INVESTIGATION 6

CELLULAR RESPIRATION*

What factors affect the rate of cellular respiration in multicellular organisms?

BACKGROUND

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,

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

The chemical oxidation of glucose has important implications to the measurement of respiration. From the equation, if glucose is the energy source, then for every molecule of oxygen consumed, one molecule of carbon dioxide is produced.

Suppose you wanted to measure the overall rate of cellular respiration.

- What specific things could you measure?
- Which of these might be easier or harder to measure?

In Procedures, you will learn how to calculate the rate of cellular respiration by using a respirometer system (either microrespirometers or gas pressure sensors with computer interface). These measure relative volume (changes in pressure) as oxygen is consumed by germinating plant seeds. As oxygen gas is consumed during respiration, it is normally

* Transitioned from the *AP Biology Lab Manual* (2001)

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 CO_2 to form the solid potassium carbonate (K_2CO_3) 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. What would be a good control for this procedure? Talk with another student for a minute, and come up with at least one possible control you could use.

As you work through Procedures, think about this question: What factors can affect the rate of cellular respiration? In Designing and Conducting Your Investigation, you will design and conduct an experiment(s) to investigate at least one of your responses to this question or some other question you have. Your exploration will likely generate even more questions about cellular respiration.

The investigation also provides an opportunity for you to apply and review concepts that you have studied previously, including the relationship between cell structure and function (mitochondria); enzymatic activity; strategies for capture, storage, and use of free energy; diffusion of gases across cell membranes; and the physical laws pertaining to the properties and behaviors of gases.

Learning Objectives

- To learn how a respirometer system can be used to measure respiration rates in plant seeds or small invertebrates, such as insects or earthworms
- To design and conduct an experiment to explore the effect of certain factors, including environmental variables, on the rate of cellular respiration
- To connect and apply concepts, including the relationship between cell structure and function (mitochondria); strategies for capture, storage, and use of free energy; diffusion of gases across cell membranes; and the physical laws pertaining to the properties and behaviors of gases

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. Follow your teacher's instructions when using the hot glue gun to seal microrespirometers. Do not work in the laboratory without your teacher's supervision.

THE INVESTIGATIONS

Getting Started

Your teacher may assign the following questions to see how much you understand concepts related to respiration before you design and conduct your own investigation:

1. Why is it necessary to correct the readings of the respirometers containing seeds with the readings taken from respirometers containing only glass beads? Your answer should refer to the concepts derived from the general gas law:

$$PV = nRT$$

Where

P = pressure of the gas

V = volume of the gas

n = number of moles of the gas

R = the gas constant (its value is fixed)

T = temperature of the gas

2. What happens to the volume of the gas being measured (O_2 consumption or CO_2 production) when the temperature or pressure changes during the experiment? If pressure and temperature remain constant, will the volume of gas in the respirometers increase or decrease? Please explain.

Hint: Several tutorials and animations explaining the general gas law are available online (e.g., ***http://www.nclark.net/GasLaws***).

3. 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. Predict the rate of oxygen consumption (i.e., cellular respiration) for these seeds and explain your reasons.

4. Imagine that you are asked to measure the rate of respiration for a 25 g reptile and a 25 g mammal at 10°C. Predict how the results would compare, and justify your prediction.

5. 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, and explain your prediction in terms of the metabolism of the animals.

6. What difficulties would there be if you used a living green plant in this investigation instead of germinating seeds?

Procedures

The rate of cellular respiration can be measured by several methods, and two reliable methods are detailed below. Your teacher will tell you which method you will use to measure the rate of respiration in germinating plant seeds at room temperature.

