PHOTOSYNTHESIS AND RESPIRATION IN DEVELOPING FRUITS
I. $C^{14}O_2$ UPTAKE BY YOUNG ORANGES IN LIGHT AND IN DARK [1]

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The effect that photosynthesis in a green fruit may have on the growth of that fruit has not been extensively studied. In another paper of this series (Todd, Bean, and Propst, unpublished results) a study was made of the magnitude of photosynthesis in cucumbers, lemons, oranges, and avocados at different ages and under varying conditions. Photosynthesis in these fruits could completely compensate for respiratory loss of carbon dioxide, under some conditions. With normal growth situations, however, it may be assumed that photosynthesis in the fruit itself does not supply a large proportion of the material needed for growth of the fruit.

Assimilation of carbon dioxide might also affect the type of materials produced and stored within a given tissue. Even though photosynthetic assimilation might account for a relatively small percentage of the total stored material, the compounds formed in fixation of carbon dioxide might have a strong effect on the quality of the fruit. To help determine what role carbon dioxide assimilation may play in formation of storage products, a study has been made of the materials formed by fixation of carbon dioxide in young fruit in the light and in the dark, using radioactive tracers. The present paper gives some of the results found with young oranges. It appears from some of these results that carbon dioxide fixation in the dark by juice vesicles of these small oranges may be involved extensively in the formation and storage of the organic acids of the orange juice.

MATERIALS AND METHODS

A single, intact Valencia orange (13.4 g – 15.7 g) was placed in each of the two chambers of the carbon dioxide fixation apparatus pictured in figure 1. Each chamber also contained 100 mg samples of each of the three major tissues of the orange, the flavedo (outer, green layer of the peel), albedo (inner, white peel layer), and the juice vesicles. The vesicles, or juice sacs, were prepared by carefully teasing them loose from the carpellary membrane. Flavedo and albedo were prepared by dividing diametric sections of the fruit (about 1.5 mm thickness) into the two tissues. The chambers were immersed in a 25° C water bath and illuminated from below by fluorescent lamps (ca 1,000 ft-c). After reducing pressure in the system slightly, carbon dioxide was generated by adding lactic acid to $BaC^{14}O_3$ (3.4 mg, 82 μc/mg). The circulating pump kept a constant flow of gas through the apparatus during the experimental period at a rate such that the gas should completely recycle about four times per minute (flow rate 1200 ml/min, apparatus volume 280 ml). Thus, both light and dark samples were exposed to identical atmospheres. The dark flask was wrapped with black plastic tape to keep light out.

At the end of the experimental period (220 min) the air from the chambers was withdrawn rapidly into an evacuated 2,000 ml flask containing standardized, carbon dioxide free sodium hydroxide. Subsequent titration of the alkali allowed a determination of the final carbon dioxide concentration in the system. Each of the tissue samples was dropped directly into separate vessels of rapidly boiling ethanol. The intact fruits were rapidly frozen in crushed, frozen CO_2. They were allowed to warm to −20° C, separated into the flavedo, albedo, and pulp, and each tissue was inactivated and extracted in boiling ethanol.

After centrifuging and decanting the initial alcohol extracts, extraction of each of the samples was continued by adding a small amount of water to each sample and grinding while heating over steam. After

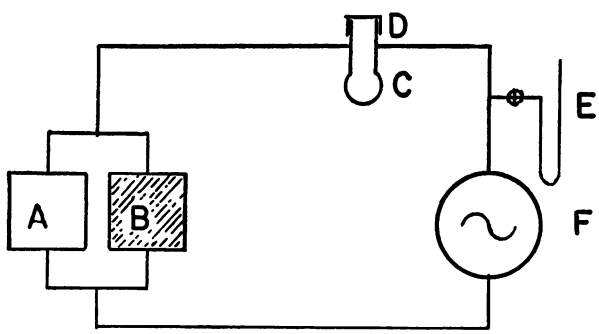

FIG. 1. Carbon dioxide assimilation apparatus. A. Light chamber. B. Dark chamber. Wrapped with plastic electrical tape. C. $C^{14}O_2$ generating chamber containing $BaC^{14}O_2$. D. Ampoule cap. To allow addition of lactic acid to $BaC^{14}O_2$ with a syringe. E. Manometer. F. Circulating pump. Chambers were immersed in a 25° C water bath and illuminated from below by fluorescent lamps.

