Cellular Respiration in Germinating Peas

Investigation Manual

CAROLINA
DISTANCE LEARNING

CELLULAR RESPIRATION IN GERMINATING PEAS

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Some amount of preparation takes place 1 and 2 days before the experiment. See the Preparation section for specific preparation steps.

Overview

All organisms require captured and stored energy to fuel the demands for cellular processes that are essential for life. This laboratory activity will guide your investigation of cellular respiration using garden peas as an experimental system. A respirometer (a device that measures oxygen consumption) will be constructed and used to observe, measure, and compare the rate of cellular respiration in both germinating and dormant pea seeds. The rate of cellular respiration of the pea seeds will be compared with the respiration rate of a blank control.

Objectives

- Describe the three stages of cellular respiration.
- Explain the roles oxygen and carbon dioxide play in cellular respiration.
- Prepare and execute an experiment to observe and measure the rate of cellular respiration in both germinating and dormant pea seeds.
- Analyze data collected using a simple respirometer.

Time Requirements

- Preparation (First Day) ..... 5 minutes
- Preparation (Second Day) ..... 15 minutes
- Activity 1: Experimental System Set-Up for Cellular Respiration ..... 60 minutes
- Activity 2: Conducting the Cellular Respiration Experiment ..... 40 minutes

Key

Personal protective equipment (PPE)

goggles gloves apron

follow link to video

photograph results and submit

stopwatch required

warning

corrosion

flammable

toxic

environment

health hazard

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Background

Plants and some algae are the only organisms that can harness the energy of the sun in a process called **photosynthesis**, which synthesizes sugars and other molecules that support cellular metabolism. The sugars produced during photosynthesis are transported throughout the plant and stored in roots and seeds. How do plants acquire energy when they germinate underground, out of the reach of sunlight? The answer: they metabolize the stored sugars, much like humans do, through the process of **cellular respiration**.

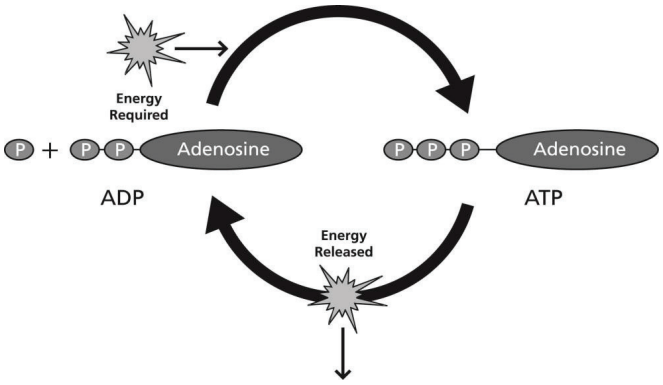
All cells need energy. Energy is contained in the chemical bonds of organic compounds such as carbohydrates, proteins, and fats. Simple carbohydrates, such as sugars, are a primary source of chemical energy. When the chemical bonds of a sugar molecule are broken down (metabolized) by specific enzymes in a series of small steps, the energy stored in those bonds is used to synthesize a molecule called **adenosine triphosphate** (ATP).

ATP is the main form of energy used by cells. The energy is stored in the chemical bonds of its three-phosphate tail. When one of these phosphate bonds is broken and the phosphate is removed from the ATP molecule to produce **adenosine diphosphate** (ADP), it releases a large amount of energy that can be harnessed to power almost all metabolic and cellular processes (Figure 1). Within each cell there is constant cycling between ATP and ADP for respiration, fermentation, and other metabolic processes.

This laboratory activity focuses on aerobic cellular respiration. The chemical reactions that occur during aerobic cellular respiration are grouped into four processes called glycolysis, acetyl-coenzyme A (acetyl-CoA) synthesis, Krebs cycle, and electron-transport chain. The complete metabolism of one sugar molecule during cellular respiration is summarized by the following equation:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 36\ ATP$$

Figure 1.



Glycolysis

Glycolysis is a series of 10 reactions that converts a 6-carbon glucose molecule into two 3-carbon molecules called **pyruvate**. Glycolysis occurs in the cell cytoplasm. Four ATP molecules and two NADH molecules are produced during glycolysis. Two ATP molecules are required to start the series of reactions; therefore, there is a net gain of two ATP molecules.