Option 1: Using Microrespirometers to Measure the Rate of Cellular Respiration

Materials

- Germinating/nongerminating Wisconsin Fast Plants seeds or seeds of several species of plants, including grasses; small animals, such as crickets or earthworms; small glass beads; or dry, baked seeds
- Safety goggles or glasses, aprons, and gloves
- 1 mL plastic tuberculin syringes without needles
- Thin-stem plastic dropping pipettes
- 40 µL plastic capillary tubes or plastic microhematocrits
- Hot glue gun; absorbent and nonabsorbent cotton
- 3 or 4 one-quarter inch flat metal washers
- Celsius thermometer, centimeter rulers, permanent glass-marking pens
- Constant-temperature water bath
- Manometer fluid (soapy water with red food coloring)
- 15% solution of KOH, potassium hydroxide solution (or NaOH, Drano)

Figure 1. Materials

Figure 2. Microrespirometer Assembly

Constructing a Microrespirometer

Measuring the rate of respiration is more technically challenging than many lab procedures because there are many places for potential error in the assembly and use of equipment. The advantages of the microrespirometer method as described by Richard E. Lee in *American Biology Teacher* include low cost, reliability, simplicity, and rapid response. A modification of the Lee method is described at ***http://www.elbiology.com/labtools/Microrespirometers.html***. However, for the sake of convenience, the procedure is outlined below. **Hint**: Read each step before doing it! You need to assemble two microrespirometers: one for measuring the rate of respiration in germinating seeds and the other for the control.

Step 1 Plug in the hot glue gun and allow it to heat up.

Step 2 Take a tuberculin syringe (without a needle) and make sure that its plunger is pushed all the way in.

Step 3 Carefully insert a 40 µL plastic capillary tube into the syringe where the needle normally would be. Insert it as far as the plunger tip but no farther. This will help prevent the capillary from becoming plugged with glue.

Step 4 While holding the capillary tube straight up, add a small amount of hot glue around its base (where it meets the syringe) to seal the capillary to the syringe. Keep the capillary pointed straight up until the glue cools — this should not take long. If needed, add a bit more glue to ensure an airtight seal between the capillary and syringe. (See Figure 3.)

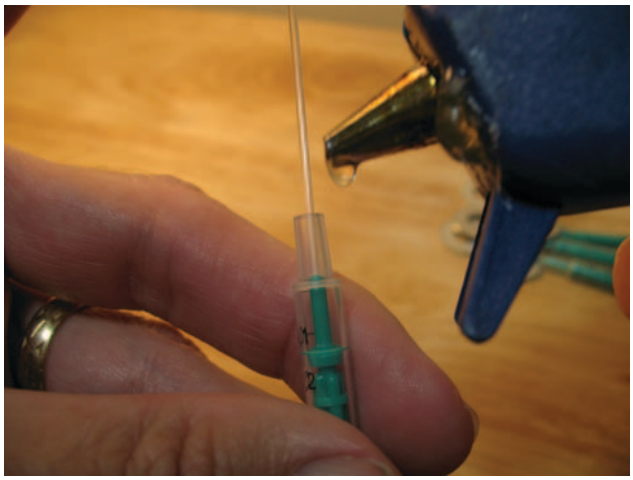

Figure 3. Hot Glue Added to Capillary Tube Base

Step 5 After the glue has cooled, pull back on the plunger and make sure that the glue has not plugged the capillary. If the capillary is plugged, carefully remove the glue and capillary and start over.

Preparing the Microrespirometer

Step 1 Draw a small quantity of manometer fluid (soapy water with red food coloring) into the full length of the microrespirometer's capillary tube. Then eject the fluid back out of the capillary. This coats the inside of the tube with a thin soapy film that helps prevent the manometer fluid from sticking.

Step 2 Carefully insert a small plug of absorbent cotton into the barrel of the microrespirometers, all the way into the 0 mL or cc mark. You can pack this cotton to the end with the barrel of a clean thin-stem pipette. (See Figure 4.)

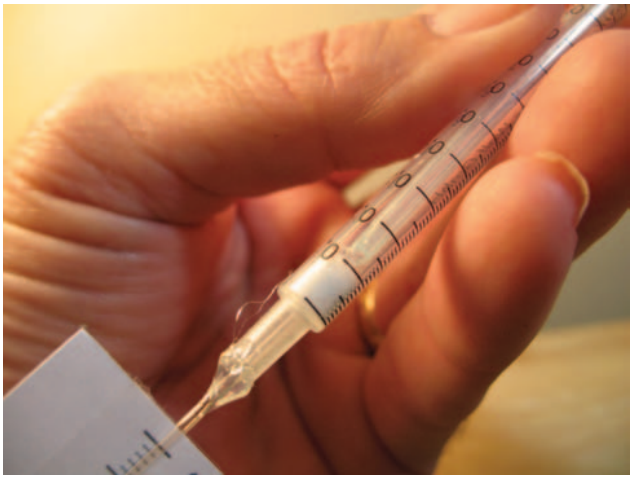

Figure 4. Cotton Inserted into Microrespirometer Barrel

Step 3 Add one small drop of 15% KOH (or NaOH, Drano) to the cotton in the microrespirometers. Do not add too much! **CAUTION: Make sure you are wearing gloves and safety goggles to protect your eyes because KOH is caustic.**

