How-To-Do-It

Radioactive CO_2 Fixation in *Geranium* Leaves

An Experimental Series to Show Quantitative Results

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This experiment was devised for advanced biology classes, with the students working individually on a specific factor that affects photosynthesis. Each student functions as a member of a research team investigating the various factors affecting photosynthetic rate.

The simplicity of the experiment yields quantitative data that often is lacking in the traditional methods of investigating photosynthesis. With the use of $^{14}CO_2$ a basic experimental setup is used to investigate the various factors. This apparatus was developed for a series of experiments on radioactivity by the AEC in the late 1950s. Throughout the investigation students are introduced to techniques and instrumentation that yields reproducible data.

A source of healthy leaves is essential for this series of radioactivity detections. Although *Geranium* leaves are used, many other species should yield similar results. If detached leaves are to be used, they need to be kept in water to prevent wilting.

In exposing the leaves to an atmosphere of $^{14}CO_2$, the experimenter must be protected from the radioactive atmosphere and still allow the leaves to carry on photosynthesis. In this experiment, $^{14}CO_2$ will be generated by the reaction of hydrochloric acid and C-14 labeled barium carbonate. The presence of carbon-14 will be detected by making a radiation image on X-ray film and with an end-window Geiger-Muller scaler. Because of the low-level radioactivity used, the experiments are perfectly safe providing one follows the normal safety procedures in handling radioactive materials.

The apparatus (Figure 1) is a 1-liter beaker covered with a layer of plastic wrap. The petioles of the leaves must be sealed with a thin coating of petroleum jelly. Next, the leaves are taped to a support with their jelly-sealed petioles up.

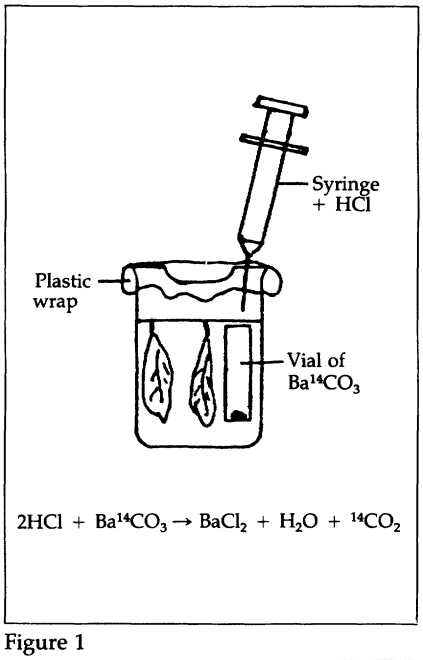

Figure 1

Experimental Procedure Using the Apparatus

Seal the petioles of the fresh leaves with petroleum jelly and secure them to the support in the beaker. Tape the uncapped vial containing 0.1 g of radioactive barium carbonate to the inside of the beaker. Cover the top of the beaker with a sheet of plastic wrap and secure it to the sides of the beaker with rubber bands or transparent tape.

Have a small piece of transparent tape handy, about 2 cm long, in order to seal the puncture hole from the syringe in the next step. Draw 3 ml of 0.1 N HCl into the syringe. Insert the needle through the plastic wrap into the vial containing the barium carbonate and slowly introduce the acid (see Figure 1). Withdraw the needle and cover the puncture hole with the tape. Allow the leaves to photosynthesize until the next laboratory period (approximately 24 hours). Illuminate the leaves with a 100-watt bulb, placed 6-12 inches above the beaker. Rinse and dry the syringe and needle.

At the next laboratory period, in order to prevent any unused $^{14}CO_2$ from escaping into the atmosphere when the beaker is opened, inject 3 ml of 0.1 N NaOH solution into the reaction vial. Withdraw the syringe and seal the hole with transparent tape. Leave the apparatus in bright illumination (the exposure period) for 5-10 minutes to absorb any remaining $^{14}CO_2$. Wash and dry the syringe and needle.

After the exposure period, wearing plastic gloves, open the apparatus and remove the leaves. Remove the residual $Ba^{14}CO_3$ solution to a waste container by wrapping the vial, plastic wrap, etc. in newspaper.

Using a small cork borer (number 6) cut discs from one of the leaves. Cut at least three discs from the leaf; do not touch the leaf with your bare hands during this operation. Take the discs and the other leaf. Sandwich them between two paper towels and place

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them between two glass plates. Place the plates in an oven at 100°C under heavy pressure for one hour or until the leaf and discs are dry. This kills the leaf tissue, fixes the labeled compound in place and makes the leaf suitably flat for autoradiography.

