CAROLINA

Student Guide

Name/Group # _____

Date _____

AP® Biology Laboratory 5
Cell Respiration

Objectives

- Measure the consumption of oxygen by respiring seeds
- Compare respiration rates at two different temperatures

Background

Many cellular processes require energy. Aerobic cellular respiration supplies energy by the oxidation of glucose. This is a complex process involving a number of enzyme-mediated reactions; however, we can summarize the process in terms of input and output products with a very simple equation:

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

Introduction

You will use a respirometer to measure the rate of respiration of germinating and nongerminating pea seeds at two different temperatures. The respirometer consists of a vial that contains the peas and a volume of air. The mouth of the vial is sealed with a rubber 1-hole stopper that has a pipet inserted in it. The respirometer is submerged in water. If the peas are respiring, they will use oxygen and release carbon dioxide. Since 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. (**Avogadro's Law:** At constant temperature and pressure, 1 mole of any gas has the same volume as 1 mole of any other gas.) You will alter this equilibrium by placing a solution of potassium hydroxide (KOH) in the vial. Potassium hydroxide reacts with carbon dioxide to form potassium carbonate, which is a solid.

$CO_2 + 2\ KOH \rightarrow K_2CO_3 + H_2O$

Since the carbon dioxide produced is removed by reaction with potassium hydroxide, as oxygen is used by cellular respiration the volume of gas in the respirometer will decrease. As the volume of gas decreases, water will move into the pipet. You will use this decrease of volume, as read from the scale printed on the pipet, as a measure of the rate of cellular respiration.

Measuring Respiration of Germinating and Nongerminating Peas

Materials

Room-temperature water bath, cold water bath, container of ice, paper (white or lined), water, germinating peas, nongerminating peas, glass beads, respirometers, graduated tube, absorbent cotton balls, nonabsorbent cotton, 15% potassium hydroxide (KOH) solution, dropping pipets, forceps, thermometers, stopwatch or timer or clock with second hand, calculators (optional).

Procedure

Setup of Respirometers and Water Baths

You will use two water baths (trays of water) to buffer the respirometers against temperature change and to provide two temperatures for testing: room temperature and a colder temperature (approximately 10°C). Place a sheet of paper in the bottom of each water bath. This will make the graduated pipet easier to read. Next, place a thermometer in each tray. If necessary, add ice to the cold-temperature tray to further cool the water to get it as close to 10°C as possible. While waiting for the cold-water temperature to stabilize at 10°C, two of you should prepare three respirometers to test at room temperature, and two of you should prepare an identical set of three respirometers to test at the colder temperature.

Preparing Peas and Glass Beads

You will need a set of peas and/or beads for testing at each temperature.

Respirometer 1: Put 25 mL of H_2O in your 50-mL graduated plastic tube. Drop in 25 germinating peas. Determine the volume of water that is displaced (equivalent to the volume of peas). Record the volume of the 25 germinating peas. Remove these peas and place them on a paper towel.

Respirometer 2: Refill the graduated tube to 25 mL with H_2O. Drop 25 dry, nongerminating peas into the graduated cylinder. Next, add enough glass beads to equal the volume of the germinating peas. Remove the nongerminating peas and beads and place them on a paper towel.

Respirometer 3: Refill the graduated tube to 25 mL with of H_2O. Add enough glass beads to equal the volume of the germinating peas. Remove these beads and place them on a paper towel.

Respirometer Assembly

You will need three respirometers for room-temperature testing and three respirometers for cold-temperature testing.

To assemble a respirometer, place an absorbent cotton ball in the bottom of each respirometer vial. Use a dropping pipet to saturate the cotton with 2 mL of 15% KOH. (**Caution:** *Avoid skin contact with KOH. Be certain that the respirometer vials are dry on the inside. Do not get KOH on the sides of the respirometer.*) Place a small wad of dry, nonabsorbent cotton on top of the KOH-soaked absorbent cotton. The nonabsorbent cotton will prevent the KOH solution from contacting the peas. It is important that the amounts of cotton and KOH solution be the same for each respirometer.

- Place 25 germinating peas in your respirometer vial(s) 1.
- Place 25 dry peas and beads in your respirometer vial(s) 2.
- Place beads only in your respirometer vial(s) 3.

