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. In cellular respiration, free energy becomes available to drive metabolic pathways vital to cellular processes primarily by the conversion of ADP → ATP. 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,

$$C_6H_{12}O_6 + 6O_{2\,(g)} \rightarrow 6CO_{2\,(g)} + 6H_2O + \frac{\text{686 kilocalories of energy}}{\text{mole of glucose oxidized}}$$

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 one molecule of oxygen consumed, one molecule of carbon dioxide is produced. To determine the rate of cellular respiration, one could measure any of the following:

- Consumption of O_2 during the oxidation of glucose (How many moles of O_2 are consumed when one mole of glucose is oxidized?)
- Production of CO_2 during aerobic respiration (How many moles of CO_2 are produced when one mole of glucose is oxidized?)
- Release of energy in the form of heat as one mole of glucose is oxidized

In Getting Started, students conduct prelab research on the process of cellular respiration and review concepts they may have studied previously.

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

In Procedures, students learn how to calculate the rate of cellular respiration by using a respirometer system (microrespirometers or gas pressure sensors with computer interface) that measures the relative volume (changes in pressure) as oxygen is consumed by germinating plant seeds at room temperature (20°C). As oxygen 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, one would expect no change in gas volume to result from this experiment. However, the CO_2 produced is removed by 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 O_2 is consumed, the overall gas volume in the respirometer decreases, and this change 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, students need to use a control respirometer containing nonliving matter (e.g., glass beads) instead of germinating seeds to measure and correct for changes in temperature and pressure.

Once students learn how to measure the rate of cellular respiration, questions should emerge about the process that lead to investigation, including the following:

- What is the difference, if any, in the rate of cellular respiration in germinating seeds versus 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?
- What problems would arise if students used a living, green plant instead of germinating seeds?

In Designing and Conducting Your Investigation, students design and conduct an experiment(s) to investigate one or more questions that they raised in Procedures. Their exploration will likely generate even more questions about cellular respiration.

The lab also provides an opportunity for students to apply, review, and/or scaffold concepts that they 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.

PREPARATION

Materials and Equipment

Complete details of the procedure for assembling and using microrespirometers or gas pressure sensors to measure the rate of cellular repiration are found in the Student Manual. However, the following materials should be available.

- Germinating/nongerminating Wisconsin Fast Plant seeds or seeds of several species of plants, including grasses; small insects, 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, and 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)

As part of an experimental setup, more than one syringe size can be used depending on the size of organisms. Students then can pick barrel diameters that match the organism(s) being tested. Having various sizes or syringes available also mitigates the problem of seeds getting stuck after germinating. Larger syringes can be disassembled, cleaned, and reused. Students can then compare species — plants versus animals, annelids versus arthropods, slow versus fast moving, flying versus not flying, etc. Students also can examine the effects of different temperatures or light levels on respiration rates. Table 1 indicates appropriate syringe sizes for various organisms.

Table 1. Syringe Sizes for Various Organisms

Syringe Size	Organisms
1 mL (tuberculin)	radish, broccoli seed; *Drosophila*
3 mL	rye, oats; mealworms, ladybugs
5 mL	flower and vegetable seed; small worms, ants
10 mL	peas, beans; crickets, large worms, bessbugs, cockroaches

Timing and Length of Lab

The prelab questions and online preparation and review activities suggested in Getting Started can be assigned for homework.

The investigation requires approximately four lab periods of about 45 minutes each — one period for students to assemble microrespirometers, if they choose that system; one period to conduct Procedures (using respirometers to measure respiration); and approximately two periods to conduct their own investigations (Designing and

Conducting Your Investigation). If gas pressure sensors are available and students know how to use them, they can assemble them in about 10 minutes and proceed directly to Procedures. Alternatively, students can design their experiment(s) as a homework assignment, and lab groups can communicate through various social networking sites or by email. Teachers should allow time for students to share their results and conclusions with the class by appropriate means, such as a mini-poster session or traditional lab report. Students can work in pairs or small groups to accommodate different class sizes.

