METABOLIC STUDIES WITH BANANA FRUIT SLICES

I. CHANGES IN THE INCORPORATION OF ^{14}C-LABELLED COMPOUNDS IN RESPONSE TO CUTTING

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Summary

Transverse slices of green banana fruit were vacuum-infiltrated with aqueous solutions of ^{14}C-labelled metabolites and the non-metabolite α-methyl-D-glucoside immediately after cutting, and at 17 hr after cutting—i.e. near the peak of "induced" respiration.

The rates of release of $^{14}CO_2$ from freshly cut slices infiltrated with [1-^{14}C]-pyruvate, [1-^{14}C]- and [2-^{14}C]acetate, [1,5-^{14}C]citrate, [5-^{14}C]2-oxoglutarate, and specifically labelled D-glucose show that both the tricarboxylic acid cycle and the pentose phosphate pathway are active in this tissue and that there is little change in the relative contribution of the latter pathway during the development of induced respiration. An active tricarboxylic acid cycle is also indicated by the rapid distribution of carbon from D-[U-^{14}C]glucose through the organic acids of the cycle.

The rate of uptake of acetate and malonate is rapid in slices both at 0 and 17 hr after cutting, wheras the rate of uptake of citrate and D-glucose is low at 0 hr but rapid at 17 hr after cutting. α-Methyl-D-glucoside is taken up at a low and similar rate at both times. The patterns of release of $^{14}CO_2$ from ^{14}C-labelled glucose, acetate, and malonate show that the metabolic responses induced by cutting begin within 3 hr after cutting.

I. Introduction

When green bananas are cut into transverse slices, respiration immediately increases but largely subsides within 2 hr, followed by a gradual rise in rate to a broad peak at 15–20 hr (Palmer and McGlasson 1969). The latter rise has been termed "induced" respiration (Laties 1963; ap Rees 1966). In addition, when the bananas are cut, ethylene production immediately increases about 10-fold and then rises to a peak after 6–8 hr (McGlasson 1969). Many types of plant tissue show a similar pattern of induced respiration after cutting (ap Rees 1966; Laties 1967), and up to the present a period of induced ethylene synthesis has been observed only in fruit (Galliard *et al.* 1968*b*; McGlasson 1969; Lee, McGlasson, and Edwards 1970).

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When thick banana slices ripen, changes in ethylene synthesis, respiration rate, starch and sugar content, cell permeability, peel colour, and production of flavour volatiles occur as in whole fruit (Palmer and McGlasson 1969; Brady, O'Connell, Smydzuk, and Wade 1970). Before a more detailed biochemical study of ripening in slices is undertaken, the effect of cutting on metabolism must be considered. This paper concerns the increase in uptake and oxidation of ^{14}C-labelled sugars and organic acids observed during the development of induced respiration in banana slices.

II. Materials and Methods

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

Slices were prepared from green fruit of the Williams strain of the Dwarf Cavendish variety obtained from Avoca, N.S.W., and incubated under fluorescent laboratory lighting at 20°C. The methods of preparation, measuring respiration rates, administering ^{14}C-labelled metabolites, and collection and measurement of $^{14}CO_2$ were described by Palmer and McGlasson (1969).