Insert a stopper fitted with a calibrated pipet into each respirometer vial. The stopper must fit tightly. If the respirometers leak during the experiment, you will have to start over.

Placement of Respirometers in Water Baths

Place a set of respirometers (1, 2, and 3) in each water bath with their pipet tips resting on one lip of the tray. See Figure 1. Wait five minutes before proceeding. This is to allow time for the respirometers to reach thermal equilibrium with the water. If any of the respirometers begins to fill with water, you have a leak and must start over.

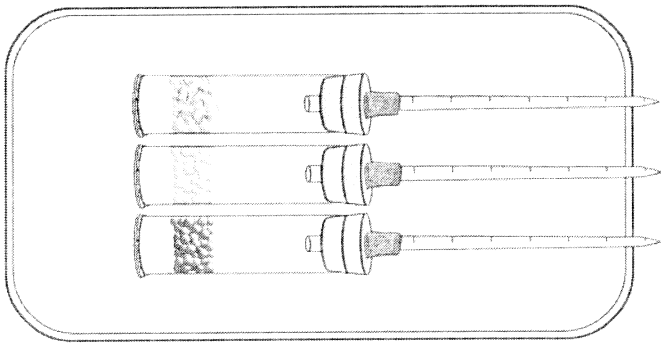


Figure 1. Respirometers in the water bath.

After the equilibration period, immerse all respirometers (including pipet tips) in the water bath. Position the respirometers so that you can read the scales on the pipets. The paper should be under the pipets to make reading them easier. Do not put anything else into the water bath or take anything out until all readings have been completed.

Take Readings

Allow the respirometers to equilibrate for another five minutes. Then, observe the initial volume reading on the scale to the nearest 0.01 mL. Record the data in Table 1 for Time 0. Also, observe and record the temperature. Repeat your observations and record them every five minutes for 20 minutes.

Table 1: Respiration of Peas at Room Temperature

		Respirometer 1 Germinating Peas			Respirometer 2 Dry Peas + Beads			Respirometer 3 Beads Only	
°C	Time (Min)	V of Pipet	ΔV	Corrected ΔV	V of Pipet	ΔV	Corrected ΔV	V of Pipet	ΔV
	0		–	–		–	–		–
	5								
	10								
	15								
	20								

ΔV = V at Time 0 – V at time of current reading
Corrected ΔV = ΔV (for Respirometer 1 or Respirometer 2) – ΔV of Respirometer 3

Table 2: Respiration of Peas at Colder Temperature

		Respirometer 1 Germinating Peas			Respirometer 2 Dry Peas + Beads			Respirometer 3 Beads Only	
°C	Time (Min)	V of Pipet	ΔV	Corrected ΔV	V of Pipet	ΔV	Corrected ΔV	V of Pipet	ΔV
	0		–	–		–	–		–
	5								
	10								
	15								
	20								

ΔV = V at Time 0 – V at time of current reading
Corrected ΔV = ΔV (for Respirometer 1 or Respirometer 2) – ΔV of Respirometer 3

Analysis of Results: Measuring Respiration of Germinating and Nongerminating Peas

Graph the data for respirometers 1 and 2 from your group's tables. Title the graph and supply the following information:

a. The independent variable is _____.

b. The dependent variable is _____.

Plot the independent variable on the x-axis, and the dependent variable on the y-axis. Label each plotted line.

1. Write two hypotheses that this experiment is designed to test.

_____

_____

_____

_____

_____

_____

2. In this experiment, you measured the change in volume of the gas inside the respirometers. The *general gas law* describes the state of a gas under given conditions:

   $pV = nRT$

   where p = pressure of the gas
   V = volume of the gas
   n = kmoles (number of molecules) of gas
   R = universal gas constant [8314 joules/(kmole)(K)]
   T = temperature of the gas in K

Since you have been measuring changes in volume, restate the general gas law to solve for volume:

$$V = \frac{nRT}{p}$$

Using the general gas law and your experience in this lab, give the variables that had to be controlled for your data to be valid. State the controls used for each variable and any means used to correct for the influence of a variable(s).