If oxygen is present, cells may undergo aerobic cellular respiration to produce more energy by metabolizing the derivatives of glucose. All higher organisms and many microorganisms have the necessary enzymes to perform aerobic cellular respiration.

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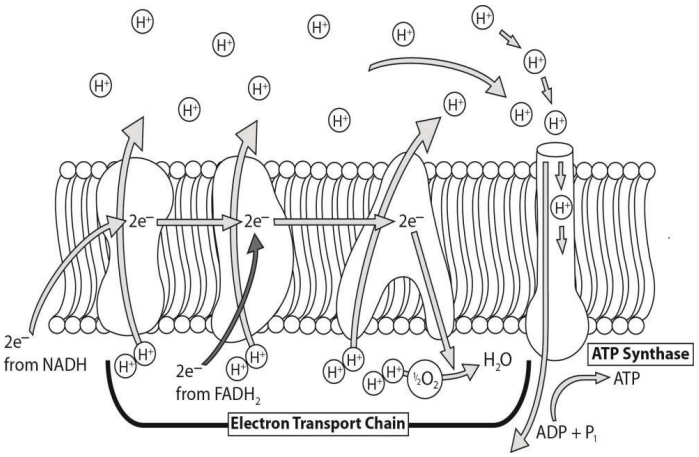
Background continued

Process	Starting Material	Net Energy Output
Glycolysis	1 Glucose	2 NADH, 2 ATP
Acetyl-CoA Synthesis and Krebs Cycle	2 Pyruvate	8 NADH, 2 $FADH_2$, 2 ATP
Electron-Transport Chain	10 NADH, 2 $FADH_2$	32 ATP

Acetyl-Coenzyme A Synthesis and Krebs Cycle

In the presence of oxygen, the pyruvate synthesized during glycolysis enters mitochondria and is initially converted into a molecule called **acetyl-CoA**. One molecule of carbon dioxide (CO_2) and one molecule of NADH are produced during the conversion of pyruvate to acetyl-CoA. The acetyl-CoA is then metabolized through a series of six reactions called the **Krebs cycle** or the citric acid cycle. Each molecule of acetyl-CoA is metabolized to produce two molecules of CO_2, and some of the released energy is used to synthesize ATP. Some of the released energy is in the form of electrons, which are transferred to the electron-carrier molecules NAD^+ and FAD to yield the reduced forms NADH and $FADH_2$. The electrons held by these electron carriers represent energy that can be used to synthesize ATP in the **electron-transport chain**.

Figure 2.



The NADH and $FADH_2$ molecules are called electron carriers because they transport electrons and associated hydrogen atoms to a series of membrane-embedded proteins called the electron-transport chain. Here, the hydrogens and electrons are stripped from the electron carriers, so NADH is oxidized to NAD^+ and $FADH_2$ is oxidized to FADH. These specialized electron-carrier molecules constantly cycle between reduced and oxidized forms within the cell.

When these electrons are removed from NADH and $FADH_2$, and transferred from one protein to the next protein, they release energy that is used to shuttle hydrogen ions into the intermembrane space of the mitochondria. As the ions accumulate, they form an ionic gradient across the membrane. This ionic gradient creates an electrochemical imbalance across the membrane that is harnessed to produce ATP. In this process, hydrogen ions are allowed to pass through a membrane channel formed by a protein called **ATP synthase**. ATP synthase

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functions somewhat like a windmill. The flow of hydrogen ions through the membrane channel causes one part of the ATP synthase complex to spin, which drives the production of ATP. In this reaction, catalyzes the phosphorylation of ADP by adding another phosphate to produce ATP (Figure 2). In eukaryotic cells, one electron donated from each NADH yields three ATP molecules, and one electron donated from each $FADH_2$ yields two ATP molecules. The electron-transport chain produces 16 ATP molecules for each pyruvate molecule. Each glucose molecule produces two pyruvate molecules. Therefore, aerobic cellular respiration of each glucose molecule produces approximately 36 ATP molecules.

Oxygen enters the equations during the final step of the electron-transport chain. When the electrons reach the last protein, they are transferred, along with hydrogen atoms, to oxygen, resulting in the formation of water (H_2O). For this reason, oxygen (O_2) is called the terminal electron carrier. The transfer of electrons to oxygen at the end of the transport chain enables more electrons to enter the start of the chain. If no oxygen is available, no more electrons can be transferred from NADH and $FADH_2$ to enter the start of the transport chain. In the absence of oxygen, the electron-transport chain and Krebs cycle cease to function, and the cell becomes starved of ATP and other organic compounds.

