A and B. Figure 1A shows that in coupled slices CLAM fails to inhibit respiration in the absence of CN. The uninhibited respiration with the residual respiration subtracted represents the respiratory flux through the Cyt path. In the presence of CN, however, CLAM inhibits respiration about 70%.

The data in Figure 1A were replotted according to equation 2 and the results are shown in Figure 2A. A horizontal line with slope (ρ) of 0 is obtained, indicating that the contribution of the alternate path is nil. Titrations similar to those shown in Figure 1A were carried out with uncoupled preclimacteric avocado slices,
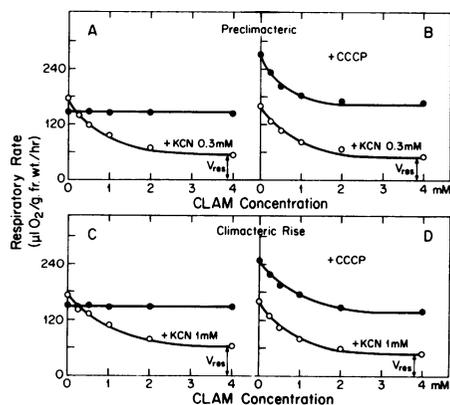
and the results are presented in Figure 1B. In the absence of CN, CLAM partially inhibits the respiration, respiratory rates reaching a high plateau with increasing CLAM concentrations. In the presence of CN almost complete inhibition is obtained. The data in Figure 1B were replotted according to equation 2 and the results are also shown in Figure 2A. A straight line with slope (ρ) of 1 indicates maximum engagement of the alternate path.

V_{cyt}, V_{alt}, and V_{res} in Coupled and Uncoupled Preclimacteric Avocado Slices. The respiratory fluxes of coupled and uncoupled preclimacteric avocado slices are summarized in Table II. In coupled slices (experiments 1 and 3) V_T, which represents preclimacteric slice respiration, is the sum of V_{cyt} and V_{res}. The contribution of the alternate path is 0, since ρ is 0. Uncouplers stimulate preclimacteric avocado slice respiration, with the result that V_T increases (compare experiment 1 with 2, or experiment 3 with 4) and the alternate path is engaged, as indicated by $\rho = 1$. The activity of the Cyt path increases but slightly with CCCP (compare the V_{cyt} values of experiments 1 and 2), indicating that in coupled preclimacteric avocado slices the Cyt path is almost fully saturated.

Values of ρ in Banana. The results of titrating coupled and uncoupled preclimacteric banana slices with CLAM in the presence and absence of CN are shown in Figure 3, A and B. The results in Figure 3, A and B, are reminiscent of those obtained with coupled and uncoupled preclimacteric avocado slices, respectively (Fig. 1, A and B). When the data of Figure 3A were replotted according to equation 2 a horizontal line with slope (ρ) equal to 0 was obtained (Fig. 4A), indicating that the alternate path does not contribute to preclimacteric banana slice respiration. On the other hand, when the data of Figure 3B were replotted in Figure 4A according to equation 2, a straight line with slope (ρ) of 0.7 was obtained, indicating 70% engagement of the alternate path.

V_{cyt}, V_{alt}, and V_{res} in Coupled and Uncoupled Preclimacteric Banana Slices. The respiratory fluxes comprising the respiration of fresh preclimacteric banana slices are summarized in Table III. V_T in coupled slices (experiments 1 and 3) is the sum of V_{cyt} and V_{res}. The contribution of the alternate path is 0, since ρ is 0. In the presence of uncoupler (experiments 2 and 4) V_T increases, and the alternate path is engaged extensively as indicated by $\rho = 0.55$ to 0.7. The activity of the Cyt path increases 1.6 to 2.8 times with CCCP (compare the V_{cyt} values of experiment 1 with 2 or experiment 3 with 4), indicating that in coupled preclimacteric banana slices the potential capacity of the Cyt path is not fully realized.
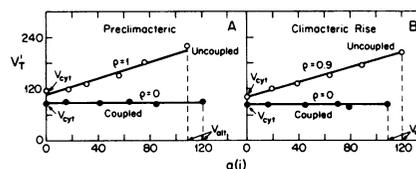
FIG. 2. V_T' as function of g(i) in avocado slices. A: coupled and uncoupled preclimacteric slices; B: coupled and uncoupled climacteric rise slices.