(*b*) *Analytical Methods*

To determine the extent of incorporation of ^{14}C following administration of D-[U-^{14}C]-glucose, single slices (about 6 g) were killed in 75 ml of boiling aqueous 80% (v/v) ethanol acidified with formic acid (1 ml 6M formic acid to 75 ml ethanol). Extraction was completed as described by MacLennan, Beevers, and Harley (1963). Lipids were removed by partitioning into ethyl ether and acidic, basic, and neutral fractions recovered from the aqueous phase by sequential passage through ion-exchange resins (Permutit De-acidite FF, acetate form, 52–100 mesh, and Permutit Zeokarb 255, H^+ form, 52–100 mesh). The acidic fraction, which included aspartic and glutamic acids, was separated by ion-exchange column chromatography (Wills and McGlasson 1968). The amounts of each acid were measured by titration with 0·02N NaOH. To separate the sugars, aliquots of the neutral fraction were chromatographed on Whatman No. 1 paper [n-butanol–acetic acid–water, 4 : 1 : 1 (v/v/v), descending for 16 hr at 25°C]. The sugars were located with alkaline silver nitrate (Trevelyan, Proctor, and Harrison 1950). Radioactivity in aqueous samples was measured by counting aliquots in dioxane scintillation solution (Herberg 1960). Radioactivity in the lipid fraction and in sugars separated on paper was counted in a toluene scintillation solution (Palmer and McGlasson 1969).

To measure uptake of labelled metabolites, individual 6-mm slices were taken 1 hr after infiltration and cut into three equal slices. These were immersed in aerated distilled water at 0°C for 30 min. Uptake of metabolites was estimated from the distribution of ^{14}C between the bathing solution and the tissue (Brady, O'Connell, Palmer, and Smillie 1970). Radioactivity in the bathing solution includes that from ^{14}C which diffused from the intercellular spaces of the tissue as well as some intracellular ^{14}C released by leakage and damage from cutting. To correct for this release of ^{14}C, ethanol-soluble carbohydrates in the bathing solutions and remaining in the tissue were measured (Pinnegar 1965).

(*c*) *Radiotracer Supplies*

Labelled compounds were obtained as follows: sodium [1-^{14}C]acetate, 44·4 mCi/mmole; sodium [2-^{14}C]acetate, 38 mCi/mmole; sodium [1-^{14}C]malonate, 14·5 mCi/mmole; sodium [1-^{14}C]pyruvate, 9·3 mCi/mmole; sodium [5-^{14}C]2-oxoglutarate, 9·3 mCi/mmole; [1,5-^{14}C]citric acid, 15·4 mCi/mmole; D-[U-^{14}C]glucose, 2·8 mCi/mmole; D-[1-^{14}C]glucose, 39·7 mCi/mmole; D-[2-^{14}C]glucose, 33·4 mCi/mmole; and D-[6-^{14}C]glucose, 35·2 mCi/mmole from the Radiochemicals Centre, Amersham, England; D-[3(4)-^{14}C]glucose, 13·8 mCi/mmole from the New England Nuclear Corporation, Mass., U.S.A.; and [U-^{14}C]α-methyl-D-glucoside, 4·86 mCi/mmole from Nichem Incorporation, Maryland, U.S.A. All compounds were infiltrated in aqueous solution at the above specific activities except that the solutions of specifically labelled D-glucose were adjusted to 13·8 mCi/mmole by addition of unlabelled D-glucose.

III. Results

Solutions of ^{14}C-labelled acetate, pyruvate, malonate, citrate, 2-oxoglutarate, and D-glucose were vacuum-infiltrated into 2-mm and 6-mm thick slices immediately

Table 1

UPTAKE IN 1 HR OF ^{14}C-LABELLED COMPOUNDS BY BANANA FRUIT SLICES*

Slices vacuum-infiltrated with labelled compounds 0 and 17 hr after cutting

Compound	Uptake (%)†	
	0-hr Slices	17-hr Slices
[2-^{14}C]Acetate	80·2±1·1	84·7±0·8
[1-^{14}C]Malonate	60·6±0·7	71·8±0·6
[1,5-^{14}C]Citrate	14·8±0·6	61·7±0·8
D-[U-^{14}C]Glucose	14·6±0·4	66·6±0·5
[U-^{14}C]α-Methyl-D-glucoside	23·9±1·0	22·6±0·5

* From 5 to $\cdot 10 \times 10^4$ disintegrations/min/g tissue were infiltrated in aqueous solution.