Using a Respirometer

This laboratory activity uses a respirometer to measure the respiration rate of germinating and dormant pea seeds (and uses beads as a blank control sample). The respirometer is composed of a vial containing the peas and a volume of air; the mouth of the vial is sealed with a one-hole rubber stopper with a pipet inserted into the hole. To perform the experiment, the entire respirometer is completely submerged in water. If the peas respire, they will use oxygen (O_2) and release carbon dioxide (CO_2). Because 1 mole of carbon dioxide is released for each mole of oxygen consumed, there is no change in the volume of gas in the respirometer. However, we can experimentally alter this equilibrium by placing a cotton ball that has been saturated with a solution of potassium hydroxide (KOH) into the bottom of the vial. Potassium hydroxide reacts with carbon dioxide to form potassium carbonate (K_2CO_3), which is a solid. The following reaction occurs:

$$\mathbf{CO_2 + 2KOH \rightarrow K_2CO_3 + H_2O}$$

Avogadro's Law: At constant temperature and pressure, 1 mole of any gas has the same volume as 1 mole of any other gas.

Inside the respirometer, solid K_2CO_3 is not observed because it dissolves within the saturated cotton ball as rapidly as it is formed. Therefore, in this experimental setup, cellular respiration consumes oxygen and produces carbon dioxide, which is removed by its reaction with potassium hydroxide, leading to a reduction in the volume of gas inside the submerged respirometer. As the volume of gas inside the vial decreases, water moves from the bath into the submerged pipet. The gas volume decrease is measured from the scale printed on the pipet, and can be used to calculate the rate of respiration.

CELLULAR RESPIRATION IN GERMINATING PEAS

Materials

Included in the materials kit:

Garden peas, ¼ lb

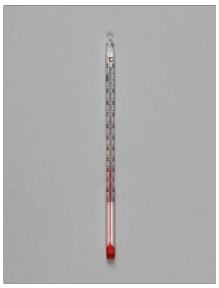
Nonabsorbent cotton

3 Glass pipets, 1 mL

3 Flat-bottom glass vials

Needed from the equipment kit:

Graduated cylinder, 100 mL



Thermometer

2 Plastic graduated pipets

30 mL Potassium hydroxide (KOH), 15%

4 Parafilm® squares, 2 × 2"

3 Rubber stoppers with holes

Forceps

200 Beads

10 Cotton balls

Needed but not supplied:

- Disposable aluminum baking pan, two 9 × 13" pans, or a single larger roasting pan (preferred)
- 3 Nickels
- Timing instrument (cell phone, stopwatch, or timer)
- Food coloring
- Plastic cup, 10 oz (for germinating seeds)
- Paper towels
- Resealable bag, sandwich size
- Scissors
- Water, nonchlorinated (bottled spring water or distilled water)

Reorder Information: Replacement supplies for Cellular Respiration in Germinating Peas investigation, item number 580118, can be ordered from Carolina Biological Supply Company.

Call 800-334-5551 to order.

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Safety

Wear your safety goggles, gloves, and apron at all times while conducting this investigation.

Read all the instructions for this laboratory activity before beginning. Follow the instructions closely and observe established laboratory safety practices, including the use of appropriate personal protective equipment (PPE) as described in the Safety and Procedure sections.

Potassium Hydroxide is a corrosive material that causes severe skin burns and eye damage. It is harmful to aquatic life. Use this material near a source of running water that can be used as a safety eye wash or safety shower if any corrosive material comes in contact with skin or eyes.

Do not eat, drink, or chew gum while performing this activity. Wash your hands with soap and water before and after performing the activity. Clean up the work area with soap and water after completing the investigation. Keep pets and children away from lab materials and equipment.

Preparation

Two days before conducting Activity 1

1. Place 50 garden pea seeds (dormant seeds) into a small plastic cup.
2. Cover the peas with nonchlorinated water to a depth of at least three times their height in the cup. This depth allows for expansion of the seeds as they swell.
3. Allow the seeds to soak overnight at room temperature.

Common tap water contains chlorine for sanitation, which may negatively affect seed germination. For best results, use bottled spring water, distilled water, filtered water (if it removes the chlorine), or add an aquarium chlorine removal agent to tap water.