Safety and Housekeeping

Safety goggles or glasses, aprons, and gloves must be worn because KOH (or the alternative, NaOH in Drano) is caustic. Keep the KOH solution in cotton, using a limited amount of KOH, inside the barrel of the syringe, and you'll minimize accidental exposure to KOH. When charging the microrespirometers, point the capillary into a sink in case there is excess KOH that might be expelled from the capillary under pressure. Students must be careful when using the hot glue gun to seal microrespirometers. Students should be supervised at all times while working in the laboratory.

ALIGNMENT TO THE AP BIOLOGY CURRICULUM FRAMEWORK

This investigation can be conducted during the study of concepts pertaining to cellular processes (big idea 2) — specifically, the capture, use, and storage of free energy — or interactions (big idea 4). In addition, some questions students are likely to connect to evolution (big idea 1) if students explore cellular respiration — a conserved core process — in a variety of plants or insects. As always, it is important to make connections between big ideas and enduring understandings, regardless of where in the curriculum the lab is taught. The concepts align with the enduring understandings and learning objectives from the AP Biology Curriculum Framework, as indicated below.

Enduring Understandings

- 1B1: Organisms share many conserved core processes and features that evolved and are widely distributed among organisms today.
- 2A1: All living systems require constant input of free energy.
- 2A2: Organisms capture and store free energy for use in biological processes.
- 2B3: Eukaryotic cells maintain internal membranes that partition the cell into specialized regions (e.g., mitochondria).
- 4A2: The structure and function of subcellular components, and their interactions, provide essential cellular processes.
- 4A6: Interactions among living systems and with their environment result in the movement of matter and energy.

Learning Objectives

- The student is able to describe specific examples of conserved core biological processes and features shared by all domains or within one domain of life, and how these shared, conserved core processes and features support the concept of common ancestry for all organisms (1B1 & SP 7.2).
- The student is able to justify the scientific claim that organisms share many conserved core processes and features that evolved and are widely distributed among organisms today (1B1 & SP 6.1).
- The student is able to justify a scientific claim that free energy is required for living systems to maintain organization, to grow, or to reproduce, but that multiple strategies exist in different living systems (2A1 & SP 6.1).
- The student is able to use representations to pose scientific questions about what mechanisms and structural features allow organisms to capture, store, and use free energy (2A2 & SP 1.4, SP 3.1).
- The student is able to use representations and models to describe differences in prokaryotic and eukaryotic cells (2B3 & SP 1.4).
- The student is able to construct explanations based on scientific evidence as to how interactions of subcellular structures provide essential functions (4A2 & SP 6.2).
- The student is able to apply mathematical routines to quantities that describe interactions among living systems and their environment, which result in the movement of matter and energy (4A6 & SP 2.2).

ARE STUDENTS READY TO COMPLETE A SUCCESSFUL INQUIRY-BASED, STUDENT-DIRECTED INVESTIGATION?

Before students investigate cellular respiration, they should be able to demonstrate understanding of the following concepts:

- The relationship between cell structure and function (mitochondria)
- Enzymatic activity and the effects of environmental variables, such as temperature and pH, on enzyme-catalyzed reactions
- Strategies for capture, storage, and use of free energy
- Interdependence of photosynthesis and cellular respiration
- Aerobic respiration versus fermentation
- Diffusion of gases across cell membranes

These concepts may be scaffolded according to level of skills and conceptual understanding. For example, a number of physical laws relating to gases are important to understanding how the respirometer systems used in the investigation(s) measure respiration rate. In particular, the laws are summarized in the general gas law, and students should be able to manipulate the equation PV = nRT, where

P = pressure of the gas

V = volume of the gas

n = number of molecules of the gas

R = the gas constant (its value is fixed)

T = temperature of the gas

Students can be directed to several online resources to review the gas laws, including ***http://www.phschool.com/science/biology_place/labbench/lab5/intro.html***, which offers activities to introduce key concepts pertaining to cellular respiration, and ***http://www.nclark.net/GasLaws***, which provides myriad tutorials and animations to introduce or review the gas laws.