† Means of three 6-mm slices and estimates of the standard deviation of the population.

these were cut (0-hr slices), or 17 hr later when the maximum induced respiration had developed (17-hr slices). Frequent measurements of ^{14}C in the respired CO_2 were

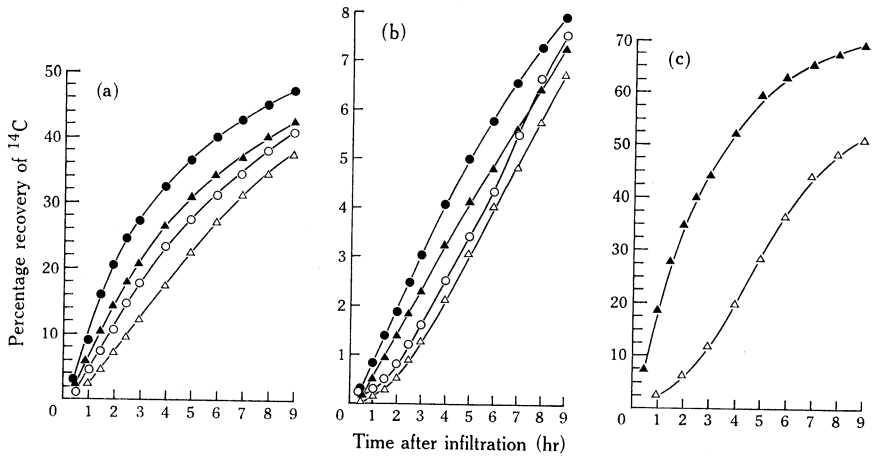

Fig. 1.—Percentage recovery of ^{14}C (as $^{14}CO_2$) evolved by 2-mm (○, ●) and 6-mm (△, ▲) banana slices infiltrated with [1-^{14}C]acetate (*c.* 5×10^{-4} disintegrations/min/g) (*a*) or with [2-^{14}C]acetate (*c.* 10^5 disintegrations/min/g) (*b*) immediately (○, △) and 17 hr (●, ▲) after cutting. (*c*) Percentage recovery of ^{14}C as $^{14}CO_2$ evolved by 6-mm slices infiltrated with [1-^{14}C]malonate (*c.* $2 \cdot 5 \times 10^4$ disintegrations/min/g) immediately (△) and 17 hr (▲) after cutting.

made. The uptake of the non-metabolite, [U-^{14}C]α-methyl-D-glucoside, was also measured (Table 1).

(*a*) [*1*-^{14}*C*]- *and* [*2*-^{14}*C*]*Acetate;* [*1*-^{14}*C*]*Pyruvate*

Acetate was taken up rapidly by the cells of freshly cut slices, and there was little difference between 0- and 17-hr slices when uptake was measured 1 hr after infiltration (Table 1).

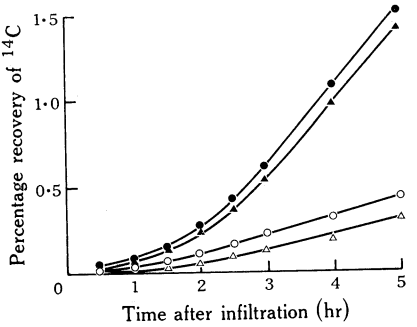

Fig. 2.—Percentage recovery of ^{14}C (as $^{14}CO_2$) evolved by banana slices infiltrated with [1,5-^{14}C]citrate (10^5 disintegrations/min/g). Symbols as for Figures 1(*a*) and 1(*b*).

Evolution of $^{14}CO_2$ from both [1-^{14}C]- and [2-^{14}C]acetate was more rapid in 17-hr slices, and more rapid from 2-mm than from 6-mm slices [Figs. 1(*a*) and 1(*b*)]. $^{14}CO_2$ evolution from [1-^{14}C]pyruvate was more rapid than from [1-^{14}C]acetate. The recovery of ^{14}C as $^{14}CO_2$ from 0-hr slices (6-mm) in the first hour after infiltration was 14% from [1-^{14}C]pyruvate, 2·5% from [1-^{14}C]acetate, and 0·2% from [2-^{14}C]-acetate. The corresponding values for 17-hr slices were 41, 6, and 0·5% respectively. These results indicate a functional tricarboxylic acid (TCA) cycle in both fresh and aged slices.

