DID YOU KNOW?

A respirometer is an apparatus for measuring the amounts of gases used during respiration.

OBJECTIVES

- Demonstrate the relationship of gas laws to the function of a respirometer,
- Investigate the effect of temperature and germination or non-germination on cell respiration, and the relationship between dependent and independent variables
- Explain the significance of a control and calculate the rate of cell respiration using derived data
- Develop a hypothesis and design an experiment to measure cellular respiration using a respirometer

MATERIALS

MATERIALS NEEDED PER GROUP

20	Germinating peas
20	Dry peas
225	Glass beads (approx.)
6 ml	Potassium hydroxide, 15%
2	Waterbaths
6	Respirometers (glass vials, graduated pipets, one-hole stoppers, washers)
6	Absorbent cotton balls
6	Nonabsorbent cotton balls
1	Graduated cylinder, 100 ml
1	Thermometer
1	Pipet
1	Glass marking pen
1	Stopwatch
	Ice
	Masking tape
	Petroleum jelly
	Food coloring
	Paper towels

DID YOU KNOW?

It is necessary to keep the temperature within the respirometer stable so the enclosed gas does not expand or contract as in a thermometer.

Cellular respiration:
The process in which cells use oxygen to burn sugar for fuel.

DID YOU KNOW?
Potassium hydroxide (KOH), otherwise known as caustic potash, is used in making soaps, detergents, potassium carbonate and other potassium chemicals.

PROCEDURE

Gloves, goggles, and apron should be worn while performing this lab. Potassium hydroxide is corrosive.

1. Set up an ice waterbath in a large tray and keep the tray filled with ice at all times. Add a thermometer. Chill the water to less than 10°C and maintain this temperature throughout the experiment. If available, place a Styrofoam pad under the ice waterbath to insulate it from the benchtop.

2. Obtain six vials with steel washers on the bottoms. Number the vials 1 through 6 with a glass marking pen.

3. Fill a 100 ml graduated cylinder with 50 ml water. Add 10 germinating peas and take a reading of the displaced water. This is the volume of the germinating peas. Record the volume in the space below. Decant the water, remove the peas and place them on a paper towel; pat the peas dry and set aside.

   Volume of germinating peas for vial 1_____
   Volume of germinating peas for vial 4_____

4. Refill the graduated cylinder with 50 ml water. Add 10 dry non-germinating peas. Add glass beads until the water level is the same as that of the germinating peas. Remove the peas and beads and place them on a paper towel; pat the peas and beads dry and set aside.

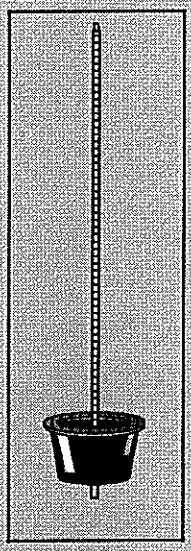
5. Refill the graduated cylinder with 50 ml water. Add glass beads until the water level is the same as that of the germinating peas. Remove the beads and place them on a paper towel; pat the beads dry and set aside.

6. Repeat steps 3 through 5 with more germinating peas, non-germinating peas and beads, and beads. Set this set aside for vials 4-6.

7. Place an absorbent cotton ball in each of the six vials and push each down to the bottom using a pipet or pencil tip. Be sure to use the cotton balls and NOT the non-absorbent rayon.

Potassium hydroxide is corrosive.

Figure 2



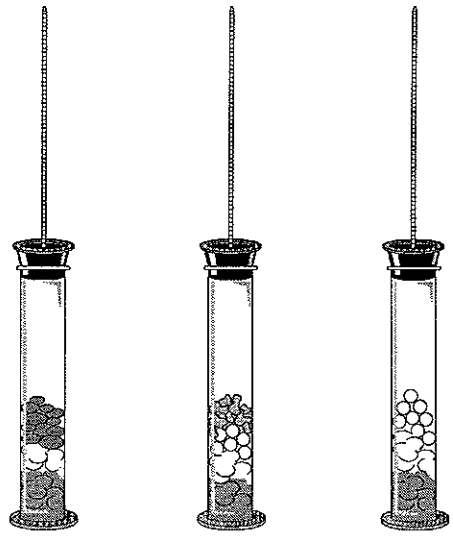
8. Without getting any liquid on the sides of the respirometers, use a pipet to add 1 ml 15% potassium hydroxide (KOH) to the cotton. Add a piece of non-absorbent rayon that is slightly smaller than that of the cottonball and place it on top of the KOH-soaked cotton.