Total volume of the apparatus air space was 280 ml.

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the samples were fairly well macerated, alcohol was added to give a concentration of 80 per cent and the samples boiled for a few minutes. This process was repeated several times with centrifuging and decanting between each repetition. The residues were subjected to continuous extraction by alcohol in a Bailey-Walker apparatus to remove the last traces of soluble material. This latter step removed only a very small percentage (less than 1 % of the total alcohol extractable activity but it was required to obtain consistent results on the activity of the insoluble residues.

All the extracts from each sample were combined and diluted to known volumes. Aliquots were taken and plated directly on stainless steel planchets for determination of total activity fixed in each tissue. Larger aliquots were evaporated to dryness at 30° to 40° C to obtain samples for chromatographic analysis. These samples were redissolved in known amounts of water and applied to filter paper. One dimensional chromatograms were prepared with water-saturated phenol, butanol-acetic acid-water (52:13:35), or butanol-ethanol-water (52.5:32:15.5). Two dimensional chromatograms were also prepared with the first two solvents. The radioactive compounds were located by radioautography. Counting of activity was done directly on the chromatographic paper, using previously determined conversion factors to obtain figures comparable to those found with counting on steel planchets.

Labeled compounds found on the chromatograms were tentatively identified by chromatographic positions and by reaction to spray reagents (1 % *p*-anisidine hydrochloride in butanol for sugars, 0.1 % ninhydrin in acetone as a dipping agent for amino acids, 0.04 % bromcresol green in alcohol for organic acids). For confirmation of the identity of glucose, the glucose area was eluted and treated with fungal glucose oxidase and the reaction mixture rechromatographed to identify gluconic acid. Sucrose was checked by hydrolysis with invertase and chromatographic identification of the resulting monosaccharide components. Rechromatography of the reaction products resulting from treatment of the eluted fructose area with calcium hydroxide showed a normal distribution of activity between the epimers, fructose, mannose, and glucose. Amino acids and organic acids were mixed with authentic samples of the suspected acid and rechromatographed in other solvents. Coincidence in several solvents of the radioactive spot with the colored spot resulting from the indicator reaction was considered adequate identification for the purposes of this study. The amount of activity lost upon treatment of the radioactive spots with ninhydrin also contributed to confirmation of the identity of the amino acids. Organic phosphates were in such low concentration that identification was made only upon the original chromatographic characteristics.

The alcohol-insoluble residues were ground finely and alcohol suspensions of the powder were plated for counting. Approximate absorption factors were obtained on the assumption that the residue powder would have the same absorption as a similar thickness and weight of filter paper.

All radioactivity determinations were made using a Nuclear-Chicago, D-47 gas-flow tube with a thin end window. For counting on paper chromatograms, this tube, without shielding, was supported on a ring about 2 mm from the paper.

To determine the relative distribution of activity in the carboxyl groups of malic acid, the β-carboxyl was obtained as carbon dioxide by treatment with *Lactobacillus arabinosus* (9). Both carboxyl groups were obtained by adapting the method of Friedman and Kendall (5). The α-carboxyl activity was taken as the difference between the two determinations.

Carbon dioxide concentrations during the experiment were estimated from the initial carbonate introduced and titration of the trapped carbon dioxide at the end of the experiment.

Results and Discussion

The total fixation of carbon dioxide per unit weight of total solid material in the alcohol-soluble and alcohol-insoluble fractions of all tissues of the fruit is compiled in table I. Preliminary experiments indicated that fixation was relatively uniform when ex-

Table I

C^{14}-Activity in Alcohol-Soluble and Alcohol-Insoluble Fractions of Orange Tissues Exposed to $C^{14}O_2$ [1, 2]

	Alcohol-soluble extract[3]				Alcohol-insoluble residue			
	Intact fruit		Tissue preparation		Intact fruit		Tissue preparation	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Flavedo	34,700	1,540	54,000	3,700	15,400	280	6,670	490
Albedo	3,400	1,150	2,500	1,950	235	160	280	280
Vesicle	2,580	2,500	13,600	16,500	284	360	2,580	1,680

[1] Initial carbon dioxide concentration was about 0.2 %; final 2.5 %.