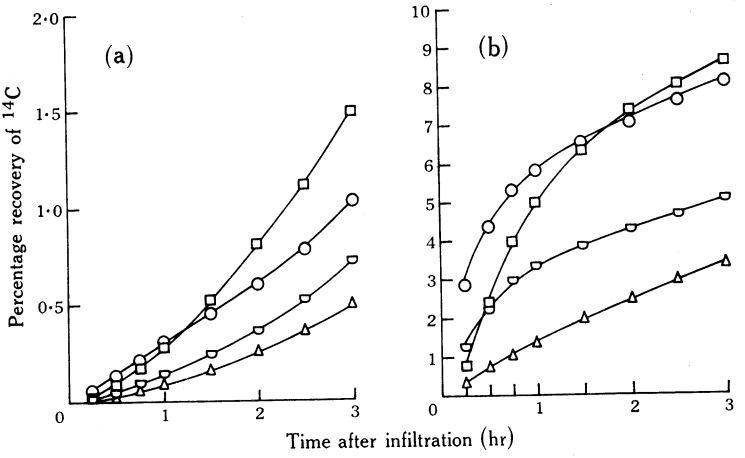

Fig. 3.—Percentage recovery of ^{14}C (as $^{14}CO_2$) evolved by 6-mm banana slices infiltrated with D-[^{14}C]glucose (*c.* 8×10^4 disintegrations/min/g) labelled at carbon atoms 1(○), 2 (△), 3 and 4 (□), and 6 (⌓), immediately (*a*) and 17 hr (*b*) after cutting.

(*b*) [*1*-^{14}*C*]*Malonate*

Malonate was taken up rapidly by 0-hr slices, but the rate was slightly higher in 17-hr slices (Table 1). Evolution of $^{14}CO_2$ from [1-^{14}C]malonate was initially slow in 0-hr slices, but the rate increased greatly between 2 and 4 hr after slicing [Fig. 1(*c*)].

In 17-hr slices, $^{14}CO_2$ evolution from [1-^{14}C]malonate was very rapid within 30 min of infiltration.

(*c*) [*1,5-^{14}C*]*Citrate and* [*5-^{14}C*]*2-Oxoglutarate*

Citrate was taken up only slowly by the cells of 0-hr slices, but much more rapidly by those of 17-hr slices (Table 1). In both 0- and 17-hr slices, the rate of $^{14}CO_2$ evolution from [1,5-^{14}C]citrate increased with time to a nearly constant rate 3 hr after infiltration (Fig. 2). This rate presumably represents an equilibration of added [^{14}C]citrate with the tissue citrate; in 17-hr slices this final rate was about four times that in 0-hr slices. Evolution of $^{14}CO_2$ from [5-^{14}C]2-oxoglutarate resembled in pattern and amount that from [1,5-^{14}C]citrate.

TABLE 2

CONTRIBUTION OF INDIVIDUAL CARBON ATOMS OF GLUCOSE TO RESPIRED CARBON DIOXIDE*

Time after Infiltration (hr)	Slices	Contribution to CO_2 (%)					
		C1	C2	C3	C4	C5	C6
1	0-hr	25·4	7·6	23·7	23·7	7·6	11·9
	17-hr	26·7	6·1	22·9	22·9	6·1	15·2
2	0-hr	19·5	8·1	26·3	26·3	8·1	11·7
	17-hr	22·7	7·95	23·8	23·8	7·95	13·8
3	0-hr	18·0	8·6	26·1	26·1	8·6	12·6
	17-hr	21·8	9·0	23·2	23·2	9·0	13·7

* Percentages were calculated from the data presented in Figures 3(*a*) and 3(*b*). C3 and C4 were assumed to contribute equally and the contribution of C5 was taken to equal that of C2.

