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.

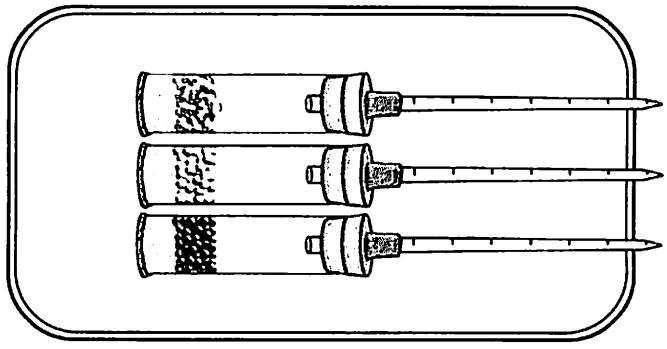


Figure 1. Respirometers in the water bath.

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

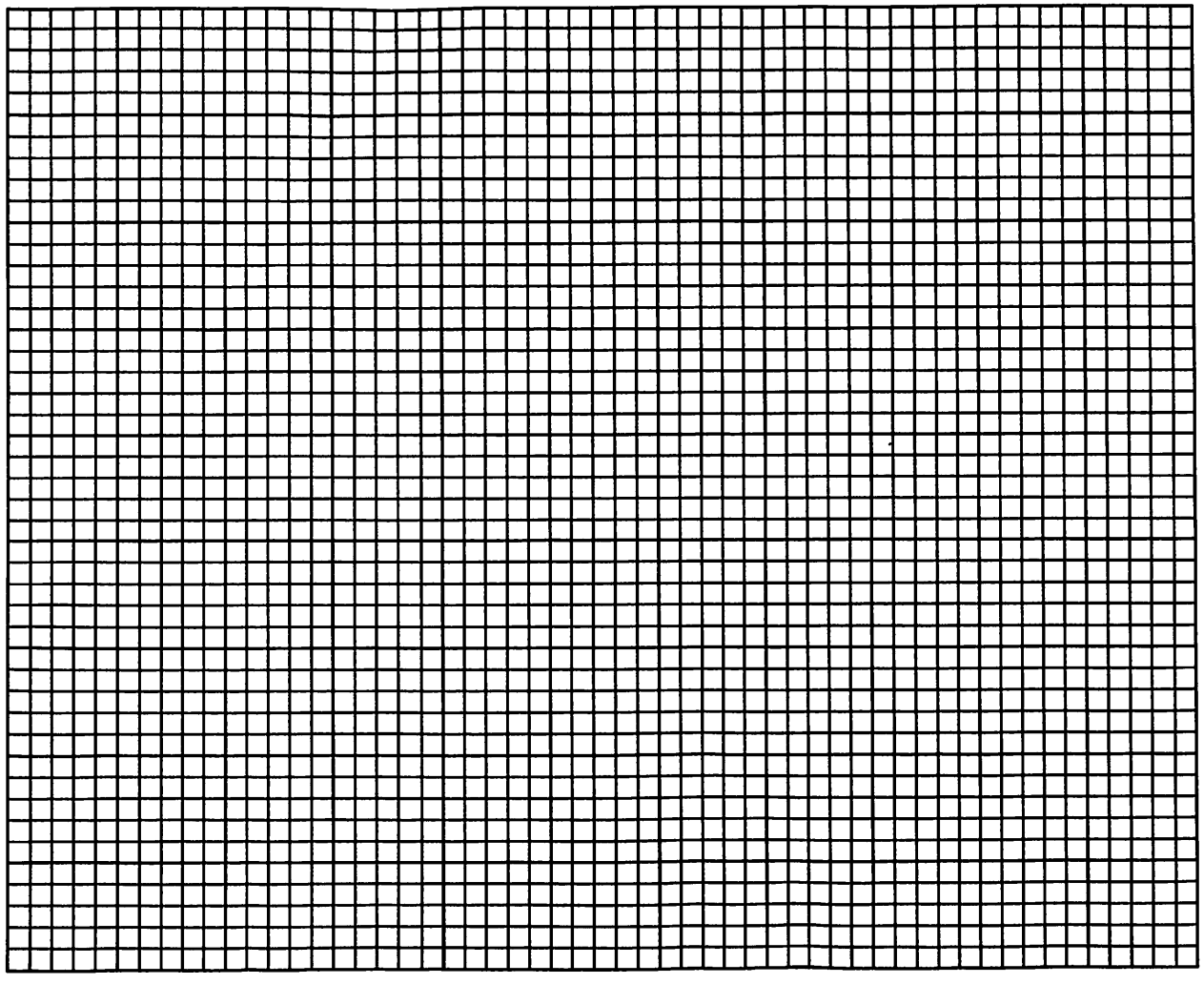
Blackline Master

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.



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