3. Which of the respirometers (1, 2, or 3) serves as a negative control? Explain your answer.

4. In reference to the general gas law, and assuming your control measures worked, a change to which of the variables led to the observed change in volume (Corrected ΔV in Tables 1 and 2)? Explain your answer.

5. Using your graph and data tables, summarize your findings, comparing results from respirometers 1 and 2, and results obtained at room temperature vs. results at the colder temperature. Speculate as to the cause(s) of any differences between the treatments.

6. From your graph, calculate the rate of oxygen consumption for each treatment:

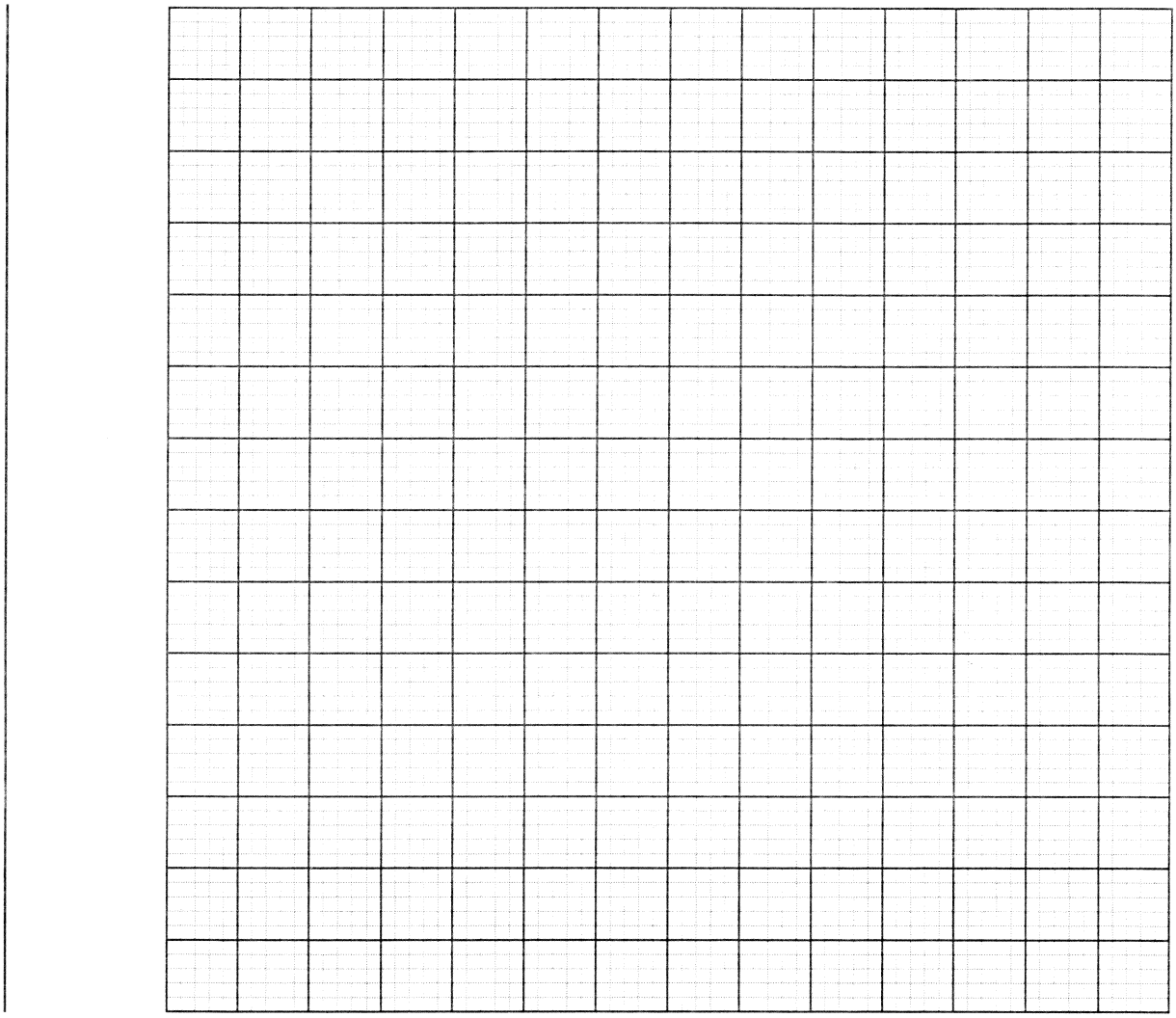
   a. germinating seeds at room temperature = _____ mL/min

