Quantitative Determination of $^{14}$C-Ethylene Produced by Plant Tissues

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Since Gane’s proof$^1$ that ethylene is produced from ripening fruits, ethylene has been identified as a normal metabolic product of a number of plant tissues and it is now established that almost all of plant tissues produce the gas. Amount of ethylene produced by plant tissues is in many cases exceedingly small, yet it has been shown that exogenous ethylene supplied in a comparable amount is capable of initiating many physiological alterations$^2,3$ such as fruit ripening, induction of epinasty and triple response of etiolated pea seedlings, and in recent years ethylene has become recognized as a plant hormone. Consequently investigations on biosynthesis of ethylene as well as regulation mechanism of its formation by plant tissues are of great importance, as those problems are still obscure. For those investigations, tracer experiments with radioactive compounds are indispensable technique. Since rate of conversion of administered labeled compounds to ethylene in plant tissues has been found to be very small in many materials,$^4,5$ quantitative collection of all the radioactive ethylene produced by the tissues and transfer of the collected gas to counting vials for the radioactivity measurement by a liquid scintillation spectrometer are essential experimental steps. So far several methods for ethylene collection have been reported,$^6,7$ but some were rather difficult to handle with long-term experiments and in others there were limitations in sample size and number.

In the course of investigation of ethylene biosynthesis by sweet potato root tissue, we have devised a simple and convenient technique which is suitable for 5 to 10 g of plant tissues and easy to treat with many samples in relatively short time.

Incubation and gas collection were carried out in gas-tight aluminum chambers of 300 ml volume which were sold for carrying juicy foods and obtainable from local markets. A gas absorption apparatus to be set in the aluminum chamber consisted of two decks of Petri dishes; a small Petri dish (5 cm in diameter) having three side arms was stacked over a large Petri dish (8 cm in diameter) and both dishes were detachable. In the upper dish containing a solution of radioactive compounds to be fed, plant materials were placed and the lower dish contained 5 ml of a mercuric perchlorate solution (0.25 M mercuric oxide in 2 M perchloric acid).$^8$ A size limit of plant materials was about 2 cm in height, when a

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A 300 ml-aluminum chamber was used. Tissue discs, slices, segments, seeds or small seedlings are particularly suitable. Five ml of a mercuric perchlorate solution in the lower dish is far enough to absorb ethylene produced by plant materials which can be placed in the upper dish, but a surface area of the solution provided by 8 cm Petri dish is necessary. The apparatus was set in the aluminum chamber together with a small vessel containing 1 ml of 20% KOH and a small cotton plug saturated with water. The chamber was then placed in a thermostat incubator set on a reciprocal shaker, which was run at 120 cycles per minute in 3 cm stroke, and a shaking during the incubation was necessary to absorb ethylene quantitatively. After an appropriate period of incubation, the mercuric perchlorate and KOH solutions were exchanged for new ones and the withdrawn solutions were processed for radioactivity measurement by a liquid scintillation spectrometer.

Quantitative transfer of labeled ethylene absorbed in mercuric perchlorate to a counting vial was carried out with a method illustrated in Fig. 1. Four ml of the mercuric perchlorate which absorbed ethylene was taken into a 10 ml-graduated centrifuge tube. The tube was stoppered with a silicone rubber stopper, cooled with ice, evacuated and then 4 M lithium chloride was introduced until the gas space in the tube reached 2 ml followed by heating with a hot water-bath. On the other hand, a counting vial containing 1 ml of 0.1 M mercuric acetate in methanol was stoppered with a silicone rubber and evacuated after cooling. The counting vial and the centrifuge tube were connected with a connector tubing of which both sides were attached with hypodermic needles. Then, air containing about 10 μl of ethylene as a carrier was slowly introduced into the centrifuge tube with a needle and a syringe up to an atmospheric pressure followed by immediate disconnection of the connector tubing. The connector tubing contained a small cotton plug moistened with 20% KOH in the middle of the tubing to eliminate a possible contamination of labeled carbon dioxide. The counting vial was allowed to stand in an ice-bath for at least 30 min with occasional shaking, then a scintillator solution developed by Shimokawa and Kasai was added. The scintillator solution composed of toluene : ethyleneglycol monoethylether : dioxyene (1 : 1 : 1 v/v) containing 0.4% PPO, 0.01% dimethyl POPOP and 10% naphthalene. The amount of ethylene was determined by taking 0.4 ml of mercuric perchlorate into 10 ml-graduated centrifuge tube followed by regeneration of ethylene as described previously.