Step 4 Add a small plug of nonabsorbent cotton on top of the absorbent cotton plug already inside the barrel of the microrespirometers. You can pack the cotton to the end with the barrel of a clean thin-stem pipette. (This nonabsorbent plug is needed to protect the seeds from the caustic KOH.)

Step 5 Slowly reinsert the syringe plunger. **CAUTION: Be sure to point the capillary tip into a sink or container**. There may be excess KOH in the syringe that might squirt from the end of the capillary. Push the plunger in until it reaches the cotton so that any excess KOH is removed.

Step 6 Remove the plunger to add seeds.

Step 7 Add 0.5 mL of germinating seeds to the microrespirometers. Push the plunger in to the 1.0 mL mark. This creates a sealed microrespirometer chamber with a 1.0 mL volume.

Step 8 Place three to four washers around the barrel of the microrespirometers. The washers provide weight so that the microrespirometers will sink.

Step 9 Place the microrespirometers in a room temperature (about 20°C) water bath. You must maintain the temperature of the water bath for the experiment. Adjust the level of the water bath so that the capillary tube is sticking out of the water while the barrel of the microrespirometers is completely submerged. You will not be able to read the capillary tube easily unless it is out of the water. Make sure the top end of the capillary tube is open (not sealed).

Setting Up Your Control

Because a microrespirometer is sensitive to changes in gas volume, it is also sensitive to changes in temperature and air pressure. To compensate for any changes, you will use control microrespirometers. The control respirometer is set up just like the microrespirometer except that it contains nonliving matter (e.g., small glass beads or dry, baked seeds) instead of germinating seeds.

Step 1 Add 0.5 mL of beads or baked seeds to the second microrespirometer you assembled. Reinsert the syringe plunger and push it to the 1.0 mL mark. This seals the chamber and creates a chamber that has the same volume as the experimental microrespirometer.

Step 2 Place three to four washers around the barrel of the control.

Step 3 Place the assembled control in the water bath next to the experimental microrespirometer. Adjust the level of the water bath so the capillary tube is sticking out of the water while the barrel of the control is completely submerged. In order to easily read the capillary tube, it must be out of the water. Make sure the top end of the capillary tube is open (not sealed).

The respirometers must be airtight, and they are sensitive to environmental changes, including bumping the lab table. Once the respirometers have reached equilibrium, they should not be touched or moved, nor should anything else be added to or taken out of the water baths (including your hands!).

Collecting Data

Step 1 Prepare a table like Table 1 to record your data and observations in your lab notebook. You will need to record data for both the experimental and control microrespirometers.

Table 1. Results for Option 1, Using Microrespirometers

A Total Time (Min.)	B Water Bath Temperature (20°C)	C Total Distance Fluid Has Moved (cm)	D Change in Fluid Position During Time Interval (cm)
0			
5			
10			
15			
20			
25			

Step 2 Place the experimental and control microrespirometers into the 20°C water bath. Wait 5 minutes to allow the temperature in the microrespirometers to equalize.

Step 3 Use a dropping pipette to add one small drop of manometer fluid to the tip of each capillary tube. If everything is working properly, the drop will be sucked down into the capillary tube. The manometer fluid will seal the chamber of the microrespirometers. (You should use the plunger on the control microrespirometers to get the manometer fluid into the capillary. Pull on the plunger until the manometer drop is about halfway down the capillary. See Figure 5.)

