CELLULAR RESPIRATION

■INTRODUCTION

Living systems require free energy and matter to maintain order, to grow, and to reproduce. Energy deficiencies are not only detrimental to individual organisms, but they cause disruptions at the population and ecosystem levels as well. Organisms employ various strategies that have been conserved through evolution to capture, use, and store free energy. Autotrophic organisms capture free energy from the environment through photosynthesis and chemosynthesis, whereas heterotrophic organisms harvest free energy from carbon compounds produced by other organisms. The process of cellular respiration harvests the energy in carbon compounds to produce ATP that powers most of the vital cellular processes. In eukaryotes, respiration occurs in the mitochondria within cells.

If sufficient oxygen is available, glucose may be oxidized completely in a series of enzyme-mediated steps, as summarized by the following reaction:

$$C_6H_{12}O_6 + 6O_2(g) \rightarrow 6CO_2(g) + 6H_2O + \text{energy}$$

More specifically,

$$C_6H_{12}O_6 + 6O_2(g) \rightarrow 6CO_2(g) + 6H_2O + 686\ \frac{\text{kilocalories of energy}}{\text{mole of glucose oxidized}}$$

The chemical oxidation of glucose has important implications to the measurement of respiration. From the equation, there are three ways cellular respiration could be measured:

1. Consumption of O_2
2. Production of CO_2
3. Release of energy during cellular respiration

In this experiment, we will measure the overall rate of respiration by looking at O_2 consumption based on the fact that for every molecule of oxygen consumed, one molecule of carbon dioxide is produced. 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 experiment. However, in the following procedure the CO_2 produced is removed by potassium hydroxide (KOH).

KOH reacts with CO2 to form the solid potassium carbonate (K2CO3) through the following reaction:

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

Thus, as O_2 is consumed, the overall gas volume in the respirometer decreases. The change in volume can be used to determine the rate of cellular respiration. Because respirometers are sensitive to changes in gas volume, they are also sensitive to changes in temperature and air pressure; thus, you need to use a control respirometer.

■■General Safety Precautions

You must wear safety goggles or glasses, aprons, and gloves during this investigation(s) because KOH (or the alternative, NaOH in Drano) is caustic. Do not work in the laboratory without your teacher's supervision.

■ SOME BACKGROUND INFO

A number of physical laws relating to gases are important to the understanding of how the apparatus that you will use in this exercise works. The laws are summarized in the general gas law that states: **PV = nRT**
where:

- P is the pressure of the gas
- V is the volume of the gas
- n is the number of molecules of gas
- R is the gas constant (its value is fixed)
- T is the temperature of the gas (in K^o)

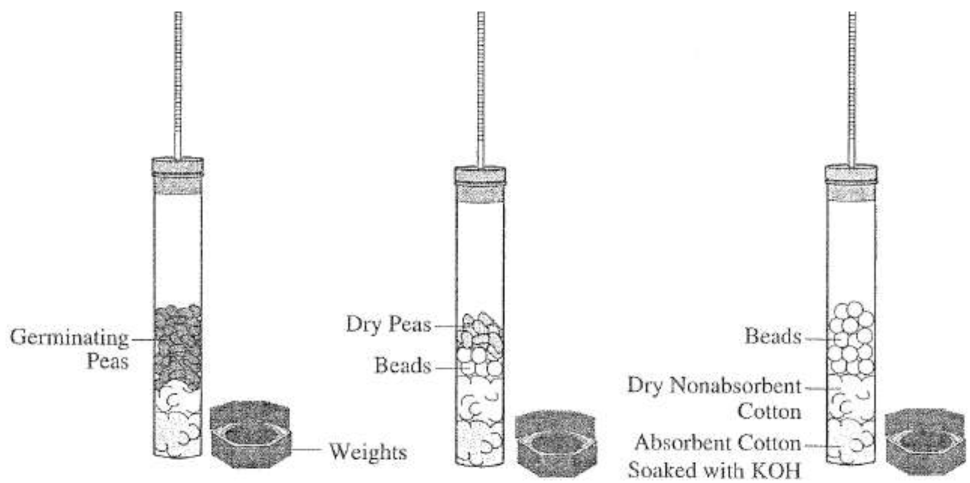
This law implies the following important concepts about gases:
1. If temperature and pressure are kept constant, then the volume of the gas is directly proportional to the number of molecules of gas.
2. If the temperature and volume remain constant, then the pressure of the gas changes in direct proportion to 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 are kept constant, then either pressure or volume (or both) will change in direct proportion to the temperature.
5. It is also important to remember that gases and fluids flow from regions of high pressure to regions of low pressure.

Since the carbon dioxide is being removed, the change in the volume of gas in the respirometer will be directly related to the amount of oxygen consumed.

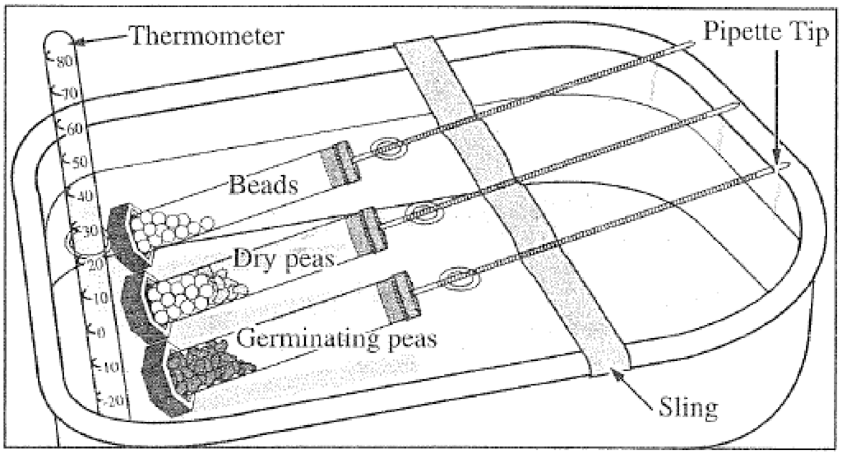
In the experimental apparatus if water temperature and volume remain constant, the water will move toward the region of lower pressure. During respiration, oxygen will be consumed. Its volume will be reduced, because the carbon dioxide produced is being converted to a solid. The net result is a decrease in gas volume within the tube, and a related decrease in pressure in the tube. The vial with glass beads alone will permit detection of any changes in volume due to atmospheric pressure changes or temperature changes. The amount of oxygen consumed will be measured over a period of time.