[2] Activity is given as cpm/mg of the dried soluble solids or of the alcohol-insoluble residue. Counting was done to within 3 % statistical error.

[3] Total activity in the various extracts from intact fruits: Light (10^5 cpm): Flavedo, 165; albedo, 18.0; vesicles, 11.0; Dark (10^5 cpm): Flavedo, 6.9; albedo, 5.7; vesicles, 7.4.

pressed on the basis of unit solid material for other sizes of fruit but total activity would vary greatly. Total activity in the extracts from tissues of the intact fruits is given as a footnote to table I. The C^{14} activity found in preparations of the illuminated flavedo is very much higher than that found in the dark flavedo or in any other tissue. The activity of extracts from the photosynthesizing flavedo range from 14 to 23 times the activity of the dark flavedo and similar ratios (14–55 fold) are found in the alcohol-insoluble material. This indicates that the green flavedo is capable of a respectable photosynthetic assimilation rate and agrees with previous determinations on the intact fruits (Todd, Bean, and Propst, unpublished results).

Illumination causes a rather large increase in activity in the albedo tissues of the intact orange (3:1 ratio as shown in table I). The difference between light and dark fixation is negligible with sections of albedo. The fact that illumination causes increased activity only in the albedo of the intact fruit and not in the albedo tissue sections would make it appear that the major part of the increase in albedo activity in the intact fruit may be due to translocation of photosynthate into the albedo from the highly active flavedo tissues. This conclusion is further supported by consideration of the activities found in individual components of the tissues (table II and discussion below).

The juice sacs show essentially no difference in total assimilation of activity in the light and the dark, whether as an intact fruit or tissue preparation (table I). There is a strong difference between assimilation in the vesicles of the intact fruit and in the isolated vesicles. This might be expected since the respiratory carbon dioxide from surrounding tissues in the intact fruit would greatly dilute the activity of the carbon dioxide reaching the vesicles. Despite showing no significant photosynthetic activity, the vesicles were capable of a very high degree of $C^{14}O_2$ fixation. The isolated juice sacs were able to assimilate from 25 to 30 per cent of the activity fixed per unit of soluble material, by the photosynthesizing, isolated flavedo. In the dark intact fruit, the unit activity of the vesicle extract is considerably higher than that of either of the two tissues which completely surround it. Comparison of the total activities fixed in extracts from different tissues of intact fruit (footnote to table I) further accents the ability of the vesicles to accumulate carbon dioxide. The vesicles in the dark were found to have fixed a total of 7.4×10^5 cpm while the flavedo only contained 6.9×10^5 cpm. These factors would suggest that the vesicles may contain a highly efficient system for exchange or dark fixation of carbon dioxide.

Table II shows some of the differences found in the fixation of activity into the individual components of the alcohol extracts of various tissues. The sugars are highly labeled in the photosynthesizing flavedo, as would be expected in the primary photosynthesizing tissue. Sucrose is found with relatively high activity in the albedo of the intact fruit but only a very small amount of activity is found in sucrose in the illuminated, isolated albedo. This tends to support the conclusion above that the major increase in activity of the albedo of the intact, photosynthesizing fruit is due to translocation of activity (in the form of sucrose) from the flavedo. A very slight amount of activity is found in sucrose in the illuminated, isolated vesicles indicating that even these tissues may be capable of a very limited amount of photosynthesis at this stage of development.