This investigation reinforces the following skills. (However, if students have not acquired these skills previously, the procedures in this lab will help students develop them.)

- Preparing a constant temperature water bath
- Measuring volume and temperature using the metric system
- Constructing data tables and graphs
- Communicating results and conclusions

Skills Development

Students will develop the following skills:

- Assembling and using microrespirometers or gas pressure sensors with computer interface
- Measuring/calculating rates of cellular respiration

Potential Challenges

Students often come to biology with the misconception that plants undergo photosynthesis (only) and animals undergo cellular respiration. Students are surprised to learn that most plant cells possess mitochondria and respire. The Procedures section, in which students measure the rate of respiration in germinating seeds, dispels the misconception.

If students have a solid understanding of the aforementioned concepts, they should be able to pose scientific questions about cellular respiration and design an experiment(s) around the effects of variables on the rate of respiration. The skills and concepts may be taught through a variety of methods in an open-inquiry investigation, and respiration rates may be measured by several means. Only two methods (microrespirometers or gas pressure sensors with computer interface) are described in the Student Manual, and alternative procedures may be equally and successfully substituted. For example, in the procedures outlined in the Student Manual, consumption of O_2 gas in respiration is measured, but students also can measure the production of CO_2 or even simultaneous changes in volumes of both gases, depending on available equipment.

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 the respirometers described in the *AP Biology Lab Manual* (2001), Lab 5. Since gas pressure sensors are expensive, the microrespirometer system described in the Student Manual provides an easier, cheaper, and more reliable method to study both plant seed and small insect metabolisms. The details of the microrespirometer method — first published by Richard E. Lee in *The American Biology Teacher* — can be found at ***http://www.elbiology.com/labtools/Microrespirometers.html***. Microrespirometers provide advantages for use in high school laboratories because they cost less than 25 cents each, have adjustable volumes, and work quickly. In addition, their small size allows them to equilibrate their temperature rapidly in water baths.