One day before conducting Activity 1

1. Pour off the remaining water in the cup of hydrated pea seeds, and then place the seeds on a paper towel saturated with nonchlorinated water. Each seed should be in direct contact with the wet paper towel. Arrange the seeds on one-half of the area of the paper towel.
2. Fold the other half of the paper towel over the seeds, and then place into a resealable bag.
3. Close the bag and store the seeds in a dark place overnight.
4. Prepare a water bath: If using two 9 × 13" pans, fill them both to a depth of approximately 1½" of tap water. If using one larger roasting pan (preferred), fill it to a depth of approximately 1½" of tap water. Allow the water to stand overnight so that it comes to room temperature.

ACTIVITY

ACTIVITY 1

A Experimental System Set-Up for Cellular Respiration

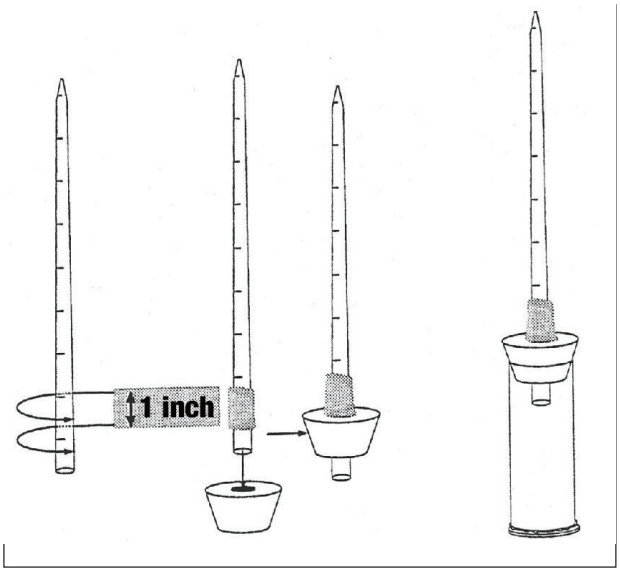
Assembling the Respirometer

The following materials are needed:

3 Flat-bottom glass vials
3 Nickels
Scissors
2 Parafilm® squares, 2 × 2"
3 Rubber stoppers with holes
3 Glass pipets, 1 mL

1. Place one nickel in the bottom of each glass vial.
2. Take two squares of Parafilm® (2 × 2"). Cut each square in half to make two 1 × 2" strips. Use one strip for each of the three respirometers.

Figure 3.



3. To assemble the top of the respirometers, gather one rubber stopper, one 1-mL glass pipet, and one strip of Parafilm®.
4. Remove the wax paper from a strip of Parafilm® and tightly wrap the strip around one of the glass pipets, approximately 1 1/2" from the blunt end.
5. Hold the pipet at the Parafilm®, and carefully insert the blunt end of the pipet through the hole in the rubber stopper. This will form an airtight seal.

Caution: Hold the pipet as close to the stopper (at the Parafilm®) as possible and push carefully. Shoving abruptly or holding the pipet too far from the stopper may cause the pipet to break, and cause cuts or puncture wounds.

Creating a Water Bath

1. Take out the water bath that was filled the previous day. The water in the bath will buffer the respirometers against temperature changes, and ensures a closed system inside the respirometers during the experiment.

Test if the seal between each stopper and pipet is airtight. After setting up the rubber stoppers and pipets, plug the top of each vial with one of the stoppers, and completely submerge the respirometer in the water bath for 20–30 seconds and check for leakage. If the contact between the stopper and the pipet or the stopper and the vial is leaking, water will begin to enter the vial. Simply tighten the stopper and/or the pipet to correct this. It is better to detect and repair any leaking respirometers at this point before the experiment has begun, to avoid having to repeat the experiment later.

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2. Place a thermometer in the water bath. Check the water temperature two or three times before starting the experiment to verify that the temperature is stable.