FIG. 1. Titration of avocado slice respiration with CLAM in presence and absence of CN. A: preclimacteric, coupled slices; B: preclimacteric, uncoupled slices; C: climacteric rise, coupled slices; D: climacteric rise, uncoupled slices.

Table II. Respiratory Rates of Coupled and Uncoupled Fresh Preclimacteric Avocado Slices.

The data for experiments 1 and 2 were obtained from Figs. 1A, B, and 2A. Experiments 3 and 4, done with the same preclimacteric fruit, were similar to 1 and 2.

Experiment	CCCP 10 μM	V_T	V_{alt}	ρ	$\rho \cdot V_{alt}$	V_{cyt}	V_{res}
		μl O_2/g fresh wt·hr				μl O_2/g fresh wt·hr	
1	–	147	121	0	0	90	55
2	+	271	109	1	109	107	51
3	–	141	122	0	0	100	43
4	+	266	104	1	104	129	33
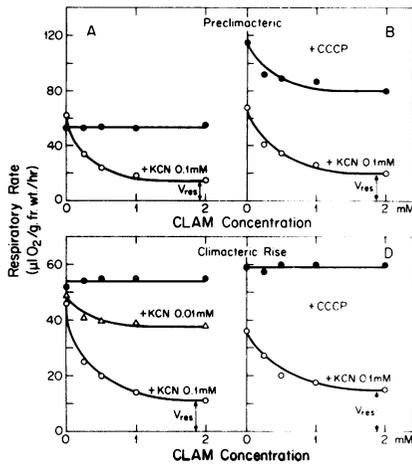
FIG. 3. Titration of banana slice respiration with CLAM in presence and absence of CN. A: preclimacteric, coupled slices; B: preclimacteric, uncoupled slices; C: climacteric rise, coupled slices; D: climacteric rise, uncoupled slices.
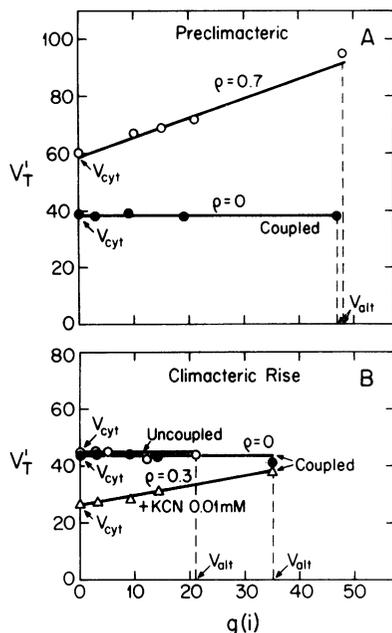
FIG. 4. V_T' as a function of $g(i)$ in banana slices. A: coupled and uncoupled preclimacteric slices; B: coupled and uncoupled climacteric rise slices.

CONTRIBUTION OF ALTERNATE PATH IN COUPLED AND UNCOUPLED SLICES FROM MIDCLIMACTERIC FRUITS: QUANTITATIVE RELATIONS OF V_{cyt}, V_{alt}, AND V_{res}

Values of ρ in Avocado Slices from Midclimacteric Fruit. The results of titrating coupled and uncoupled avocado slices from midclimacteric fruit with CLAM in the presence and absence of CN are shown in Figure 1, C and D. The slices used were prepared from a single avocado fruit in which ripening was induced with 10 μl/l ethylene (Fig. 5A). The respiratory rate of the intact fruit when sliced was 98 μl of CO_2/g fresh wt·hr. Figure 1C shows that in the absence of CCCP, CLAM alone fails to inhibit respiration. In the presence of CN, however, respiration is sensitive to CLAM. About 40% of the total respiration, V_{res}, is resistant to CN and CLAM together.