TABLE 3

DISTRIBUTION OF ^{14}C IN SLICES 1 HR AFTER INFILTRATION WITH D-[U-^{14}C]GLUCOSE*

Compound	^{14}C Recovered (%)†		Compound	^{14}C Recovered (%)†	
	0-hr Slices	17-hr Slices		0-hr Slices	17-hr Slices
$^{14}CO_2$	4·4	15·4	Lipid	1·2	0·8
Fructose	26·4	17·0	Organic acids	7·3	2·2
Sucrose	58·6	63·7	Amino acids	2·1	0·8

* About 10^5 disintegrations/min/g were infiltrated.

† In the extracts of 0-hr slices 1 hr after infiltration, 76·6% of the ^{14}C was in glucose; the corresponding value for 17-hr slices was 83·0%. In either case, about 90% of the ^{14}C infiltrated was recovered as $^{14}CO_2$ and in the aqueous ethanolic extracts. The values given show the percentage of ^{14}C in fractions derived from ethanol extracts after excluding the activity remaining in free glucose. Each value is the mean derived from duplicate tissue samples.

(*d*) D-[*^{14}C*]*Glucose*

Uptake of glucose by cells of freshly cut slices was slow but in 17-hr slices this rate had increased almost fivefold (Table 1). Delayed uptake and greater endogenous dilution may contribute to the low initial rate of $^{14}CO_2$ production by 0-hr slices infiltrated with D-[^{14}C]glucose [Fig. 3(*a*)]. The increase in the rate of $^{14}CO_2$ production

in 17-hr slices compared to 0-hr slices [Fig. 3(*b*)] far exceedeed the increase in the rate of uptake. The initial rate of $^{14}CO_2$ production from D-[1-^{14}C]glucose exceeded that from D-[6-^{14}C]glucose in both 0- and 17-hr slices [Figs. 3(*a*) and 3(*b*); Table 2].

The distribution of ^{14}C in ethanol-soluble compounds after metabolism of D-[U-^{14}C]glucose for 1 hr by 0- and 17-hr slices is shown in Tables 3 and 4. Notably higher proportions of ^{14}C were found in the acidic and basic compounds in 0-hr slices. While this distribution may have been influenced by differences in the amount of ^{14}C incorporated in the ethanol-insoluble fraction, the extent of ^{14}C accumulation in the ethanol-soluble acidic fraction suggests that ^{14}C had ready access to acids of the TCA cycle in 0-hr slices (Table 3). This was confirmed by estimates of the amounts and specific activities of the acids (Table 4).

TABLE 4*

^{14}C IN ORGANIC ACIDS 1 HR AFTER INFILTRATING SLICES WITH D-[U-^{14}C]GLUCOSE

Acid	0-hr Slices		17-hr Slices	
	m-equiv/100 g	Disintegrations/ min/mg C	m-equiv/100 g	Disintegrations/ min/mg C
Glutamic	0·025	120,000	0·023	147,000
Aspartic	0·219	45,000	0·217	45,000
Succinic	0·067	77,000	0·078	79,000
Malic	4·66	65,000	5·17	59,000
Citric	2·21	46,000	2·49	39,000

* The data in Tables 3 and 4 were obtained from the same experimental material.

IV. DISCUSSION

In many cases the "induced rise" in respiration which occurs during the aging of thin slices or disks of non-fruit tissues has been associated with "wound healing" or "rejuvenation" (MacDonald, Knight, and DeKock 1961; Laties 1963). This response has been shown to be accompanied by changes in respiratory metabolism but the extent of these changes, exemplified by the ability of the tissues to oxidize glucose and acids of the TCA cycle, varies widely. Fresh potato slices oxidize these substrates slowly but show a large increase in capacity to do so during aging (Laties 1967). However, fresh slices of other storage tissues, e.g. carrot phloem and red beet-root storage tissue, readily oxidize these substrates (ap Rees and Beevers 1960; MacLennan, Beevers, and Harley 1963). During aging in some tissues there is evidence that the proportions of glucose catabolized via the Embden–Meyerhof–Parnas (EMP) and the pentose phosphate (PP) pathways change (ap Rees and Beevers 1960; ap Rees 1966; Laties 1967). Aged slices of many tissues have an increased capacity to take up metabolites and inorganic ions from solution (Laties 1967).

