RESULTS AND DISCUSSIONS

The defluorination of wagnerite obtained by reacting the charge with the vapor of H_2O carried with a carrier gas proved to be independent of the nature of the carrier. The same amount of available P_2O_5 was obtained in air, oxygen, and nitrogen carriers, provided that the amount of H_2O was kept constant. The kinetics of defluorination at 800, 900, and 1000° in air are shown in Figure 2.

It can be seen that there is a negligible increase in soluble P_2O_5 at 800°, a greater one at 900°, and a maximum value is reached at 1000° after 4 hr. At the start of the defluorination process at 800 and 900°, there is a decrease in the solubility, the cause of which could not be determined. We assumed that in the first stage of the reaction, some of the free MgF_2 reacted with $Mg_3(PO_4)_2$ (a reaction which takes place at temperatures higher than 600°) and produced insoluble wagnerite before it had a chance to defluorinate, but this hypothesis was not confirmed by X-ray diffraction, nor did we find in the residue undissolved $Mg_3(PO_4)_2$.

The raw materials, the defluorinated ones and their insoluble residues, have been studied by X-ray diffraction. It can be seen that the main component of the raw MGP (Figure 3) which did not dissolve in the citric acid solution is wagnerite (Figure 4), which disappears completely after defluorination at 1000° for 4 hr (Figure 5). Fluorapatite, which can not be defluorinated at this temperature, is the main insoluble compound of the defluorinated MGP (Figure 6). The fluorapatite is not seen in Figure 3 due to the fact that it is present only in relatively small amounts which are masked by the other major components of the MGP. MgO is partially soluble in citric acid, as indicated in Figures 4 and 6.

CONCLUSIONS

Magnesium phosphate fertilizer, produced by the reaction between phosphate rock and molten carnallite, contains a large amount of wagnerite which is not soluble in 2% citric acid solution. The wagnerite can be completely defluorinated at 1000° for 4 hr in the atmosphere of a wet carrier gas. It decomposes according to the following reaction. The HF can be recovered and utilized.

$$2Mg_2PO_4F_{(s)} + H_2O_{(v)} \longrightarrow Mg_3(PO_4)_{2(s)} + MgO_{(s)} + 2HF_{(v)} \quad (5)$$

Some unreacted phosphate rock, mainly fluorapatite, makes up to 10% of the total amount of the fertilizer and is not defluorinated at 1000°. Therefore, the citric acid solubility of the calcined fertilizer is only about 90%.

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Distribution of [^{14}C]Ethylene and the Incorporation of Radiocarbon in "Valencia" Oranges after Exposure to [^{14}C]Ethylene

James F. Fisher

Externally applied [^{14}C]ethylene was found to distribute throughout the internal environment of the orange. Radiocarbon was incorporated into the ether-soluble material and fractions containing amino acids, organic acids, and the 80% ethyl alcohol-soluble carbohydrates. The greatest activity was in the ether-soluble and organic acid fractions. Citric acid was isolated and found to be radioactive.

Ethylene has been reported to incorporate in plant material such as avocado and pear tissue by Buhler *et al.* (1957) and Jansen (1963, 1964), in cotton and *coleus* plants by Hall *et al.* (1961), and in Japanese morning glory seedlings by Shimokawa *et al.* (1969). Burg and Burg (1962) reported that the internal content of ethylene in oranges increased when they were in the presence of external ethylene. Stewart and Wheaton (1972) have shown that ethylene enhances the synthesis of carotenoids in oranges. Recently, Maier *et al.* (1973) showed the specific effect of ethylene in accelerating limonoid metabolism in

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oranges. However, the literature contains little information pertaining to the internal distribution or incorporation of ethylene in the orange.

The purpose of this work was to study the qualitative fate of externally applied ethylene after it became part of the internal atmosphere of the orange.

MATERIALS AND METHODS

Distribution of [14C]Ethylene. Two mature blemish-free "Valencia" oranges, each weighing 240 ± 0.5 g with 8 cm of attached stem (stem weight not included) harvested during April, were placed in a 12-l. plexiglas chamber containing 200 ml of a 20% potassium hydroxide solution and 50 μCi (53 mCi/mmol) of [14C]ethylene (U) in a sealed glass ampoule obtained from Amersham/Searle. The ethylene was 99% radiochemically pure by gas–liquid radiochromatography on 3% squalene-on-alumina. The ampoule was crushed with a metal rod extending into the chamber. This produced an atmosphere of about 1.0 ppm of [14C]ethylene. After 4.0 hr at 27° in subdued light, the oranges were removed. One of the above oranges was selected as a control to determine the total free [14C]ethylene present. The other orange was pierced through the stylar scar into the central axis with a No. 15 hypodermic needle extending through a serum sleeve-type rubber stopper. This stopper was attached to a series of three mercuric acetate–ethylene traps described below. A slight vacuum was applied to the final trap and the central axis was evacuated for 1.0 min. The [14C]ethylene was measured as described below.