Quantitative nature of the procedures was checked at several points. First, the rate of absorption by the absorption apparatus was

![Figure 1](image-url)
examined. A series of the incubation chambers equipped with the absorption apparatuses were added with a known amount of ethylene (100 mℓ) through an injection hole of the chamber and incubated under shaking. At every 5 min, duplicate of the chamber were opened and the mercuric perchlorate solution was withdrawn to measure the amount of ethylene absorbed. Figure 2 showed that more than 80% of added ethylene was absorbed within 5 min and a 15 min shaking incubation accomplished a complete absorption. Second, transfer of the regenerated ethylene in a centrifuge tube to a counting vial was checked with radioactive ethylene. An aliquot of a mercuric acetate containing a known amount of $^{14}$C-ethylene was taken into a centrifuge tube and ethylene was regenerated as above. As shown in Table I, the label ethylene regenerated in the centrifuge tube was quantitatively transferred to counting vials. Finally, degree of contamination of carbon dioxide in ethylene fraction was examined, as output of carbon dioxide with high radioactivity was expected when labeled compounds were fed to plant tissues. Contamination of even a small amount of radioactive carbon dioxide will lead to a fatally erroneous result. Radioactive carbon dioxide was added to the incubation chamber followed by shaking incubation for 1.5 hr and radioactivity in ethylene fraction and in KOH solution was determined. Table II indicated that the contamination was negligible and practically negligible.

### Table I. Quantitative Transfer of $^{14}$C-Ethylene from the Centrifuge Tube to a Counting Vial

<table>
<thead>
<tr>
<th>Expt</th>
<th>Ethylene added dpm</th>
<th>Ethylene recovered dpm</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>508</td>
<td>510</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>216</td>
<td>220</td>
<td>101</td>
</tr>
</tbody>
</table>

A solution of mercury-ethylene complex of a known radioactivity was placed in a centrifuge tube. $^{14}$C-Ethylene was regenerated and transferred to a counting vial containing 1 ml of 0.1 M mercuric acetate methanol solution.

### Table II. Elimination of $^{14}$CO$_2$ Contamination from Mercuric Perchlorate Solution

<table>
<thead>
<tr>
<th>Expt</th>
<th>$^{14}$CO$_2$ Added dpm</th>
<th>$^{14}$CO$_2$ Recovered dpm</th>
<th>Contamination %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45,653</td>
<td>46,821</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>47,245</td>
<td>45,408</td>
<td>0.00</td>
</tr>
</tbody>
</table>

$^{14}$CO$_2$ of a known radioactivity was injected into an incubation chamber equipped with an ethylene absorption apparatus and a small vessel containing 5 ml mercuric perchlorate solution and 1 ml of 20% KOH respectively. After a 1.5 hr incubation, mercur perchlorate and 20% KOH were collected and process for the radioactivity measurement. Contamination was expressed as the radioactivity in mercuric acetate methanol solution after the reabsorption process.

### Table III. Incorporation of U-$^{14}$C-Glucose into Ethylene in Sweet Potato Root Slices

<table>
<thead>
<tr>
<th>Incubation period (hr)</th>
<th>0~6</th>
<th>6~12</th>
<th>12~18</th>
<th>18~2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene</td>
<td>11.2</td>
<td>5.6</td>
<td>5.6</td>
<td>5.5</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>180</td>
<td>817</td>
<td>364</td>
<td>11</td>
</tr>
</tbody>
</table>
| $^{14}$CO$_2$ of a known radioactivity was injected into an incubation chamber equipped with an ethylene absorption apparatus and a small vessel containing 5 ml mercuric perchlorate solution and 1 ml of 20% KOH respectively. After a 1.5 hr incubation, mercur perchlorate and 20% KOH were collected and process for the radioactivity measurement. Contamination of even a small amount of radioactivity of carbon dioxide will lead to a fatally erroneous result. Radioactive carbon dioxide was added to the incubation chamber followed by shaking incubation for 1.5 hr and radioactivity in ethylene fraction and in KOH solution was determined. Table II indicated that the contamination was negligible and practically negligible.
the carbon dioxide was trapped in KOH solution.

With the procedures reported here, incorporation of $^{14}$C-glucose into ethylene in sweet potato root slices was studied. As shown in Table III, even if actual amount of ethylene produced was exceedingly small, incorporation of radioactivity was satisfactorily investigated. A detailed account of the study on biosynthesis of ethylene in sweet potato root tissue will be published elsewhere.¹⁰

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