The data of Figure 1C were replotted according to equation 2 and the results are shown in Figure 2B. A horizontal line with slope (ρ) of 0 was obtained, indicating no contribution of the

Experiment	CCCP 10 μM	V_T	V_{alt}	ρ	$\rho \cdot V_{alt}$	V_{cyt}	V_{res}
		μl O_2/g fresh wt·hr				μl O_2/g fresh wt·hr	
1	-	53	47	0	0	38	15
2	+	115	48	0.70	34	60	20
3	-	52	45	0	0	35	17
4	+	145	48	0.55	26	100	20
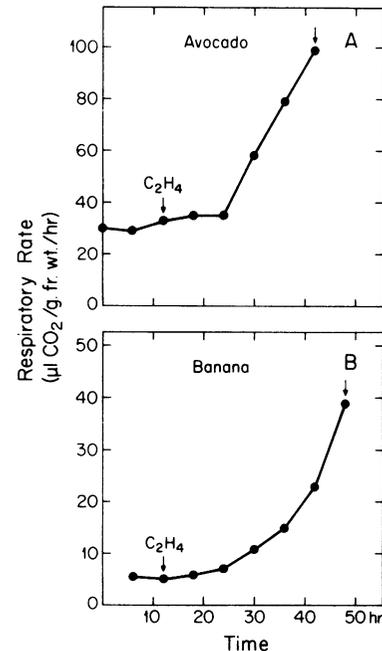
FIG. 5. Respiratory course in ripening avocado and banana fruit. A: avocado; ethylene addition (10 μl/l) and slicing where indicated by arrows. B: banana; ethylene addition and slicing where indicated by arrows.

alternate path. On the other hand, in the presence of CCCP (Fig. 1D) CLAM alone partially inhibits the respiration, while in the presence of CN almost complete inhibition is obtained. The data of Figure 1D were replotted according to equation 2 and the results are shown in Figure 2B. A straight line with slope (ρ) of 0.9 indicates almost maximum engagement of the alternate path.

V_{cyt}, V_{alt}, and V_{res} in Coupled and Uncoupled Slices from Midclimacteric Avocado Fruit. The respiratory fluxes of coupled and uncoupled avocado slices prepared during the climacteric rise and determined from Figures 1, C and D and 2B are summarized in Table IV. Data from similar experiments using a single avocado fruit from a more advanced stage of ripening are also shown (Table IV, experiments 3 and 4). In the case of coupled slices (experiments 1 and 3) the contribution of the alternate path is 0, since in equation 2 ρ is 0. The total climacteric slice respiration (V_T) is the sum of V_{cyt} and V_{res}. Uncouplers stimulate climacteric slice respiration (compare experiment 1 with experiment 2) and the alternate path is engaged extensively as shown by the values of ρ = 0.7 to 0.9. The activity of the Cyt path increases slightly with uncouplers (compare V_{cyt} of experiments 1 and 2 or 3 and 4), suggesting that coupled avocado slices at the climacteric rise have an almost fully saturated Cyt path.

Values of ρ in Banana Slices from Midclimacteric Banana Fruit. The results of titrating coupled and uncoupled midclimacteric banana slices with CLAM in the presence and absence of CN are shown in Figure 3, C and D. The slices used were made from a

single midclimacteric banana fruit (Fig. 5B). The results in Figure 3C show that in coupled slices CLAM fails to inhibit respiration in the absence of CN. In the presence of CN, however, CLAM inhibits respiration 80%. When titration with CLAM is carried out in the presence of 0.01 mM CN, a concentration which only partially inhibits the Cyt path, inhibition by CLAM is observed. The data in Figure 3C were replotted according to equation 2 and the results are shown in Figure 4A. In the absence of KCN, a horizontal line, i.e. with slope (ρ) of 0, indicates no contribution by the alternate path. On the other hand, in the presence of 0.01 mM KCN the slope (ρ) is 0.3, indicating that the alternate path is operating at 30% of its maximal capacity.

Titration experiments similar to those in Figure 3C were carried out in the presence of uncoupler and the results are shown in Figure 3D. Uncouplers do not stimulate the respiration of midclimacteric banana slices and CLAM alone is without effect. In the presence of CN there is almost complete inhibition by CLAM. The data of Figure 3D were replotted according to equation 2 and the results are shown in Figure 4B. A horizontal line with slope (ρ) of 0 indicates no contribution by the alternate path.