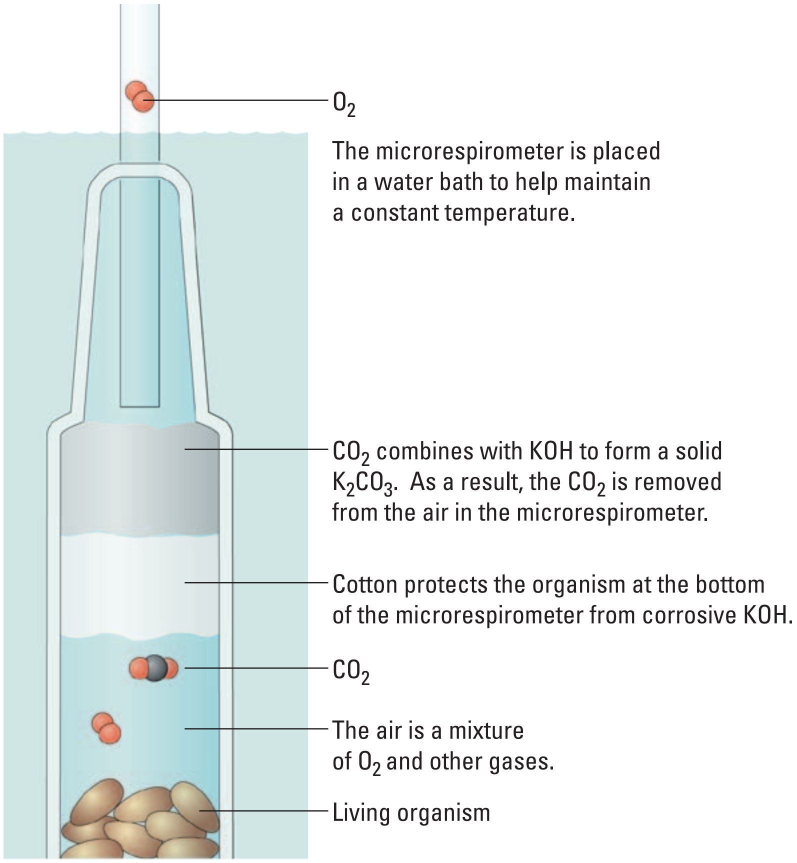

Figure 1. Microrespirometer

The respirometers must be airtight. They are sensitive to environmental changes, including movement from one's 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 students' hands!). Students should not try to simplify their investigations by leaving out the control respirometers containing glass beads only; the readings taken from these respirometers are essential for correcting the readings of the other respirometers.

As stated previously, rates of cellular respiration also can be determined using gas pressure sensors with a computer interface. Instructions, tips, and suggestions for most accurate usage of these devices can be found in the instructions that are provided with the purchase of the equipment.

THE INVESTIGATIONS

Getting Started: Prelab Assessment

You may assign the following questions for homework; as a think, pair/group, share activity, in which pairs or small groups of students brainstorm ideas and then share them with other groups; or as a whole-class discussion to assess students' understanding of key concepts pertaining to cellular respiration:

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,

$$PV = nRT$$

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 5 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 metabolism.

Visuals

Although encouraged to develop their own means of reporting data, students might find the following tables and graph helpful for recording their data/results and proposing their plan for their independent, inquiry-based investigation(s). If students use a gas pressure sensor with computer interface, the computer will generate the graph on the screen; however, you may elect to have students draw, label, and annotate any graphs.

Table 2. Results for Procedures, Using Microrespirometers

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

Table 3. Investigation Proposal

Hypothesis ("if … then … because"):
Materials and supplies:
Variable(s) manipulated:
Variable(s) held constant/controls:
Method(s) or procedure(s):

Students often having difficulty analyzing and presenting data. Following is an example of a graph of investigation results that a student might present:

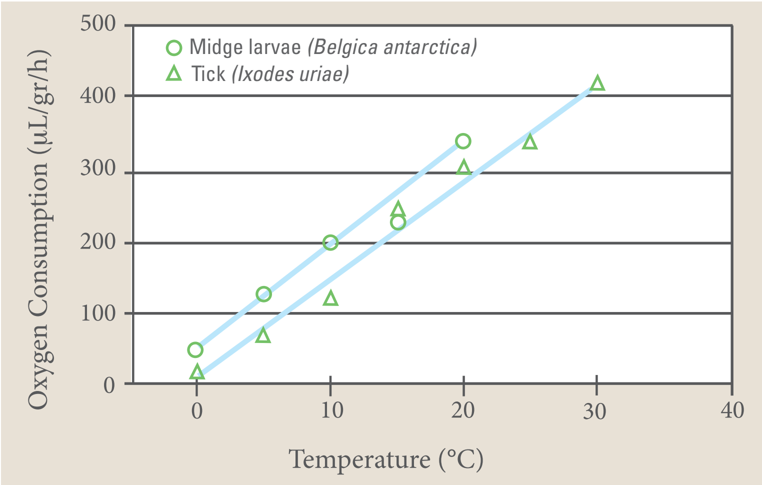

Figure 2. Effect of Temperature on the Rate of Oxygen Consumption Determined Using Microrespirometers in Two Antarctic Terrestrial Arthropods: Adult Females of the Ixodid Tick, *Ixodes uriae*, and Chironomid Larvae, *Belgica antarctica*. Data from Lee and Baust (1982 a, b).

Note that the points plotted are respiration *rates* at various temperatures. Your students might not consider the number of treatments or replications typical of even small investigations. You might consider sharing this graph or similar ones with your students to help them arrive at their own experimental design and analysis.