   b. germinating seeds at colder temperature = _____ mL/min

   c. dry seeds at room temperature = _____ mL/min

   d. dry seeds at colder temperature = _____ mL/min

Title: _____



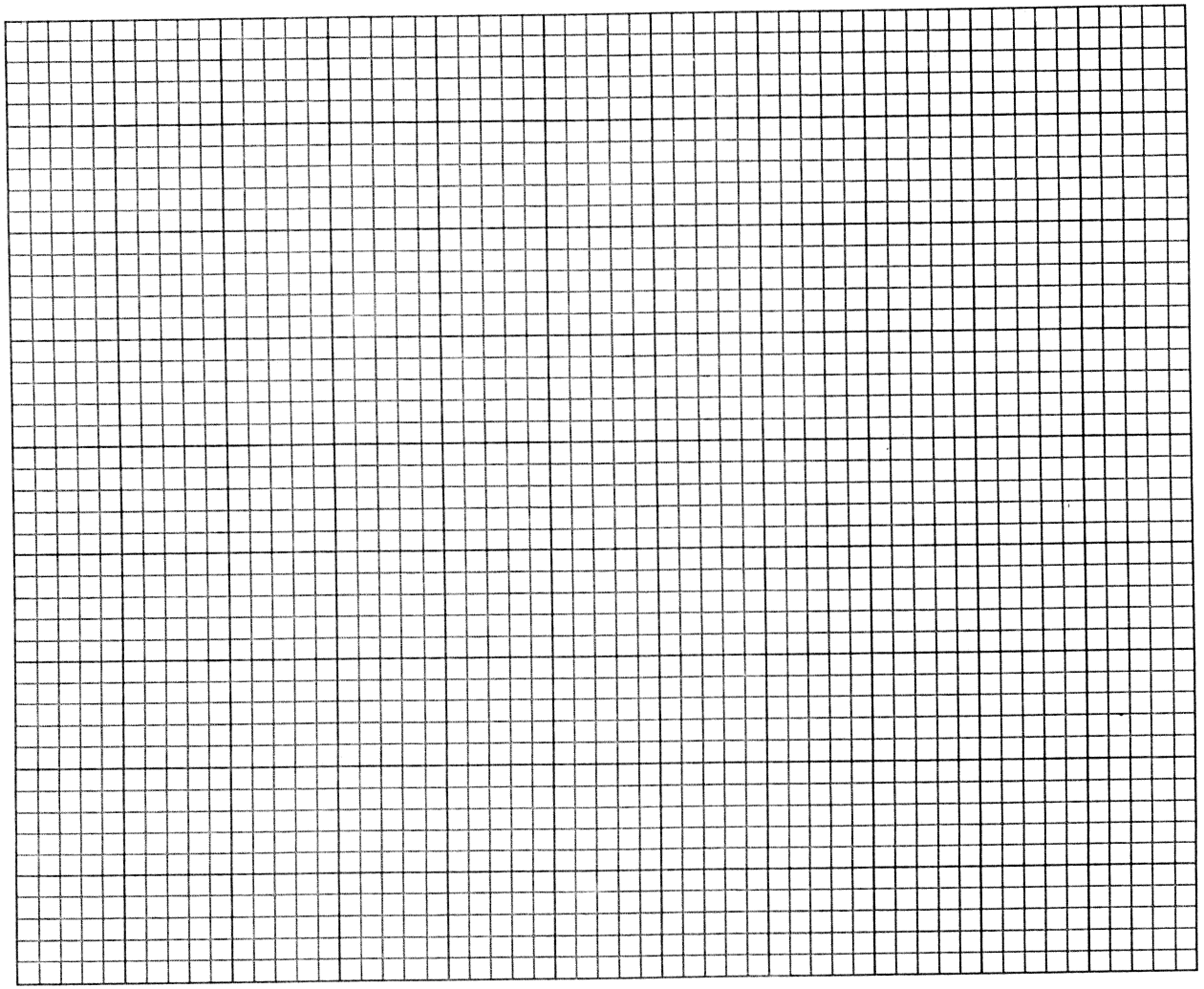
Blackline Master

Analysis of Results

Name _____

Date _____

Graph the results from the corrected difference column for the germinating peas and dry peas at both room temperature and at 10°C. Place *Time in minutes* on the x-axis and *mL oxygen consumed* on the y-axis.



