LABORATORY 5. CELL RESPIRATION

Objectives

In this laboratory, you will

- measure oxygen consumption during respiration as the change in gas volume in respirometers containing either germinating or nongerminating peas
- measure the respiration of these peas at two different temperatures

Required Knowledge

Before beginning this laboratory, you should understand

- how a respirometer works in terms of the gas laws
- the general processes of metabolism in living organisms

Expectations

At the completion of this laboratory, you should be able to

- test the effects of temperature on the rate of cell respiration in ungerminated vs. germinated seeds in a controlled experiment
- calculate the rate of cell respiration from experimental data
- relate gas production to respiration rate

Background

Aerobic cellular respiration is the release of energy from organic compounds by metabolic chemical oxidation in the mitochondria within each cell. The equation below shows the beginning and end products of the oxidation of glucose. Many enzyme-mediated reactions occur between the left and right sides of this equation.

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ + 586 kilocalories of energy/mole of glucose oxidized

The chemical oxidation of glucose has important implications in relation to the measurement of respiration. One could measure the

1. consumption of O_2 during the oxidation of glucose. (How many moles of O_2 are consumed when 1 mole of glucose is oxidized?)
2. production of CO_2 during aerobic respiration. (How many moles of CO_2 are produced when 1 mole of glucose is oxidized?)
3. release of energy in the form of heat as 1 mole of glucose is oxidized.

In this experiment, you will measure the relative volume of O_2 consumed by germinating and nongerminating (dry) peas at two different temperatures.

The Gas Law and the Design of the Exercise

Introduction

A number of physical laws relating to gases are important to the understanding of how the apparatus used in this exercise works. The laws are summarized in the general gas law that states:

$$PV = nRT$$

where

P = pressure of the gas

V = volume of the gas

n = number of molecules of gas

R = the gas constant (its value is fixed)

T = temperature of the gas

This law implies the following important concepts about gases.

1. If temperature and pressure remain constant, then the volume of the gas is directly proportional to the number of molecules of the gas.
2. If the temperature and volume remain constant, then the pressure of the gas changes in direct proportion to changes in the number of molecules of gas present.
3. If the number of gas molecules and the temperature remain constant, then the pressure is inversely proportional to the volume.
4. If the temperature changes and the number of gas molecules remain constant, then either pressure or volume (or both) will change in direct proportion to the temperature.
5. Gases and fluids flow from regions of high pressure to regions of low pressure.

As oxygen gas is consumed during respiration, it is normally replaced by CO_2 gas at a ratio of one molecule of CO_2 for each molecule of O_2. Thus, you would expect no change in gas volume to result from this reaction. However, in this experiment, the CO_2 produced during cellular respiration is removed. The respirometers (Figures 5.1 and 5.2) contain potassium hydroxide (KOH), which reacts with CO_2 to form solid potassium carbonate (K_2CO_3) through the following reaction:

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

As the seeds consume oxygen by respiration, the number of molecules of gas (n) in the respirometer decreases. The change in the number of molecules is directly related to the amount of oxygen consumed. Assuming that temperature and atmospheric pressure remain constant, then as n decreases, V will decrease (Concept 1, above.) As the volume of gas inside the respirometer decreases, water will move into the pipet of the respirometer. Therefore, the movement of water into the pipet is a measure of the volume of gaseous O_2 consumed by the respiring seeds. Set up six respirometers as described in the following section.

Procedure

1. Both a room-temperature bath (approximately 25°C) and a 10°C bath should be set up immediately to allow for time to adjust the temperature of each. Place a piece of white paper in the bottom of each water bath. This will make the graduated pipet easier to read. Add ice to adjust the temperature of the second bath to 10°C.
2. While the baths are equilibrating, put 25 mL of H_2O in your 50-mL graduated plastic tube. Drop in 25 germinating peas and determine the volume of water that is displaced (equivalent to the volume of the peas). Record the volume of the 25 germinating peas. Remove these peas and place them on a paper towel. They will be used in Respirometer 1.
3. Refill the graduated tube with 25 mL of H_2O. Drop 25 dried peas (not germinating) into the graduated cylinder and then add enough glass beads to attain a volume equivalent to that of the expanded germinating peas. Remove these peas and beads and place them on a paper towel. They will be used in Respirometer 2.
4. Refill the graduated tube with 25 mL of H_2O. Add enough glass beads to attain a volume equivalent to that of the germinating peas. Remove these glass beads and place them on a paper towel. They will be used in Respirometer 3.

5. Repeat the procedures above to prepare a second set of germinating peas, dry peas, dry peas plus beads, and glass beads for use in respirometers 4, 5, and 6, respectively.

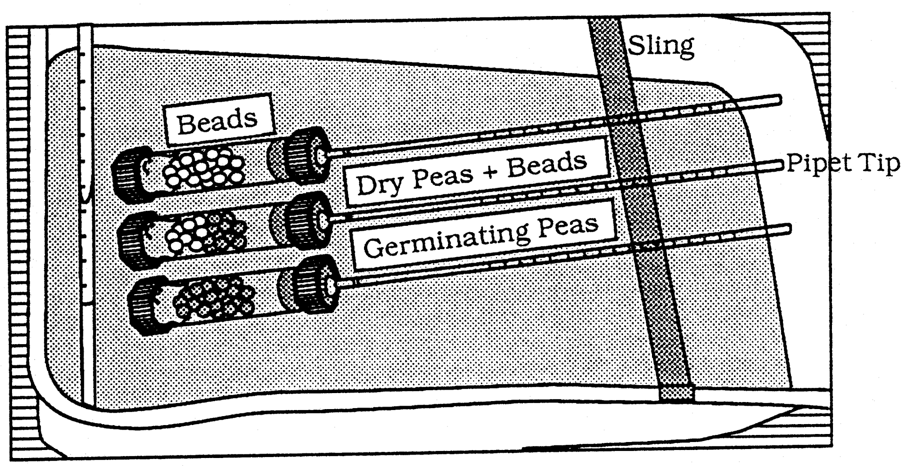
6. To assemble the six respirometers, obtain six weighted vials, each with an attached stopper and pipet. Place an absorbent cotton ball in the bottom of each vial. Using a plastic transfer pipet, saturate the cotton with 15% KOH (2–3 mL). **Caution:** Avoid skin contact with the KOH. Be certain that 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 from getting on the peas. It is important that the amounts of cotton and KOH be the same for each respirometer.