V_{cyt}, V_{alt}, and V_{res} in Coupled and Uncoupled Slices from Midclimacteric Banana Fruit. The respiratory fluxes of coupled and uncoupled banana slices from midclimacteric fruit are summarized in Table V. In coupled slices in the absence of CN (experiments 1, 4, and 6) V_T is the sum of V_{cyt} and V_{res}. The contribution of the alternate path is 0, since ρ is 0. When the activity of the Cyt path of coupled slices is decreased by low CN concentration (experiment 2), ρ is shifted to 0.3 and the contribution of the Cyt path is decreased, without any change in V_T. In the presence of CCCP (experiments 3 and 5) there is no change in V_T and the alternate path remains disengaged as indicated by ρ = 0. Table V shows that at any stage of the climacteric the respiration is mediated by the Cyt path.

CONTRIBUTION OF ALTERNATE PATH IN THIN AVOCADO SLICES RIPENED IN MOIST AIR

Tingwa and Young (34) have shown that avocado slices of various thickness show a respiratory climacteric when incubated in moist air. The respiratory rates observed by them at the climacteric peak in aged 2-mm-thick slices are similar to the rates in fresh uncoupled preclimacteric slices, where the alternate path is fully engaged (Table II). The question arises whether the respiratory climacteric in thin slices is alternate path-mediated.

Figure 6 shows the respiratory course of 1-mm-thick avocado slices incubated in moist air, in the presence and absence of 3 mM CLAM (vacuum-infiltrated at zero time). Slices aged in the absence of CLAM experienced a distinct climacteric rise. They softened within 2 days, and attained normal ripe texture. On the other hand, CLAM prevented the respiratory rise as well as softening. Titration of the respiration of thin aged slices at the climacteric peak with CLAM, in the presence and absence of 0.3 mM KCN, reveals that the alternate path is fully engaged, since the value of ρ is equal to 1 (Table VI B). Moreover, the total respiratory rate (V_T) is the same in the presence and absence of uncoupler. Table IV shows that in slices of midclimacteric avocado fruit the alternate path is inoperative. In Table VI C the ρ values of coupled and uncoupled slices prepared from fruit at the climacteric peak are shown. Here again, in contrast to ripened thin slices, the contribution of the alternate path is zero.

EFFECT OF UNCOUPLERS ON INTRINSIC ACTIVITY OF ALTERNATE PATH

When g(i) values in the presence of uncoupler are plotted against g(i) values in the absence of uncoupler in avocado and banana slices, the plots always give straight lines with slopes varying from less than 1 to 1. A slope less than 1 indicates that the activity of the alternate path (V_{alt}) has been decreased in the presence of uncoupler. In preclimacteric and midclimacteric avocado slices the capacity of the alternate path (V_{alt}) is the same in
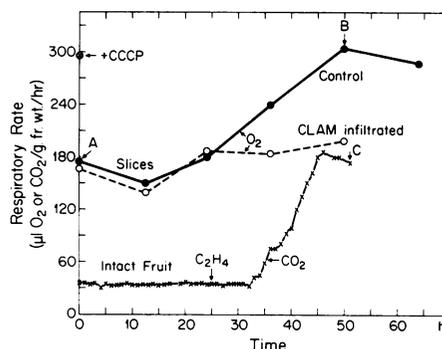
FIG. 6. Course of respiration in ripening avocado slices and in intact avocado fruit. Letters refer to data in Table VI. A: preclimacteric avocado slices; B: ripened slices at climacteric peak; C: slices from ripened fruit prepared at climacteric peak. Fresh slices infiltrated with 3 mM CLAM following slicing where indicated.

Table IV. Respiratory Rates of Coupled and Uncoupled Fresh Mid-climacteric Avocado Slices.

The data for experiments 1 and 2, done with a single fruit, were obtained from Figs. 1C,D and 2B. Experiments 3 and 4, done with one fruit, were similar to 1 and 2. The respiratory rate of the intact fruit used for experiments 3 and 4 was 128 µl O_2/g fresh wt·hr.