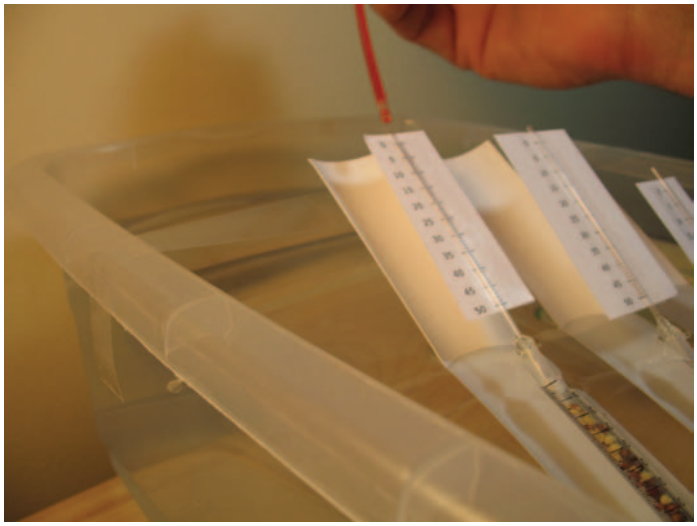

Figure 5. Manometer Fluid Added to Capillary Tube Tip

Step 4 As oxygen is consumed by cellular respiration, the manometer fluid plug will move toward the chamber. Record the starting position of each plug by marking its position on the capillary with a marker. Be sure to mark the bottom edge of the plug. These are your Time 0 marks. Begin timing once you have made the Time 0 marks.

Step 5 At 5-minute intervals, mark the position of the manometer fluid for each capillary tube. Be sure to mark the bottom edge of the fluid plug. Continue marking the positions until the fluid in the microrespirometers has traveled the entire length of the capillary, or until 25 minutes have passed.

Step 6 At the end of 25 minutes, remove the microrespirometers from the water bath. Use a centimeter ruler to measure the distance from the initial mark (Time 0 mark) to each of the 5-minute intervals marked on each capillary tube. Record your measurements in the correct column of your data table.

Step 7 Calculate the change in fluid position during each time interval. To do this, subtract the fluid position at the beginning of the time interval from the fluid position at the end of the time interval. Record your values.

Step 8 Repeat the calculations for your control microrespirometer.

Step 9 Using the values you obtained for the control microrespirometer, correct for any changes in volume that you measure that may be attributed to changes in temperature and air pressure.

Figure 6 shows how the microrespirometer works.

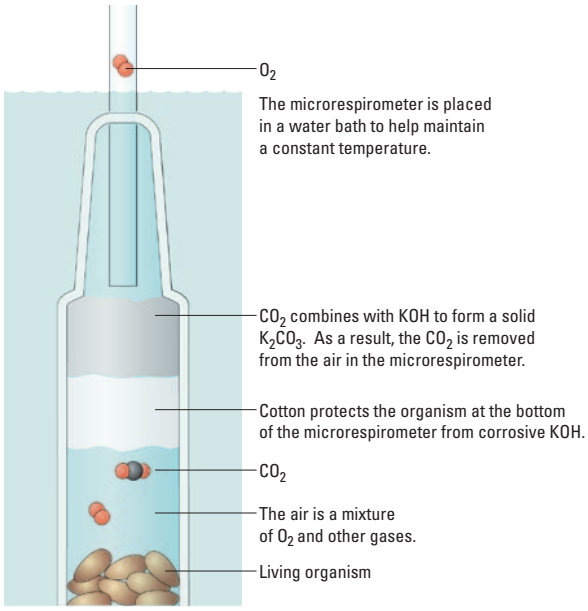

Figure 6. Microrespirometer

Analyzing Results

1. Use your data table to construct a graph. Your goal is to determine respiration rate. How should you plot your data? Which variable will be on the x-axis, and which will be on the y-axis?

2. From the graph, determine the rate of respiration for the germinating seeds at 20°C. **Hint**: Go back and think about what the units of measurement would be for respiration. How can you get a value with those units from your graph?

3. What additional questions can you explore about cellular respiration using the same respirometers from this experiment?

4. In the next part of this investigation, you will design and conduct your own experiments to answer questions that you raised in Procedures. Do you have any suggestions for improving the design of microrespirometers or procedure for measuring oxygen consumption/cellular respiration?