Preparation and Presentation

Photocopy the blackline master Student Guide for each student or group of students. Photocopy the graph template at the end of the Student Guide as needed.

Respirometer Assembly

Assemble the respirometers at least two hours before class begins. You will need these materials: metal washers, adhesive, flat-bottomed glass vials, scissors, Parafilm®, rubber stoppers, and glass pipets. Glue a metal washer to the bottom of each of the flat-bottomed glass vials (see Illustration 1a, below). Silicone adhesive is supplied in the kit.

Cut the large piece of Parafilm® supplied in the kit into 2" × 2" (4 in^2) pieces. Then, cut each square into two 2" × 1" strips. You will need one 2" × 1" strip of Parafilm® for each respirometer, and each student group needs six respirometers.

For each respirometer top, gather one rubber stopper, one 1-mL glass pipet, and one strip of Parafilm®. To assemble each top, wrap a strip of Parafilm® tightly around a glass pipet, approximately 1–1 1/2 inches from the blunt end of the pipet (see Illustration 1b, below). Next, hold the pipet at the Parafilm® and carefully insert the blunt end of the pipet through the hole in the rubber stopper until an airtight seal is formed. **Caution:** *Hold the pipet close to the stopper and push carefully. Shoving abruptly or holding the pipet too far from the stopper may break the pipet and risks stabbing broken glass into one's hand.*

Instruction Note: You may wish to have your students prepare the upper portion of the respirometers, but be aware of the safety issue noted above. Allow students to assemble the respirometer tops only if you are certain they will use caution.

The final assembly should resemble Illustration 1c, below.

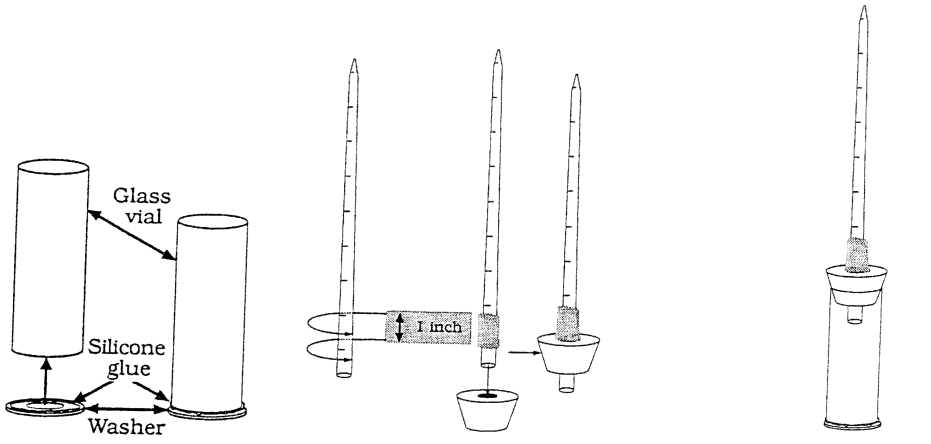


Illustration 1a **Illustration 1b** **Illustration 1c**

Illustration 1. Respirometer Assembly

Germinating Seeds

Both germinating and nongerminating peas are needed for the activity. Place the peas that are to be germinated into a cup or beaker. Cover them with water to a depth at least three times their height in the container. This compensates for the expansion of the peas as they swell. If possible, use dechlorinated water. Allow the peas to soak overnight. Once this is done, pour off any remaining water and place the peas on wet paper towels. Completely cover the peas with wet paper towels and place them in a plastic bag. Close the bag and store it in a warm, dark place over a second night. After this treatment, examine the peas. At least some of them should possess ruptured seed coats from extension of the hypocotyl. If not, return the peas to the bag for a third night before using.