■ PROCEDURE

1) Find the volume of 12 germinating seeds by filling a 100mL graduated cylinder 50mL and measuring the displaced water. NOTE this in your lab journal
2) Fill the graduated cylinder with 50mL water, 25 nongerminating seeds, and add enough glass beads to attain an equal volume to the germinating seeds. NOTE this in your lab journal
3) Using the same procedure as in the previous two steps, find out how many glass beads are required to attain the same volume as the 25 germinating seeds. NOTE this in your lab journal
4) To assemble the 3 respirometers, obtain 3 vials, each with an attached stopper and pipette. Place a small wad of absorbent cotton in the bottom of each vial and, using a dropper, saturate the cotton with 15% KOH (potassium hydroxide). It is important that the same amount of KOH be used for each respirometer.
5) Place a small wad of dry, nonabsorbent cotton on top of the saturated cotton.
6) In one vial, add your pile of germinating seeds. In the other vials, place your piles of non-germinating seeds with glass beads and glass beads only. Insert the stopper with the calibrated pipette.



7) Make a sling of masking tape attached to each side of the water baths. This will hold the ends of the pipettes out of the water during an equilibration period of 7 minutes.
8) After 7 min, put all three set-ups entirely into the water. A little water should enter the pipettes and then stop. If the water continues to enter the pipette, check for leaks in the respirometer.
9) Allow the respirometers to equilibrate for 3 more minutes and then record the initial position of the water in each pipette to the nearest 0.01mL (time 0). Check the temperature and record. Record the water level in the three pipettes every 5 minutes for 20 minutes.



10) We will collect a class set of corrected differences for the germinating and dry seeds at both room and cool temperatures.
11) Make a rough sketch graph of the class data we collect (There will be 4 lines on the graph) Complete a proper quality graph for inclusion.
12) GRAPHING REMINDERS:

a. Independent variable labels the x-axis
b. Dependent Variable labels the y-axis
c. Be sure to include units in parentheses.
d. Title the graph using your two variables.

13) SUMMARIZE and verbalize the class data in your table. What do the numbers tell you?

■ ANALYSIS

1) Identify the hypotheses being tested in this activity.
2) This activity uses a number of controls. Identify at least three of the controls, and describe the purpose of each control.
3) From the slope of the four lines on the graph, determine the rate of oxygen consumption of germinating and dry seeds during the experiments at room and cool temperatures.
SHOW your calculations based on time 0(zero) and the last time reading.
4) Why is it necessary to correct the readings from the peas with the readings from the beads?
5) Explain the effect of germination (versus nongerminating) on pea seed respiration.
6) If you used the same experimental design to compare the rates of respiration of a 25 g reptile and a 25 g mammal, at 10^{o}C, what results would you expect? Explain your reasoning and include a rough sketch of a graph of your prediction.
7) If respiration in a small mammal were studied at both room temperature (21^{o}C) and 10^{o}C, what results would you predict? Explain your reasoning and include a rough sketch graph of your prediction.
8) What difficulties would there be if you used a living green plant in this investigation instead of germinating seeds?

● EXPERIMENT RE-DESIGN

On separate paper, choose some other variable and design a controlled experiment to test that variable. (JUST LIKE WE DID FOR THE ENZYME LAB)

AP BIOLOGY PEA RESPIRATION LAB

Measurement of Oxygen Consumption by Germinating and Dry Pea Seeds

Name: ______________________

Date: ______________________

ROOM TEMPERATURE ENVIRONMENT

Time	Actual	Glass Beads			Germinated Pea Seeds				Dry Pea Seeds & Glass Beads			
(min)	Temp.	Initial (ml)	Final (ml)	Diff. (ml)*	Initial (ml)	Final (ml)	Diff. (ml)*	**Adj. Diff.****	Initial (ml)	Final (ml)	Diff. (ml)*	**Adj. Diff.****
0												
5												
10												
15												
20												
25												
30												

LOW TEMPERATURE ENVIRONMENT

Time	Actual	Glass Beads			Germinated Pea Seeds				Dry Pea Seeds & Glass Beads			
(min)	Temp.	Initial (ml)	Final (ml)	Diff. (ml)*	Initial (ml)	Final (ml)	Diff. (ml)*	**Adj. Diff.****	Initial (ml)	Final (ml)	Diff. (ml)*	**Adj. Diff.****
0												
5												
10												
15												
20												
25												
30												

* VOLUME DIFFERENCE = Initial Volume at Time 0 − Final Volume at Time X

** ADJUSTED DIFFERENCE = Pea Volume Difference at Time X − Glass Bead Volume Difference at Time X (keep your algebraic signs, + & −) Optional

Note: The ADJUSTED DIFFERENCE is the Cumulative Oxygen Consumption at Time X