Experiment	CCCP 10 µM	V_T	V_{alt}	ρ	$\rho \cdot V_{alt}$	V_{cyt}	V_{res}
		µl O_2/g fresh wt·hr				µl O_2/g fresh wt·hr	
1	–	151	109	0	0	85	64
2	+	249	120	0.9	108	105	41
3	–	170	100	0	0	90	80
4	+	258	92	0.7	64	108	88

Table V. Respiratory Rates of Coupled and Uncoupled Fresh Climacteric Banana Slices in the Presence of Low Levels of Cyanide

The data for experiments 1, 2 and 3, done with a single fruit, were obtained from Figs. 3C, D and 4B. Experiments 4, 5 and 6 were similar to 1 and 3. Experiments 4 and 5 were from the same banana fruit at the climacteric peak (44.5 µl O_2/g fresh wt·hr). Experiment 6 was from a mid-climacteric fruit.

Experiment	CCCP 10 µM	KCN 0.1 mM	V_T	V_{alt}	ρ	V_{cyt}	V_{res}
			µl O_2/g fresh wt·hr			µl O_2/g fresh wt·hr	
1	–	–	52	35	0	43	11
2	–	+	49	35	0.3	27	11
3	+	–	50	21	0	45	15
4	–	–	46	24	0	38	10
5	+	–	42	17	0	27	15
6	–	–	53	30	0	33	20

Table VI. Respiratory Components of Coupled and Uncoupled Fresh Avocado Slices at Various Stages of Ripening.

A. Slices from preclimacteric fruit

CCCP 10 µM	V_T	V_{alt}	ρ	$\rho \cdot V_{alt}$	V_{cyt}	V_{res}
	µl O_2/g fresh wt·hr				µl O_2/g fresh wt·hr	
–	184	132	0	0	144	40
+	295	120	0.85	102	156	35

B. Preclimacteric slices aged to ripeness in moist air

–	327	125	1	125	169	35
+	320	–	–	–	–	–

C. Slices from peak climacteric fruit

–	165	102	0	0	80	77
+	192	57	0	0	102	85

coupled and uncoupled slices (slope 0.85–1). On the other hand, in preclimacteric banana slices V_{alt} is unaffected by CCCP (slope = 1), whereas a 30 to 40% decrease in V_{alt} is effected by uncouplers in climacteric banana slices. As previously pointed out (32, 33), CN exerts a nonspecific negative effect on the alternate path in the presence of uncoupler, and the drop in V_{alt} is not due to uncoupler *per se.*

INHIBITOR CONSTANT OF CLAM

The inhibitor constant (K_i) was determined from Dixon plots (31, 33). Table VII shows K_i values of CLAM in preclimacteric and climacteric avocado and banana slices. The values for avocado tissue are similar to those reported for *Arum* slices (35). The K_i values in coupled banana slices are half those in aged potato slices (31). Uncouplers cause an increase in K_i in both preclimacteric and climacteric banana slices.

DISCUSSION

WOUND RESPIRATION IN PRECLIMACTERIC AND CLIMACTERIC AVOCADO AND BANANA FRUITS

Slicing intact preclimacteric avocado and banana fruits results in an immediate sharp rise in respiration reminiscent of the wound respiration in potato tubers and other fleshy organs (15). The wound respiration in fresh potato slices is CN- and antimycin-sensitive (31, 32) and qualitatively different from the respiration of the intact tuber (13, 16). On the other hand, the wound respiration in fresh preclimacteric avocado and banana slices is CN-stimulated and in the presence of CN, sensitive to CLAM. The effect of slicing on the respiration rate is gradually diminished during the course of the climacteric. In contrast to potato (30) membrane lipid breakdown on slicing an avocado or banana fruit is nil (Theologis and Laties, unpublished).

The respiratory rate of a climacteric slice (*i.e.* a slice from a climacteric fruit) is similar to that of a preclimacteric slice, and to that of the intact climacteric fruit. This observation indicates that two independent processes, slicing on the one hand, and natural or ethylene-induced ripening on the other, activate the latent respiratory capacity of the preclimacteric fruit, although not necessarily by identical means.