Setting Up the Peas and Beads

1. Add 25 mL of nonchlorinated water to the 100-mL graduated cylinder.
2. Slowly drop 25 germinating peas into the graduated cylinder.
3. Observe the final volume (V_F) after adding the peas to the graduated cylinder. Record this value in Data Table 1 for germinating peas.
4. Determine the volume of water that has been displaced, by subtracting the initial volume of water (V_i = 25 mL) from the final volume (V_F) after adding 25 germinating peas. The difference in volume is equivalent to the volume of germinating peas. Record the volume of the germinating peas in Data Table 1.
5. Remove the germinating peas and place them on a paper towel saturated with nonchlorinated water.
6. Refill the graduated cylinder with 25 mL of nonchlorinated water.
7. Slowly drop 25 dry, dormant peas into the graduated cylinder.
8. Gradually add beads to the graduated cylinder containing dormant peas so that the final volume equals the final volume measured previously for the germinating peas. Adding beads to the graduated cylinder with the smaller, dormant seeds ensures that the respirometers contain equivalent volumes at the start of the experiment.

Tap the graduated cylinder to displace any air bubbles trapped between the pea seeds and the beads during this process.

9. Remove the dormant peas and beads, and place them on a dry paper towel (keep them separate from the sample of germinating peas).
10. Record the volume of the dormant peas and beads in Data Table 1. The volume should be the same as that entered for the germinating peas.
11. Refill the graduated cylinder with 25 mL of water.
12. Gradually add beads so that the final volume equals the final volumes of the germinating peas and the dormant peas plus beads. All three groups should have equivalent volumes at the start of the experiment.
13. Remove these beads and place them on a dry paper towel (keep them separate from the sample of dormant peas plus beads).

Caution: It is essential to wear personal protective equipment for all subsequent steps of the activity. First, put on the apron, then the protective gloves, and then the safety goggles. Do not proceed to the next step without wearing this equipment.

Preparing the Samples

Caution: Avoid eye and skin contact with the potassium hydroxide (KOH) solution.

1. Place an absorbent cotton ball on top of the nickel in the bottom of each respirometer vial.

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ACTIVITY 1 continued

2. See how to use a calibrated pipet.
http://bcove.me/fpgi7b4j

   Use a plastic graduated pipet to saturate the cotton with 2.0 mL of 15% potassium hydroxide (KOH) solution. Carefully expel the solution from the pipet to avoid getting KOH on the sides of the glass vials. If KOH does end up on the sides of a vial, dry the vial with a paper towel and dispose of the paper towel in the trash. It is important that the amount of cotton and KOH solution added to the vials are the same for all three respirometers.

3. Place a small wad of dry, nonabsorbent cotton on top of the KOH-soaked absorbent cotton. This will prevent the peas from being in contact with the KOH solution, which would damage the peas and could affect the results.

4. Place the 25 germinating peas into the one of the vials.

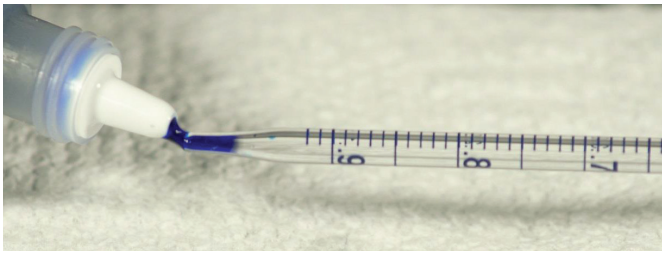
5. Place the 25 dormant peas and beads into another vial.

6. Place the equivalent volume of beads into the third vial.

7. Insert a stopper with pipet into each of the three respirometer vials. The stopper must fit tightly. If any of the respirometers leak during the experiment, the experiment will have to be started over.

8. Carefully lay the three assembled respirometers on a table or other flat surface without disturbing the moistened cotton ball and seeds, and position the tip of the respirometer pipet over a paper towel. Add some food coloring to the tip of the pipet by hanging a drop of food coloring from a food coloring bottle, so that the drop just touches the tip of the pipet, and allow capillary action to pull the drop into the pipet. The food coloring in the pipet will help you observe volume changes in the pipette during the experiment (Figure 4).

Figure 4.

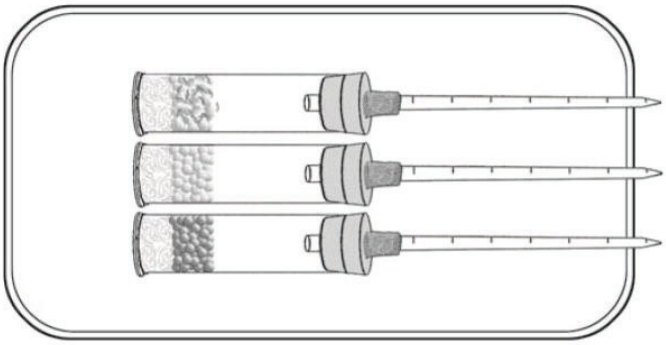


ACTIVITY 2

A Conducting the Cellular Respiration Experiment

1. Place the three respirometers into the water bath in an upright position with the pipet tips resting on the lip of the water bath (Figure 5).