A line of best fit is a straight line that best represents data on a scatterplot. Lines of best fit are plotted, but there is no indication of the correlation coefficient or the equation for either line. Moreover, you do not know whether these are single measurements or means that are plotted. These should be indicated if these data are used to support a hypothesis. Likewise, if these points are means, standard errors bars for each point should be indicated. (In the example above, Lee was demonstrating what the data *might* look like when plotted. You would need to go to Lee's original paper to view how these data were used to support a conclusion.)

Designing and Conducting Independent Investigations

Now that students have learned how to measure the rate of cellular respiration in germinating seeds, they have a tool for exploring questions on their own. They begin by thinking about the process of cellular respiration. Several questions about cellular respiration should emerge, including the following:

- 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 cellular respiration in germinating seeds versus 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 Students are asked to design an experiment to investigate one of their own questions about cellular respiration or one of the questions above, using microrespirometers or gas pressure sensors. When identifying their design, students should 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 Students should make a hypothesis, which should include a prediction about the effect of the factor(s) they chose to investigate on the rate of cellular respiration.

Step 3 Then students conduct their experiment(s) and record data and any answers to their questions in their laboratory notebook.

Step 4 Students should record their data using appropriate methods, such as the example table provided in Visuals. They should then graph the results to show the effect of the factors/variables they investigated on the rate of cellular respiration. Students should calculate the rate(s) of cellular respiration for each factor/variable.

Summative Assessment

The following are suggested as guidelines to assess students' understanding of the concepts presented in the investigation, but you are encouraged to develop your own methods of postlab assessment:

1. Revisit the learning objectives. Based on students' answers to the analysis questions, do you think students have met the objectives of the laboratory investigation?

2. As a result of this lab, did students demonstrate evidence of what they knew and could do within the context of the learning objectives?

3. Have students record their experimental design, procedures, data, results, and conclusions in a lab notebook or have them construct a mini-poster to share with their classmates.

4. Have students develop a list of concepts that they had difficulty understanding about the process of cellular respiration before conducting their investigations.

5. Did students have sufficient mathematical skills required to calculate the rate(s) of cellular respiration?

6. If you used the gas pressure sensors to measure O_2 consumption or CO_2 production, were students able to navigate through the computer interface to the lab investigation without much difficulty? If students had difficulty, ask them to teach each other how to use the equipment.

7. Did students have an adequate understanding of the general gas law as it applies to the concepts in this lab?

8. Released AP Exams have several multiple-choice and free-response (essay) questions based on the concepts studied in this investigation. These could be used to assess your students' understanding.

Where Can Students Go from Here?

Students can explore answers to other questions that might have been raised as they conducted their experiment(s). For example, if they originally investigated the effect of temperature on metabolic rate in plant seeds, they 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.

SUPPLEMENTAL RESOURCES

Prelab Activities

http://www.phschool.com/science/biology_place/labbench/lab5/intro.html

This resource provides an interactive tutorial on the structure and function of mitochondria and the process of cellular respiration.

http://www.nclark.net/GasLaws

This resource provides myriad tutorials and animations that review the gas laws.

http://vcell.ndsu.edu/animations/

This resource introduces students to the concepts of cellular respiration. By walking through the still images and movie included for each topic, students are in control of choosing the learning style that best fits their needs.

Procedural Resources

AP Biology Lab Manual, Lab 5: Cell Respiration, The College Board, 2001.

Although this laboratory protocol is teacher directed, students can use the resource to glean information about the process of cellular respiration as they design experiments to investigate factors, including environmental variables such as temperature, that affect the rate of respiration.

http://www.elbiology.com/labtools/Microrespirometers.html.

This resource describes the procedure and tips for assembling microrespirometers.

Lee, Richard E. "Using Microrespirometers to Measure O_2 Consumption by Insects and Small Invertebrates." *The American Biology Teacher,* vol. 57, no. 5, 284–85, 1995.

This resource provides information about the use of microrespirometers in measuring respiration rates in insects and small invertebrates such as crickets and earthworms.

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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,

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

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.)