Option 2: Using Gas Pressure Sensors with Computer Interface to Measure the Rate of Cellular Respiration

Gas pressure sensors can be used to measure the rate of cellular respiration by measuring the amount of O_2 consumed, the amount of CO_2 produced, or both simultaneously. Your teacher will provide written instructions or perhaps ask you to download information from the manufacturer's website or another online resource. If you are unfamiliar with the use of probes with a computer interface, you will need to spend time learning how to collect data using the equipment.

General Procedure

1. Use a gas pressure sensor to measure the rate of cellular respiration in germinating seeds at 20°C over a 25-minute time interval or as per instructed by your teacher.

2. What additional questions can you explore about cellular respiration from this experiment?

3. In the next part of this investigation, you will design and conduct your own experiments to answer questions that you raised in the first part of the investigation. Do you have any suggestions for improving the procedure provided for measuring oxygen consumption/cellular respiration using a gas pressure sensor with computer interface?

Designing and Conducting Your Investigation

Now that you have learned how to measure the rate of cellular respiration in germinating seeds, you have a tool for exploring questions on your own. Think about the process of cellular respiration.

- When does it occur? Are there any situations when living cells are not respiring?
- Why might some living cells respire more than others?
- Are there differences between major groups of organisms in how fast they respire?
- What is the difference, if any, in the rate of cellular respiration between germinating seeds and nongerminating seeds?
- Does the temperature of germinating seeds affect the rate of cellular respiration? Do plant seeds consume more oxygen at higher temperatures than at lower temperatures?
- Do germinating seeds just starting to germinate consume oxygen at a greater rate than seeds that have been germinating for several days (age dependence)?
- Do seeds such as Wisconsin Fast Plant seeds (which store energy as oil) respire at a different rate from small grass seeds (which store energy as starch)?
- Do small seeds of spring flowers, weeds, or grasses respire at a different rate from seeds from summer, fall, or winter plants?
- Do seeds from monocot plants respire at different rates from dicot plants?
- Do available nutrients affect the rate of respiration in germinating seeds?
- Can the same respirometer system be used to measure the rate of respiration in small invertebrates, such as insects or earthworms?

Step 1 Design an experiment to investigate one of your own questions about cellular respiration or one of the questions above using microrespirometers or gas pressure sensors. When identifying your design, be sure to address the following:

- What is the essential question being addressed?
- What assumptions are made about the question(s) being addressed? Can those assumptions be verified?
- Will the measurements you choose to make provide the necessary data to answer the question under study?
- Did you include a control in your experiment?
- What are possible sources of error in the experiment(s)?

Step 2 Make a hypothesis, which should include a prediction about the effect of the factor(s) you chose to investigate on the rate of cellular respiration.

Step 3 Conduct your experiment(s) and record data and any answers to your questions in your laboratory notebook or as per instructed by your teacher.

Step 4 Record your data using appropriate methods, such as the example table provided in Procedures. Then graph the results to show the effect of the factors/variables you investigated on the rate of cellular respiration. Calculate the rate(s) of cellular respiration for each factor/variable.

Analyzing Results

1. Your teacher may suggest that you perform statistical analysis of your data, comparing results of the experimental variable(s) to the controls. You should at least express the uncertainty of your measurements with error bars. You may want to review Chapter 3 for more information about statistical analysis.

2. How was the rate of cellular respiration affected by the experimental variable(s) you chose as compared to the control(s)?

3. Compare class data to explain how different variables affect rates of cellular respiration.

Evaluating Results

1. Was your initial hypothesis about the effect of your factor on the rate of cellular respiration supported? Why or why not?

2. What were some challenges you had in performing your experiment? Did you make any incorrect assumptions?

3. Were you able to perform without difficulty the mathematical routines required to analyze your data? If not, what calculations were challenging or required help from your classmates or teacher?

Where Can You Go from Here?

If time is available, ask your teacher if you can extend the investigation to explore answers to other questions that might have been raised as you conducted your experiment(s). For example, if you originally investigated the effect of temperature on metabolic rate in plant seeds, you might want to explore a different aspect, such as the effect of temperature on metabolic rate in small invertebrates, such as insects or earthworms, or the relationship between the mass of an organism and its rate of respiration.