Setting Up Water Baths

In the activity, students compare the respiration rates of seeds that are at room temperature to the respiration rates of seeds that are below room temperature. At least two hours before the activity (overnight is better) set out the plastic trays and fill them about ¾ full with water. The water must be deep enough to completely submerge the respirometers. Keep all materials (peas, glass beads, respirometers, etc.) in the same area of the classroom to insure that everything is at the same temperature when the activity begins. The room-temperature water baths need no further preparation, but the water in the cold-water baths will need to be chilled. Fifteen minutes before the lab, add ice to each of these baths. Use thermometers to check their temperature and stir the water once or twice to help with mixing. The AP® lab manual specifies a temperature of 10°C for these water baths, but do not be concerned if you cannot consistently obtain (or maintain) that exact temperature. The cold-water baths should be roughly 10–15°C colder than the room-temperature baths.

Station Setup

Following is a list of the materials needed for one group of students to perform the activity in this lab. Prepare as many setups as needed for your class.

2 water baths (room temperature and cold water)
50 germinating pea seeds
50 dry pea seeds
100 glass beads (approx.)
6 respirometers
50-mL graduated plastic tube (for measuring peas)
6 absorbent cotton balls
nonabsorbent cotton
15% KOH solution, 15 mL (approx.)
dropping pipet
forceps
*thermometer, °C
*container of ice
*stopwatch, timer, or clock with second hand
*2 sheets of ~8.5 × 11-in. white paper or lined notebook paper
*calculator (optional)
*food coloring (optional)

*Not supplied.

Troubleshooting

The respirometers must be airtight except for the pipet tips; otherwise, the data collected will be variable and useless. Check the students' respirometers to be certain that the stoppers are firmly seated. If you suspect leakage, wrap the stopper with Parafilm® and have the group start over.

Give the respirometers time to reach equilibrium in the water bath. The times given in the Student Guide to reach equilibrium may not be sufficient in all cases. It is important not to rush this lab; you can extend the equilibration time as needed, on the basis of your experience. If a group records negative values after correcting their data with readings from Respirometer 3, or if a group's data shows large up-and-down variations, suspect problems with thermal equilibrium.

Respirometers are extremely sensitive. Advise students not to bump the lab table and instruct them that, once the respirometers have reached equilibrium, the instruments should not be touched or moved. Students should keep their hands out of the water bath. Once the respirometers have been placed in the water bath, nothing else should be added to or taken out of the bath. No attempt should be made to adjust the water temperature after the respirometers are placed in the water bath.

Students may accidentally disturb the respirometers while taking temperature readings. Use a thermometer clamp, or position the thermometer in the water bath so that it can be read without being touched.

Do not try to simplify this lab by leaving out the respirometers containing glass beads. The readings taken from these respirometers are essential for correcting the readings of the other respirometers. Also, large or erratic changes in these respirometers may indicate problems with the general setup.

It can be difficult to see the water/air interface when taking readings from the pipets. To help with this, some teachers have their students touch a drop of food coloring to the tips of the pipets just before immersing them.

Finally, the respiration rate of the germinating peas is variable. If you begin the germination process too near the beginning of the lab, respiration may be so low that it is difficult to distinguish the data from the germinating and the nongerminating peas. If the peas are too active, the volume of the pipets may be exhausted before all readings can be taken. If possible, perform a trial run of the germinating peas an hour or so before the lab is to begin. You can then have your students use more or fewer than 25 peas, as needed. To minimize these problems, follow the recommended schedule of preparing the peas for germination two days before the lab. If you have saved peas from a previous year, test them before using them for the lab.

Alternative Setup of Respirometers

Some teachers report success replacing the plastic-tray water baths with large, insulated coffee cups. The respirometers sit vertically, washer-end-down, in the cups, which contain either room-temperature water or 10°C water. After equilibrium, students touch one or two drops of food coloring to the tip of each pipet. This approach may make taking readings from the pipets somewhat easier; the cups provide insulation, which helps maintain water temperature. The major disadvantage of this setup is that the cups can easily be overturned. (**Note:** Cups and food coloring not supplied.)