Determination of the nature of the changes in respiratory metabolism induced by cutting is critical if thick slices of banana tissue are to be used as a model system for studying ripening. Because it is impracticable to make direct comparisons with the intact fruit the respiratory pattern of the tissue before induced respiration develops was determined in slices immediately after cutting. Infiltrated substrates do not appear to provide carbon for the short burst of respiration which results from cutting (Palmer and McGlasson 1969). Figures 1(*a*) and 1(*b*) show that the release

of $^{14}CO_2$ from acetate proceeds at a steady or an increasing rate during the first hour after cutting although the respiration rate is declining rapidly; also they show that this pattern of $^{14}CO_2$ release is established well before the detection at 4–5 hr of the rise in respiration which denotes the development of induced respiration (Palmer and McGlasson 1969). The high relative yield of CO_2 from C3 and C4 of glucose, the movement of carbon from glucose to acids of the TCA cycle, the oxidation of citrate and 2-oxoglutarate, and the relative rates of release of C1 of pyruvate and C1 and C2 of acetate during the first hour after cutting and infiltration show that the EMP sequence and the TCA cycle function before the development of induced respiration.

Between 4 and 17 hr after cutting respiration in 6-mm slices rises to a peak about three times the rate of the intact fruit. This peak (induced respiration) is associated with a large increase in the rate at which citrate and glucose are taken up by the tissue (Table 1). Since there is no accompanying change in the relative yield of CO_2 from C3 and C4 of glucose the rate of catabolism of glucose via the EMP sequence and the TCA cycle must also increase during the development of induced respiration. The fact that in 17-hr slices glucose is catabolized to CO_2 with less accumulation in organic and amino acids (Table 3) points to a particular stimulation of the TCA cycle.

The more rapid release of CO_2 from C1 than from C6 of glucose (Table 2) shows that an appreciable proportion of glucose is catabolized *via* the initial steps of the PP pathway. The development of induced respiration is not accompanied by a significant change in the relative contributions of individual carbons of glucose to CO_2. Thus there is no evidence in banana slices of an increase in the proportion of carbon entering the PP shunt as seems to be the case during the development of induced respiration in a number of other plant tissues (ap Rees 1966; Laties 1967).

Infiltration of banana slices with malonate (0·02M, pH 5·0) does not inhibit ripening (McGlasson, Palmer, Vendrell, and Brady, unpublished data). Results presented in this paper show that the TCA cycle operates in both freshly cut and aged slices. Thus, either ripening is independent of the operation of the TCA cycle, or the tissue can by-pass malonate inhibition or metabolize the inhibitor. Malonate is readily taken up by cells of the slices (Table 1). The capacity to decarboxylate malonate develops 2–4 hr after slicing, and is well established by 17 hr [Fig. 1(*c*)] It seems likely therefore that this activity reflects the induction of the fatty acid synthetase system (Hatch and Stumpf 1962; Willemot and Stumpf 1967; Galliard *et al.* 1968*a*).

During ethylene-induced ripening of aged slices of banana fruit the rate of amino acid uptake increases (Brady, O'Connell, Palmer, and Smillie 1970). This probably represents the further development of a capacity which increased during aging. Ethylene production, protein synthesis (Brady, O'Connell, Palmer, and Smillie 1970), and respiration also increase as aged slices begin to ripen. These changes may be separated in time from those initiated by cutting and further work may establish that some at least of the ripening responses are distinct.

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