Figure 5.1: Respirometers and Contents

Respirometer	Temperature	Contents
1	Room	Germinating seeds
2	Room	Dry Seeds + Beads
3	Room	Beads
4	10°C	Germinating Seeds
5	10°C	Dry Seeds + Beads
6	10°C	Beads

7. Place the first set of germinating peas, dry peas plus beads, and beads in vials 1, 2, and 3, respectively. Place the second set of germinating peas, dry peas plus beads, and glass beads in vials 4, 5, and 6, respectively. Insert the stoppers fitted with the calibrated pipets into the vials. The stoppers must fit tightly. If the respirometers leak during the experiment, you must start over.

Figure 5.2: Respirometers in the Water Bath



8. Make a “sling” of masking tape, attached to each side of each of the water baths, to hold the pipet tips out of the water during an equilibration period of seven minutes. Make sure that the white paper is placed so that it will be under the pipets.

9. Place vials 1, 2, and 3 in the room-temperature water bath (approximately 25°C) with the pipet tips resting on the sling. Place vials 4, 5, and 6 in the 10°C water bath in the same manner. Allow the respirometers to equilibrate in the baths for seven minutes.

10. After the equilibration period, immerse all six respirometers **entirely** in their respective water baths. Water will enter the pipets for a short distance and then stop. If the water continues to move into a pipet, check for leaks in the respirometer. Work swiftly to arrange the pipets on the white paper so that they can be read through the water at the beginning of the experiment. Do not shift the pipets during the experiment. Keep your hands out of the water bath after the experiment has started. Maintain the water bath at a constant temperature.

11. Allow the respirometers to equilibrate for three more minutes and then record, to the nearest 0.01 mL, the initial position of water in each pipet (initial time 0). Check the temperature in both baths and record it in Table 5.1. Every five minutes for 20 minutes, take readings of the water's position in each pipet. Record the data in Table 5.1.

Table 5.1 Measurement of O_2 Consumption by Soaked Dry Pea Seeds at Room Temperature (25°C) and 10°C Using Volumetric Methods

	ACTUAL TEMP (°C)	TIME (MIN)	Beads Alone		Germinating Peas			Dry Peas and Beads		
			Reading at Time X	Diff.*	Reading at Time X	Diff.*	Corrected diff.Δ	Reading at Time X	Diff.*	Corrected diff.Δ
ROOM TEMP		Initial:0								
		0–5								
		0–10								
		0–15								
		0–20								
LOW TEMP		Initial:0								
		0–5								
		0–10								
		0–15								
		0–20								

* Difference = (initial reading at Time 0) – (reading at Time X)

Δ Corrected difference = Difference germinating peas (or dry peas and beads) at Time X – Difference beads alone at Time X.

Questions

1. State two hypotheses that you will test by performing this activity.

a. ____.

b. ____.

2. Graph the results from the corrected difference column for the germinating peas and dry seeds at both room temperature and at 10°C.

a. The independent variable is ______________________________.
(Use to label the horizontal x-axis.)

b. The dependent variable is ________________________________.
(Use to label the vertical y-axis.)

Graph 5.1

Title: ____

Label (y-axis): ____________

Label (x-axis): ______________________________

3. From Graph 5.1, describe the relationship between the amount of O_2 consumed and time for the germinating seeds at room temperature.

____

____

____

____

____

____

4. From the slope of the four lines on Graph 5.1, and using your data from Table 5.1, determine the rate of O_2 consumption of germinating and dry peas during the experiments at room temperature and at l0°C. Show calculations and rates in Table 5.2.

Show your formula here: **Rate of O_2 Consumption =** _______________

Table 5.2

Condition	Calculation	Rate
Germinating Peas Room Temperature		
Germinating Peas 10°C		
Non-germinating Peas Room Temperature		
Non-germinating Peas 10°C		

5. Why was it necessary to correct the readings of respirometers with peas with the readings taken from respirometers with glass beads only? Your answer should refer to the concepts derived from the general gas law and your answers to Question 1, above.

6. What was the effect of germination vs. non-germination on pea seed respiration?

7. What was the effect of temperature on pea seed respiration?

8. Imagine that you are given 25 germinating pea seeds that have been placed in boiling water for five minutes. You place these seeds in a respirometer and collect data as before. Predict the rate of oxygen consumption for these seeds and state your reasons.

9. Imagine that you are asked to use respirometers to measure the rate of oxygen consumption for a 25-g reptile and a 25-g mammal at 10°C. Predict how the results would compare.

10. Imagine that you are asked to repeat the reptile/mammal comparison of oxygen consumption, but at a temperature of 22°C. Predict how these results would differ from the measurements made at 10°C.

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