Using a potato peeler, the flavedo and albedo were successively removed from this same orange. These two fractions and the remaining endocarp, as well as the whole control orange, were all separately reduced to a fine suspension in a Waring Blendor containing 500 ml of a 10° solution of 0.25 *M* mercuric perchlorate in 2.0 *M* perchloric acid. This forms a reversible ethylene–mercury complex (Young *et al.*, 1952).

The [14C]ethylene was prepared for counting as described by Gibson (1963, 1964), with the following modifications. The contents of the Waring Blendor were transferred to a 2-l. three-necked boiling flask equipped with a magnetic stirrer, nitrogen flow system, and a 250-ml cylindrical separatory funnel. A gas outlet tube was connected to a Dry Ice–acetone trap, followed by a series of three traps, each containing 50 ml of a solution of 0.1 *M* mercuric acetate and 0.05 ml of glacial acetic acid in dry methyl alcohol. Ethylene forms a stable addition compound with this system. A gas dispersion tube was used in each trap. The traps were immersed in ice water baths. The system was flushed with nitrogen and a 10 ml/min nitrogen flow rate established. A 4.0 *N* lithium chloride solution (150 ml), which releases the ethylene, was added from the separatory funnel to the stirred 45° ethylene–mercuric perchlorate complex at such a rate as to maintain approximately 10 ml/min gas flow through the mercuric acetate–ethylene traps. A slight positive nitrogen pressure was maintained in the separatory funnel during the lithium chloride addition. The nitrogen sweep was continued for 7.0 hr.

The contents of the three mercuric acetate traps were allowed to reach 25° and, where necessary, adjusted to a volume of 50 ml by adding 1–2 ml of methyl alcohol. Five milliliters from each of the traps was separately dissolved in 14 ml of Aquasol (New England Nuclear, liquid scintillation counting cocktail) for direct counting with a Beckman liquid scintillation spectrometer (Model LS-100). The contents of the third trap were only slightly above background. The above experiments were replicated over a 2-year period.

Incorporation of Radiocarbon. *Sample Preparation.* The following experiments were replicated over a 2-year period. Five immature blemish-free "Valencia" oranges, harvested during November and December, were used. These oranges were exposed to [14C]ethylene for 3 days, as previously described.

Table I. Distribution of [14C]Ethylene in Mature "Valencia" Oranges

Fraction	Mean,[a] cpm	Standard deviation	% of control
Control (whole fruit)	269,513	13,582	
Flavedo	22,010	992	8.2
Albedo	5,923	423	2.2
Central axis	56,109	3,612	20.8
Endocarp	129,412	10,440	48.0
		Total	79.2

[a] Mean of six samples per fraction, three samples per fraction each year.

Two of these oranges were blended to a fine consistency in 1.0 l. of methylene chloride with a Waring Blendor. This suspension was filtered and the filter cake washed with methylene chloride. The methylene chloride was removed *in vacuo* at 30°. The remaining aqueous slurry was extracted with (3 × 200 ml) ethyl ether. The ether was then removed *in vacuo*, yielding the ether-soluble fraction.

Basic Amino Acids, Acidic and Neutral Amino Acids, Organic Acids, and 80% Alcohol-Soluble Carbohydrates. The remaining three oranges were extracted in a Waring Blendor with sufficient 95% ethyl alcohol to result in 80%, assuming the oranges to be 90% water. The resulting mixture was filtered and the filter cake washed with 80% ethyl alcohol. The ethyl alcohol was evaporated *in vacuo* at 30°. The aqueous residue was extracted with methylene chloride until color was no longer removed. The aqueous layer was lyophilized. This residue was subdivided into the amino acid fractions in a manner similar to Thompson *et al.* (1959) using AG 50W-X8, 200–400 mesh resin in the ammonium form to obtain the fraction containing the basic amino acids and the same resin in the hydrogen form for the fraction containing the acidic and neutral amino acids.