CN RESISTANCE IN AVOCADO AND BANANA SLICES

Preclimacteric. Whereas the existence of the alternate path is demonstrable in preclimacteric avocado and banana slices, the respiration in coupled preclimacteric avocado and banana slices is more often than not mediated by the Cyt path alone. A similar situation has been observed in aged potato slices (31). Diversion of electrons from the alternate to the Cyt path during titration with CLAM has been ruled out (31). In uncoupled preclimacteric slices the alternate path is fully engaged in avocado ($\rho = 1$), and partially engaged in banana slices ($\rho = 0.55$–0.7). In avocado slices the respiration increment induced by CCCP is mediated exclusively through the alternate path, since the flux through the Cyt path increases only 10%. In banana slices the uncoupler-evoked respiratory increment is mediated through the Cyt and alternate paths, since V_{cyt} in the presence of CCCP increases as much as 2.8

times and ρ exceeds 0.

Climacteric. In climacteric avocado the alternate path, while present, does not contribute to the respiration in the absence of CN. In uncoupled avocado slices from midclimacteric fruit the alternate path is partially engaged, whereas midclimacteric banana slices fail to respond to uncouplers, and the alternate path is not engaged. Slices from the climacteric peak in both tissues neither respond to uncouplers nor engage the alternate path. The stimulatory effect of uncouplers is diminished during the climacteric, as is the effect of slicing. In our view the unresponsiveness of peak climacteric slices to uncoupler is due to the fact substrate mobilization is already maximal in fully ripe tissue. The engagement of the alternate path by uncouplers in preclimacteric avocado and banana slices is attributed to an increase in substrate mobilization due to the enhancement of glycolysis. When the glycolytic flux exceeds the oxidative capacity of the Cyt path, the engagement of the alternate path ensues.

COMPARISON OF V_{cyt} AND V_{alt} IN PRECLIMACTERIC AND CLIMACTERIC SLICES

Avocado. Not only is the maximal capacity of the alternate path (V_{alt}) and Cyt path (V_{cyt}) the same in preclimacteric and midclimacteric slices, but V_{cyt} and V_{alt} are essentially equal as well. The uncoupled respiration of preclimacteric slices is eight times higher than that of the intact fruit. Only 50% of the latent respiratory capacity of the unripe fruit is realized during the climacteric, and since $\rho = 0$ in climacteric slices the expressed latent capacity is primarily Cyt-mediated.

Banana. Whereas the capacities of the alternate (V_{alt}) and Cyt paths (V_{cyt}) decrease somewhat during the climacteric, they are separately sufficient to accommodate the normal preclimacteric slice respiration, the residual respiration apart. On the other hand, the alternate path in climacteric slices is insufficient to accommodate the total respiration at the climacteric peak. The wound respiration together with the uncoupler response of preclimacteric slices indicates a large latent respiratory capacity in the intact fruit which is realized with ripening primarily through the Cyt path. The values of V_{cyt} and V_{alt} in preclimacteric and climacteric slices make it unnecessary to invoke *de novo* synthesis of respiratory enzymes to account for the climacteric rise. If one considers as well that anaerobiosis stimulates glycolysis in avocado fruits (27), the presumption is reinforced that the climacteric rise in respiration results from the realization of unexpressed glycolytic and respiratory capacity in preclimacteric fruits.

RIPENING OF SLICES

Figure 6 depicts the course of the climacteric rise in thin avocado slices aged in moist air. Such slices fully utilize the capacity of the Cyt and alternate paths. Since V_{cyt} and V_{alt} at the climacteric peak are the same as in uncoupled preclimacteric slices, slices ripened in air must utilize the full respiratory capacity present in preclimacteric tissue. The respiratory increment in thin avocado slices during aging in moist air fully engages the alternate path, the value of ρ being 1. In avocado slices the wound respiration is independent of slice thickness whereas the induced respiration is inversely proportional to slice thickness (34). A similar situation has been observed in potato slices (15). Thus, it is to be expected that the engagement of the alternate path with aging of avocado slices will be inversely related to slice thickness, and that ρ values at the climacteric peak will decrease with slice thickness. In effect, thickness is a regulatory parameter in the engagement of the alternate path in ripening slices.