Next, take the dry leaf and place it in the special pocket of the X-ray film pack. Keep the leaf and film pack under heavy pressure for one week before developing the film. At the end of one week, develop the film by following the procedure outlined in the American Nuclear Products-Instruction Booklet. With the use of the easy process style film packs of American Nuclear Products no darkroom is needed for developing the film.

Place the dry discs on planchets and count each disc for two minutes with an end-window counter. Average these counts and subtract the background.

Table 1 shows typical results from two advanced biology classes for a given year. Similar results have been achieved for the past 10 years using the above procedures.

Table 1. Class data from the various experiments. The count per minute of activity are taken at the closest shelf position to the end-window Geiger-Muller tube.

Experiment	*Factor*	*Period 3* CPM	*Period 7* CPM
Light Quality	Red Light	157	251
	Orange Light	94	219
	Yellow Light	11	106
	Green Light	3	52
	Blue Light	119	207
	Violet Light	77	138
	Normal Light (sunlight)	179	239
Light Requirement	Foil Covered Surface	48	44
	Non-foil Surface	125	143
Chlorophyll vs. Non-chlorophyll	Variegated Green Region	298	230
	Variegated White Region	230	198
Stomata	Greased Lower Surface	48	56
	Greased Upper Surface	286	387
Transport	Green Region	73	88
	White Region	47	46
Light Intensity	Dark	0	0
	10 cm	159	251
	100 cm	78	96
Temperature	4°C	10	13
	30°C	169	184
	100°C	0	3
Inhibitors	Hydroxylamine	23	15
	Alcohol	0	5
	Formaldehyde	2	0
	NaF	33	42

Experiments

A. Light Quality

Cover the reaction vessel with two layers of cellophane, using the color assigned. Make sure to cover the top of the plastic wrap with the colored cellophane after injecting the acid.

An alternative method is to wrap the reaction vessel with aluminum foil, cover the top with plastic wrap and place a light filter over the top.

After exposure to C-14 the discs are cut from one leaf and dried with the other leaf. The radioactivity is then determined from the discs. An autoradiogram is produced from the other leaf.

B. Light Requirement

An easy way to demonstrate the requirement for light is to cover half of each leaf with aluminum foil. After exposing them, cut three discs from the lighted half and three from the foiled half. Dry the discs and count their radioactivity. Produce an autoradiogram from the other dry leaf.

C. Chlorophyll vs. non-chlorophyll (Variegated leaf)

This experiment demonstrates the requirement of chlorophyll in photosynthesis. Select two healthy variegated *Geranium* leaves and expose to C-14. Discs are cut from the chlorophyll region and nonchlorophyll re-

gion, three discs from each. The discs are dried and the radioactivity determined. The dry leaf is used for an autoradiogram.

D. Stomata

The experiment demonstrates the importance of stomata and their distribution between the upper and lower surfaces of the leaf. Cover the lower surface of two leaves with a thin layer of petroleum jelly. Treat the top surface of two other leaves in a similar manner. Expose the leaves in separate setups, and determine the activity as before. Wipe as much jelly as possible from the leaf before drying, then autoradiogram it, placing the ungreased surface next to the film.

E. Transport

Take two variegated leaves as in experiment C and expose to C-14 for 30-60 minutes. After exposure cut three discs from each region. Dry the discs and the other leaf. Count the activity of the discs. Produce an autoradiogram from the dry leaf. Compare results to results of experiment C.

F. Influence of Light Intensity

Place the reaction vessels at a considerable distance away from the light source into dim light (several assemblies may be used here, placed at different distances from the light source).

An assembly is kept under dark conditions, e.g., covered with a dark cloth or put into a closed cupboard.

Follow the experimental procedure, determine activity count and produce an autoradiogram.

G. Influence of Temperature

Run comparative tests (using standard lighting) with the reaction vessels in (a) crushed ice (b) water maintained at 30°C and (c) other temperatures.

Follow the experimental procedure, determine the activity count and produce an autoradiogram.

H. Influence of Inhibitors

Presoak the leaves for 30 minutes in the inhibitor assigned. Various inhibitors can be used, e.g., 0.1 M NH_2OH (hydroxylamine), 0.10 M NaF, 90% ethanol, formaldehyde, etc.

After exposure, cut discs from one leaf and dry them with the other leaf. Determine activity of the discs and produce an autoradiogram from the other leaf.

Record your measurements, plot the data as graphs where warranted, and obtain the results of experiments performed by other class members. On the basis of all information collected, draw conclusions as to how the rate of photosynthesis can be affected by given conditions. Interpret these conclusions.

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