Figure 5.



2. Allow the respirometers to equilibrate for 5 minutes. This will allow the internal temperature of each vial and its contents to reach thermal equilibrium with the water

continued on next page

in the bath and with each other. **If any of the respirometers begin to fill with water, there is a leak and the experiment must be started over.**

3. After the equilibration period, submerge all the respirometers (including the pipet tips) in the water bath. Position the respirometers so that the scale can be read on each of the pipets. Do not put anything into or take anything out of the water bath until all the necessary measurements have been recorded.
4. Allow the respirometers to equilibrate for another 5 minutes, or until the the dye on the respirometer containing the germinated peas has passed the zero mark on the pipet, whichever is later.
5. After these equilibrium periods, observe the initial volume reading on the pipet scale of each respirometer to the nearest 0.01 mL. Record this information in Data Table 2 for "Time 0." Observe and record the temperature of the water bath.

If the dye has not moved into the pipet sufficiently far enough to be able to record the initial volume, mark the location of the dye on the edge of the dye farthest from the tip. In subsequent steps, estimate the change in volume.

6. Take observations of the volume on the scale of each respirometer every 5 minutes for 20 minutes, and record this information in Data Table 2. Record the temperature of the water bath for each of the 5-minute intervals.
7. Use the collected data to perform the calculations necessary to complete Data Table 2.

If using two 9 x 13" pans, place two respirometers in one bath and one in the other. To submerge the respirometers, angle each so that it lays diagonally in the pan with all respirometers fully submerged.

Disposal and Cleanup

Caution: Keep all personal protective equipment on during the cleanup.

1. Take the stoppers out of the respirometers and pour the water from the vials down a sink drain. Hold the seeds and/or beads with a gloved hand to keep them from falling into the drain.
2. Rinse gloved hand, seeds, and beads with water.
3. Empty the water from the water bath into the sink and rinse the sink.
4. Discard the peas and beads in the trash.
5. Remove the nonabsorbent cotton, KOH-soaked cotton, and nickel from the vial using a gloved hand. Rinse these items well with water. Dispose of the cotton in the trash.
6. Remove the calibrated glass pipets from each stopper by holding the pipet and Parafilm® close to the stopper and carefully pulling the pipet out of the stopper.
7. Wrap the pipets and vials in newspaper, paper bags, or other sturdy paper, tape

continued on next page

the paper securely shut, and place it in the trash. Wrapping the glass pipets in paper will prevent anyone who handles the trash from cutting themselves should the glass break.

8. Dispose of gloves, stoppers, and any remaining disposables in the trash and wash hands thoroughly.

Data Table 1. Determination of Volume of Germinating Peas

Sample	Initial volume (V_i) (mL)	Final volume (V_F) (mL)	Total volume (V_F–V_i) (mL)
Germinating peas	25		
Dormant peas + beads	25		
Beads only	25		

Note: The values in each column for all three samples (germinating peas, dormant peas + beads, and beads only) will be identical. The experiment is designed to have equivalent volumes in each of the three respirometers, which is determined by (and equal to) the volume of the germinating peas.

Data Table 2. Measurement of Volume Changes During Respiration of Peas at Room Temperature

		Respirometer 1: Germinating peas			**Respirometer 2: Dormant peas + beads**			**Respirometer 3: Beads only**	
Water temp. (°C)	Time (min)	Volume in pipet	Change in volume	Corrected volume change	Volume in pipet	Change in volume	Corrected volume change	Volume in pipet	Change in volume
	0		–	–		–	–		–
	5								
	10								
	15								
	20								

The volume in the pipet is affected by ambient air pressure. In order to correct for any pressure changes that occur during the experiment, the change in volume in Respirometer 3 (the control) is subtracted from the change in volume in respirometers 1 and 2.

Change in volume = (Volume at time 0) – (Volume at time of current reading)

Corrected change in volume = [Change in volume (for Respirometer 1 or Respirometer 2)] – (Change in volume of Respirometer 3)

NOTES

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