Aspartic acid, serine, and malic acid are all fairly strongly labeled in the dark. Little effect of light is found on the amount of activity in these compounds except in the photosynthesizing flavedo where malic acid and serine show fairly strong increases in the light. In other plants, it has been shown that malic

TABLE II

DISTRIBUTION OF C^{14} ACTIVITY IN COMPONENTS OF ALCOHOL-SOLUBLE EXTRACTS FROM ORANGE TISSUES [1]

	Intact fruit						Tissue preparations					
	Flavedo		Albedo		Vesicles		Flavedo		Albedo		Vesicles	
Component	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Glucose	1,020	...	...	...	...	...	1,290	...	...	...	...	...
Sucrose	10,400	...	1,140	...	...	...	9,870	...	159	...	146	...
Fructose	810	...	...	...	...	...	860	...	...	...	...	...
Alanine	100	...	5	...	+	+	330	...	+	...	...	...
Glutamic Acid	1,200	+	37	+	+	+	610	13	+	...	37	+
Aspartic Acid	+	90	10	55	275	190	160	40	35	23	182	209
Serine	180	65	45	100	200	185	120	70	135	110	146	101
Malic Acid	860	435	45	57	325	355	950	420	141	120	1,210	1,340
Citric Acid	30	...	30	52	460	630	33	20	...	...	475	445
Other Acids[2]	+	+	+	+	160	160	67	67	88	+	273	283
Phosphate Esters	+	...	...	...	...	...	87	67	...	...	30	...

[1] Activity given throughout as counts per minute per milligram of dried soluble extract. Activity was negligible or too low for measurement where no figure is given.

[2] Succinate and fumarate were lost by running off the edge of the paper. Generally they were of low activity.

acid is not directly concerned with photosynthetic assimilation of CO_2 but an increase in rate of labeling of malic acid in the light occurs as an indirect effect of photosynthesis (3) since compounds in equilibrium with malic acid are becoming labeled. Citric, glutamic, glycolic, glyceric, succinic, and fumaric acids are also labeled in the dark. The distribution and total amount of activity in these compounds varied from tissue to tissue but all the same compounds are labeled throughout, except for citric acid which seems to show some erratic behavior in the albedo and flavedo. Acid fractionations have shown that citric acid is in low concentration in peel tissues although it comprises the major acid of the vesicles at this developmental stage.

A number of factors indicate that the dark products found here must be formed within each tissue rather than by extensive translocation from one tissue to another. Each tissue, individually, is capable of fixing a significant amount of activity through dark reactions with carbon dioxide as shown by the tissue preparation data in table I. In the intact fruits, despite the increase and redistribution of activity in the flavedo caused by photosynthesis, there is virtually no effect on the activity found in the vesicles. Even in the albedo of the intact fruit the changes due to photosynthesis appear to be restricted to carbohydrate components. A highly efficient transport system would be required to build up the amount of activity found in the vesicles of the intact fruit during the dark reaction if it were assumed that the labeled compounds of the vesicles arose by reactions with carbon dioxide in the peel followed by movement into the vesicles for storage. Such transport is rather unlikely in view of the lack of apparent movement of the labeled sugars from the peel into the vesicles during photosynthesis. The high unit activity of the dark vesicle extracts in comparison with those from the other tissues also argues against such a transport effect. Determinations of the distribution of activity in the carboxyl groups of malic acid from the vesicles of intact fruits showed that the β-carboxyl contained about 75 per cent of the activity whether the fruit was photosynthesizing or in the dark. This lack of effect of light on the distribution further confirms an independent fixation in the vesicles.

With only a dark fixation mechanism in operation, the vesicles have accumulated a strikingly large amount of activity, as shown in the two tables. The high accumulation of carbon dioxide could be indicative of a possible mechanism for the formation of a portion of the acids which are stored in the endocarp of citrus fruits. In some succulent plants a net uptake of carbon dioxide may occur in the dark to give rise to an increase in organic acids (4, 12, 13, 14). Fixation of carbon dioxide in the dark appears to be a general reaction for plants (7, 8) although only a few plants carry it on to such an extent as to produce a measurable uptake. In succulents the dark uptake appears to depend upon utilization of sugars to form acceptor units. Sugars are depleted during the dark reaction (13) while the acids increase. The reaction of phospho-enol pyruvate with carbon dioxide (11) appears to be one of the reactions responsible for the dark fixation in succulents and other plants (10, 15, 16). It is also possible that carboxylation of ribulose diphosphate to form phosphoglycerate may be involved in succulent metabolism (2). In citrus vesicles a high activity of phospho-enol pyruvate carboxylase has been demonstrated by Huffaker and Wallace (6). Surrounded as they are by other tissues, the carbon dioxide concentration in citrus vesicles should be relatively high leading to favorable conditions for incorporation into the organic acids since this incorporation is directly dependent upon the partial pressure of carbon dioxide (1). These factors, with the data presented here, suggest the possibility that such reactions could be in part responsible for the rather large accumulation of acids in citrus fruit pulp.