9. Using the first set of germinating peas, non-germinating peas and glass beads, and glass beads, place them in vials 1-3, respectively. Repeat this procedure using your second set of germinating peas, non-germinating peas and glass beads, and glass beads for vials 4-6.

10. Insert the non-tapered end of a graduated pipet into the wide end of a stopper so that the tapered end of the pipet is furthest from the stopper and so that the pipet extends just beyond the bottom of the stopper (Figure 2).

11. Firmly insert the stopper into the vial. The seal that has been created between the stopper and the vial should be sufficient enough to prevent the pipet from easily moving up and down in the stopper. Place a washer over the pipet tip and guide it down the pipet until it rests on the stopper. Repeat this entire step for the remaining five vials. The first set of respirometers should look like those shown in Figure 3 below.

Figure 3



Germinating peas | **Non-germinating peas and beads** | **Beads**

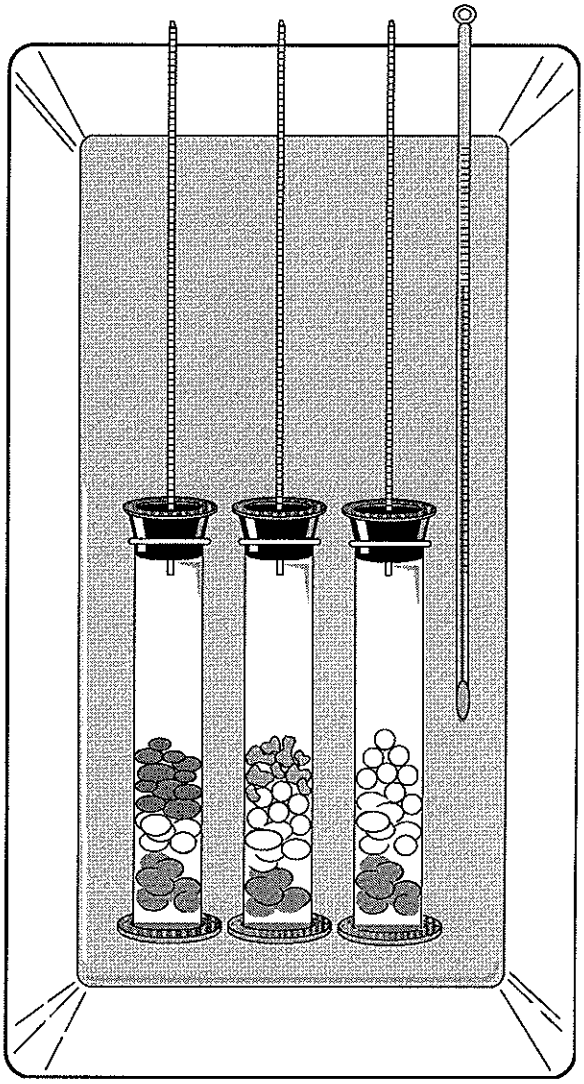
DID YOU KNOW?

Glycolysis is probably the oldest known way of producing ATP. There is evidence that it pre-dates the existence of O_2 in the Earth's atmosphere.

DID YOU KNOW?
The word aerobic means "requiring air" but the only important component of air, as far as respiration is concerned, is the gas oxygen, which makes up approximately 1/5 of the total.

DID YOU KNOW?
Seeds are dormant stages of living organisms, and contain embryos.

Figure 4



12. Place vials 1-3 in the room-temperature waterbath with the pipet tips resting on the edge of the tray as shown in Figure 4. Place vials 4-6 in the chilled waterbath in the same manner. Allow all respirometers to equilibrate for 10 minutes.

13. Add one drop of food coloring to the exposed tip of each respirometer and wait one minute. Turn each of the respirometers so that the graduation marks on the pipets are facing up. Carefully immerse all six respirometers in their waterbaths. Do not touch the respirometers once the experiment has started! Let the respirometer equilibrate for another 5 minutes before proceeding to step 14.