ALTERNATE PATH AND ETHYLENE

Contrary to earlier assertions (25, 27, 28) we must entertain the prospect that the surge of respiration comprising the climacteric rise in intact fruit is Cyt-mediated. Furthermore, it is evident that

Table VII. Inhibitor Constant (K_i) for CLAM in Coupled and Uncoupled Preclimacteric and Climacteric Avocado and Banana Slices.

Experiment	CCCP 10 µM	Avocado		Banana	
		Preclimacteric	Climacteric rise	Preclimacteric	Climacteric rise
		µM		µM	
1	–	600	680	120	130
2	+	550	550	240	–
3	–	600	–	170	170
4	+	500	–	250	425

the requisite capacity to sustain electron transport through the Cyt path at the climacteric peak is present, though unexpressed, in preclimacteric tissue. By contrast, the climacteric rise in ripening avocado slices involves the alternate path as well as the Cyt path. Inhibition of slice ripening by CLAM (Fig. 6) need not implicate the alternate path, since we have evidence that CLAM cannot be considered specific for the alternate path at concentrations as high as 3 mM.

It is doubtful that CN stimulates glycolysis and aerobic respiration in avocado and banana fruits by dint of positive modulation of the alternate path (25–28) since antimycin also stimulates aerobic respiration in avocado and banana slices. The stimulation of respiration by CN and antimycin is most readily attributable to a conventional Pasteur effect in tissue which can handle the extra traffic through the alternate path. Stimulation of respiration by CN will be observed when the capacity of the alternate path exceeds the normal Cyt-mediated respiratory rate. We have found that in tissues such as sweet potato and parsnip, where V_{alt} exceeds the observed respiration rate, CN and antimycin elicit the same degree of respiratory stimulation.

The ineffectiveness of CLAM in inhibiting respiration in the presence of antimycin has recently been attributed to a mitochondrial antimycin-resistant branch of the Cyt path (32). In all tissue slices that we have examined where the alternate path is operative, antimycin fails to inhibit the Cyt path fully.

Since ρ has been shown to be 0 in ripe banana and avocado slices, there is little reason to persist in the view that ethylene engenders the climacteric and ripening by positive modulation of the alternate path. Its site of action must be elsewhere, albeit the prospect of its involvement in transitional metal complexation remains attractive (24). The fact remains that the unexceptionable correlation between respiratory stimulation by ethylene and by CN in fruits and bulky storage organs is highly suggestive (29). Withal, the correlation may simply reflect the fact that tissues responsive to ethylene and CN are subject to substrate mobilization, and in having the alternate path are able to reflect such mobilization whether or not the CN path is inhibited.

The respiration of fresh slices (e.g. sweet potato slices) is not per se stimulated by ethylene (14). Presumably slicing in and of itself releases glycolysis sufficiently to preempt an ethylene effect. In fruits and bulky storage organs ethylene and CN seemingly act alike in stimulating respiration—but the full response takes many hr. In slices, where ethylene is ineffective, CN may still elicit limited respiratory stimulation by dint of its immediate evocation of a Pasteur effect. When thin avocado slices are aged (in distinction to slices taken from aged fruit) the respiration rate rises beyond the capacity of the Cyt path, and the alternate path is involved in sustaining the respiration of the climacteric peak.

Glycolysis stimulation is an invariable concomitant, and conceivably a cause of the climacteric rise, although enhancement of glycolysis per se will not induce ripening (25, 27, 28). Aerobic metabolism is requisite to ripening, but we have been unable to stem the ripening of avocado halves with CLAM at concentrations which inhibit the alternate path (Christoffersen and Laties, unpublished). CLAM concentrations beyond the minimal necessary for full synergistic inhibition with CN are suspect, since CLAM is nonspecific at higher concentrations.

Although the evidence we have presented mitigates against the obligatory participation of the alternate path in the climacteric and ripening, the fact remains that alternate path activity has been reported to cause peroxide formation (20), and peroxide formation both attends and promotes the climacteric rise (9). Whether one or another free radical (superoxide, hydroxyl radical) intermediates is an open question, but free radical scavengers inhibit ethylene production (2) and, it is to be expected, ripening. Thus,

while alternate path involvement in ripening, perhaps at a low level, remains a possibility, the question is at best moot, there being various ways (7, 12) of generating peroxide.

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