Summary and Conclusions

A comparison has been made of amount of $C^{14}O_2$ fixed in tissues of intact, small oranges and in tissue preparations of oranges in the light and the dark. Studies were made of the compounds into which the C^{14}-activity was incorporated.

The flavedo tissue (outer green peel) was found to be capable of rapid photosynthetic fixation of carbon dioxide. The albedo (inner white peel) and the juice vesicles showed very slight photosynthetic capacities.

In the dark, the juice vesicles were found to assimilate far more activity than the other tissues, whether in the intact fruit or as isolated tissues. Malic acid, serine, aspartic acid, and citric acid were the major labeled compounds in the orange tissues in the dark reaction.

On the basis of the high dark fixation activity of the vesicles, a proposal has been made that incorporation of CO_2 may have an important function in the formation and storage of acids in the citrus pulp.

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CARBOHYDRATE METABOLISM OF CITRUS FRUITS I. MECHANISMS OF SUCROSE SYNTHESIS IN ORANGES AND LEMONS[1]

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The basis for controlling the accumulation of metabolites during growth of plants is of special interest in the study of fruit growth since the flavor and quality of the fruit may depend on the stored materials. Oranges and lemons are two fruits having wide differences in characteristic sugar storage patterns. The mature lemon contains little or no sucrose in any of its tissues whereas sucrose constitutes the major storage sugar of the orange. It would be of interest to determine if such variations are reflected in synthetic mechanisms, synthetic capacities, or in utilization of sucrose. It would also be valuable to find the stage of growth where such differences become manifest. The synthesis of sucrose in these fruits has been compared by chemical and radioisotope techniques using enzyme and tissue preparations as well as intact fruits. Results of the study suggest that differences in sucrose storage may not be due to any deficiency of sucrose synthesizing mechanisms within the lemon, since active systems were found to exist in these fruit at all stages of growth. Pronounced differences were found, however, in the synthetic capacities of different tissues of the fruit; it appears that the albedo (white peel layer) has the highest capacity for synthesis in the older fruit.

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MATERIALS AND METHODS

Sucrose synthesis was studied in three ways. A. Reaction of fructose or fructose-6-phosphate with uridine diphosphate glucose (UDPG) was determined in vitro with extracts or particulate preparations from the fruit. B. Formation of sucrose in isolated tissue preparations was traced by incorporation of activity from glucose-U-C^{14} or fructose-U-C^{14}. C. The tracer technique was also extended to examine the formation of sucrose from labeled monosaccharides in small, intact fruits. Experimental procedures and materials are given below for each approach.

I. STUDIES WITH EXTRACTS AND HOMOGENATES. Mature Eureka lemon and Valencia orange fruits were obtained fresh from local groves. The lemons and oranges were juiced by hand with a glass reamer. The lemon juice was divided immediately into two parts. One part was neutralized to pH 7 with 1 *N* NaOH before carrying out subsequent steps while the remainder was treated as initially obtained. The juices were filtered through birdseye cloth and centrifuged at approximately 25,000 × G for 20 minutes. The sediments were resuspended in two volumes of 0.01 M phosphate buffer, pH 7, and dialyzed overnight against the same buffer. Supernatants from centrifuging were saturated with ammonium sulfate at pH 5 and the precipitates were collected by centrifuging. This precipitate was redissolved in the pH 7 phos-