DID YOU KNOW?

Hibernation is a form of adaptation where an animal's respiration slows down to very low levels in response to cold temperatures. Common hibernators include woodchucks, hedgehogs, and shrews. Less commonly known are bats, whose respiration slows from eight breaths per second to eight breaths per minute during hibernation.

It is normal for a small amount of water to enter the pipets when they are first immersed and for a small amount of food coloring to enter the water. However, if a pipet begins to fill with water, that respirometer has a leak that should be repaired immediately in the following manner:

Remove the vial from the water and remove the stopper assembly. Blot the end of the pipet on a paper towel to remove all liquid. Reassemble the respirometer in the same manner as in Steps 10 and 11 of this procedure. Be sure to firmly insert the stopper to prevent leaks. Submerge the vial portion of the respirometer and add one drop of food coloring to the tip. Carefully submerge the tip of the respirometer in the same manner as previously mentioned.

14. Read all of the respirometers to the nearest 0.01 ml and take the temperature of each waterbath. Record the initial readings and the temperature of each waterbath in Table 1 in the Analysis section of the lab.

15. Take additional readings every five minutes for 30 minutes and record the readings and temperature in Table 1.

16. When all of the readings have been taken, calculate the difference and corrected difference for each result and record each value in Table 1.

    Difference = (initial reading at time 0) – (reading at time X)

    Corrected difference = (initial pea reading at time – pea seed reading at time X) – (initial bead reading at time 0 – bead reading a time X)

The corrected difference is being used because this procedure is very sensitive and may be influenced by factors such as an increase in ambient temperature or varying barometric pressure from passing weather.

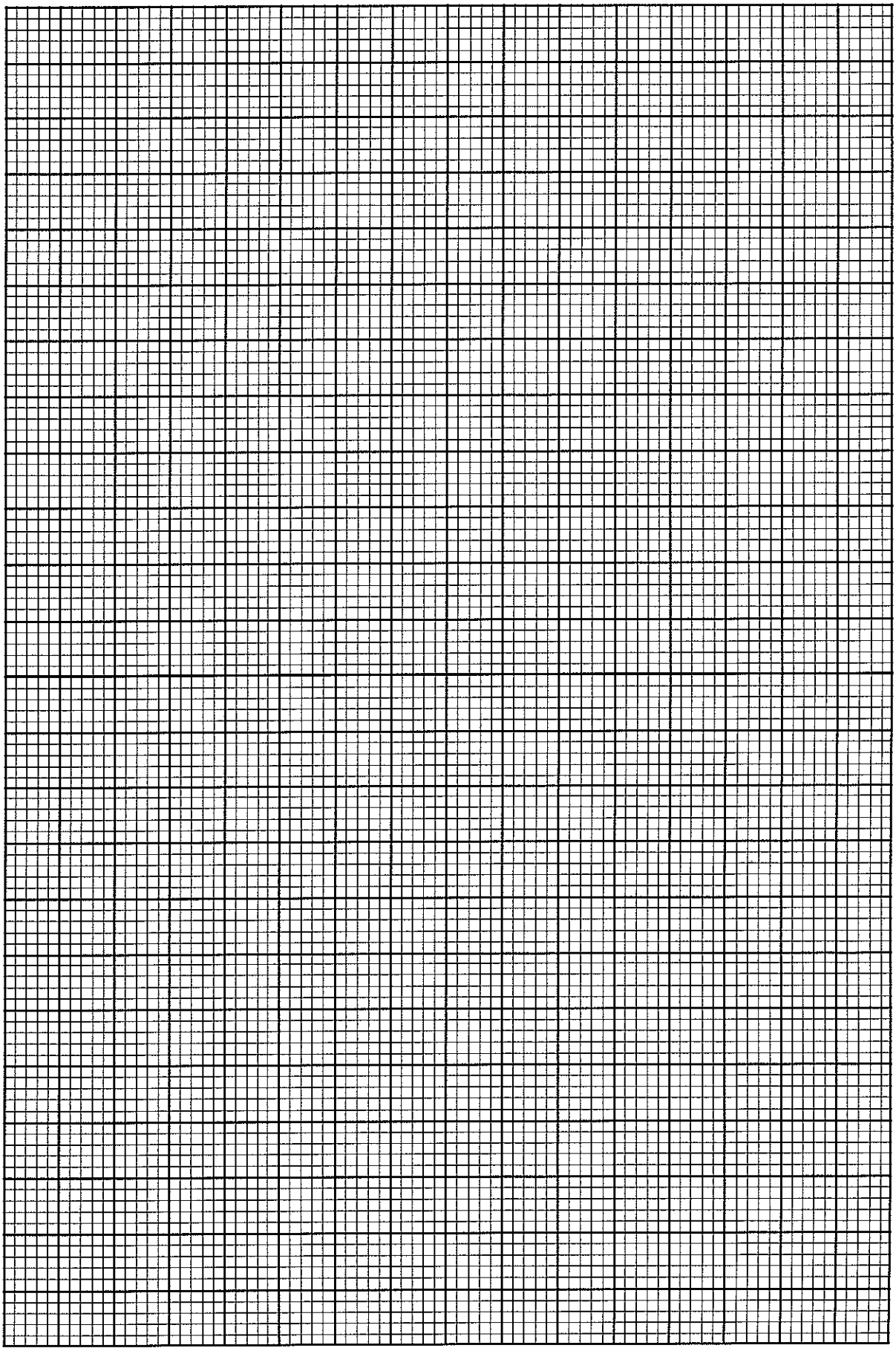
17. On the graph paper provided, graph your results from the corrected difference column in Table 1 for the germinating peas and dry peas, in both the room temperature and chilled waterbaths. Plot the time in minutes.