Organic Acids. The water effluent, from the above resin in the hydrogen form, containing the organic acids and carbohydrates was concentrated *in vacuo* at 40° to 100 ml. This solution was allowed to pass into a column of AG 1-X8, 200–400 mesh resin in the formate form. The column was then eluted until a negative Molisch test was obtained, which required 2.0 l. of deionized water. This eluate contains the carbohydrates.

The formate resin was then eluted with 2.0 l. of 6 *N* formic acid. The formic acid was removed by azeotropic distillation with water. The final aqueous solution was lyophilized, affording the organic acids.

Carbohydrates Soluble in 80% Ethyl Alcohol. The 2.0 l. of deionized water eluate from the formate column were reduced, *in vacuo* at 35°, to 100 ml and then lyophilized to give the carbohydrates.

Determination of Radioactivity Levels. An aliquot from each of the above fractions was separately combusted in a Thomas-Ogg infrared igniter according to Davidson and Oliverio (1968). A 15-ml aliquot of the CO_2 scintillation solvent was directly counted with a Beckman liquid scintillation spectrometer (Model LS-100).

Isolation and Identification of Radiolabeled Citric Acid. Citric acid was separated from the lyophilized fraction containing the organic acids on a column of AG 1-X8, 200–400 mesh resin in the formate form. Elution was carried out with increasing concentrations of formic acid (Rasmussen, 1964). The fraction containing the citric acid was determined by paper chromatography in a manner similar to Ting and Vines (1966). The authenticity of the citric acid obtained from the column was established by paper chromatography in four different solvent systems

Table II. Incorporation of Radiocarbon into Various Fractions of Immature Valencia Oranges after Exposure to [^{14}C]Ethylene

Fraction containing	Mean cpm/ 100 mg of dry tissue[a]	Standard deviation
Ether-soluble material	2320	183
Organic acids[b]	1394	136
Basic amino acids	358	38
Acidic and neutral amino acids	143	17
80% ethyl alcohol-soluble carbohydrates	451	45

[a] Mean of six samples, three samples each year. [b] Citric acid was isolated from this fraction and found to be radioactive.

and a comparison of its infrared spectrum with that of a critic acid standard. Autoradiography showed that the citric acid was radioactive.

RESULTS AND DISCUSSION

The 269,513 cpm found in the control orange (Table I) represents about 0.2% of the applied [^{14}C]ethylene (50 μCi or 1.11×10^8 cpm). The isolation of radiolabeled citric acid indicates the oranges' ability to utilize ethylene as a source of carbon. Since citric acid is synthesized in the mitochondria (Krebs cycle), it is reasonable to expect that other compounds synthesized in the mitochondria will also be labeled. This labeling is reflected in Table II, where the radioactivity in the fractions containing the various classes of compounds represents incorporation of ^{14}C from [^{14}C]ethylene.

The relative amounts of radioactivity associated with the fractions containing the organic acids and carbohydrates in Table II are in keeping with the low sugar–high acid content of "Valencia" oranges during November and December (Harding *et al.*, 1940). If ethylene enters the citric acid cycle more readily than the carbohydrate-synthesizing pathway, then this could in part explain the relative amounts of radioactivity found in the organic acid and carbohydrate fractions.

The combustion technique was necessary in order to eliminate solubility and color-quenching problems, as well as unusually long phosphorescence or chemiluminescence observed with some of the citrus samples.

Mature fruit was used in the distribution studies since it was readily available. However, immature oranges were employed for the incorporation studies to take advantage of their more active metabolism.

In a multicomponent system such as a citrus extract, some classes of compounds have similar enough properties that purification of any one fraction is a delicate and tedious process. Therefore, the various fractions in Table II did not exclusively contain the indicated class of compounds. The fractions and values are qualitative.

The low level of ^{14}C incorporation raises the possibility of experiment invalidation due to incorporation of a radiochemical impurity. However, the [^{14}C]ethylene was prepared by the reduction of [^{14}C]acetylene; therefore, the probability of $^{14}CO_2$ being an impurity is unlikely. The 1% radiochemical impurity is probably [^{14}C]ethane and unreduced [^{14}C]acetylene (Baker, 1973). The low incorporation of ethylene was not surprising, since ethylene was not expected to be a good carbon substrate. Buhler *et al.* (1957) reported incorporation of only 0.05% of the input [^{14}C]ethylene into ripe avocado and pear fruits, with the majority of ^{14}C appearing in the organic acid fractions. They did not find radiocarbon from [^{14}C]ethylene incorporated in ripe oranges. This may in part be due to their use of ripe fruit rather than young rapidly-metabolizing fruit.

This is believed to be the first published report of the incorporation in oranges of carbon from ethylene.

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