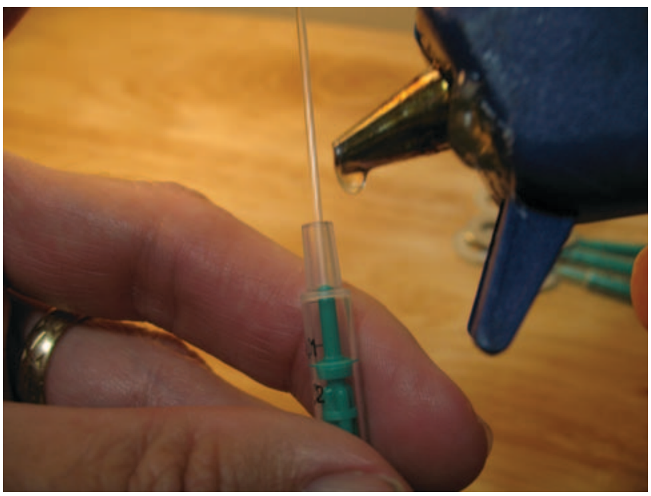

Figure 3. Hot Glue Added to Capillary Tube Base

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 (see Figure 5). 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.)

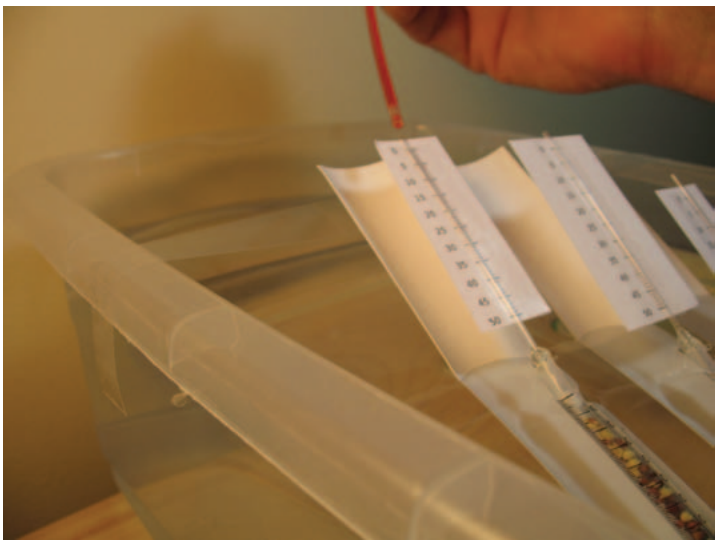

Figure 5. Manometer Fluid Added to Capillary Tube Tip

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.

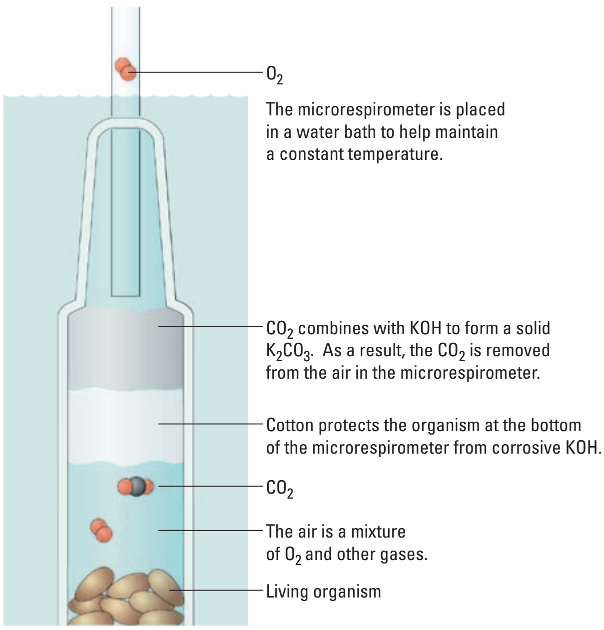

Figure 6. Microrespirometer

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.