Name: _____
Group: _____
Date: _____

ANALYSIS

Table 1
Respiration at Room Temperature

			Germinating Peas			Dry Peas and Beads			Beads Only	
Vials	Temp (°C)	Time (min)	Reading	Diff.	Corr. Diff.	Reading	Diff.	Corr. Diff.	Reading	Diff.
1-3				—	—		—	—		—
4-6				—	—		—	—		—



Name: _____
Group: _____
Date: _____

ASSESSMENT

1. According to your graph, what happens to the rate of oxygen consumed by germinating peas over time? What does this indicate to you?

2. List at least three controls in this experiment.

3. Explain why the water initially moved into the respirometer.

4. What is the role of KOH in this experiment?

5. How did the KOH affect the water movement in the respirometer?

6. Which of the two pea types, germinating or non-germinating, consumes the most oxygen? Why?

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

8. During aerobic respiration, glucose is broken down to form several end products. Which end products contain the carbon atoms from glucose? The hydrogen atoms from glucose? The oxygen atoms from glucose? The energy stored in the glucose molecules?

9. What is fermentation? What are the two types of fermentation? What organisms use fermentation?

10. Draw a Venn diagram showing how respiration and fermentation are similar and how they differ.

11. What are the three pathways involved in the complete breakdown of glucose to carbon dioxide and water? What reaction is needed to join two of these pathways? What are the substrates and products of this reaction and where does it take place?

12. Write the letter of the pathway that best fits each of the following processes.

Pathway

a. Glycolysis
b. Krebs Cycle
c. Electron Transport System

Process

1. Carbon dioxide is given off _____
2. Water is formed _____
3. PGAL _____
4. NADH becomes NAD^+ _____
5. Oxidative phosphorylation _____
6. Cytochrome carriers _____
7. Pyruvate _____
8. FAD becomes $FADH_2$ _____

13. Calculate the energy yield of glycolysis and cellular respiration per glucose molecule. Distinguish between substrate-level phosphorylation and oxidative phosphorylation. Where does the energy for oxidative phosphorylation come from?

14. You have just performed an activity using plant seeds. Prepare a system where you test respiration in a small animal. In the space provided below draw what that system would look like.

15. Your teacher has the flu and the only available substitute knows nothing about cellular respiration. You are given the responsibility to provide the substitute teacher with the important background information needed to explain this topic to the next class. Write a short letter below, explaining cellular respiration to the substitute teacher.

16. Name some other biological processes that are affected by temperature.