Respiration

Objectives

1. To describe oxidation and reduction in terms of electron and H^+ transfer.
2. To distinguish anaerobic from aerobic cellular respiration in terms of ATP, oxygen, and chemiosmosis.
3. To demonstrate that carbon dioxide is a product of cell respiration.
4. To determine the effect of boiling on the aerobic respiration of bean seeds and explain the result in terms of enzyme activity.
5. To measure the rate of oxygen consumption in germinating bean seeds.
6. To determine the metabolic rates for several small animals and relate this to body size and lifestyle.

Introduction

All organisms, whether plant or animal, bacteria, protists or fungi, carry out cellular respiration. During respiration organic food molecules are oxidized and these exergonic oxidation reactions are coupled with the synthesis of ATP, an endergonic reaction. The ATP is then used to drive the metabolic reactions necessary to maintain the organism's physical integrity and to support all its other activities.

The cytoplasm of all cells contains the enzymes needed in the ancient central pathway of glycolysis, in which glucose is oxidized to pyruvate in the absence of oxygen. The energy released in this process is used to generate ATP directly by substrate level phosphorylation, in which phosphate groups are transferred directly from organic substrates to ADP.

To obtain energy from glucose, hydrogen atoms are removed from the glucose molecule as it is metabolized. These hydrogen atoms can be removed only by hydrogen (electron) carriers, such as NAD^+. Cells contain a finite amount of NAD^+ and since each NAD^+ combines with only two hydrogens (electrons), there must be a mechanism for removing the hydrogens (electrons) from NADH so that glycolysis can continue.

In many organisms, respiration can occur under anaerobic conditions where no oxygen is present. Many bacteria, yeast, and animals ferment glucose, producing lactate or ethanol. During fermentation reactions, hydrogens are removed from glucose, passed to the electron carrier NAD^+ (forming NADH), and then on to pyruvic acid (the end product of glycolysis), converting it to lactate or ethanol. Concurrently, the NADH is oxidized to NAD^+, reconstituting the NAD^+ pool required for glycolysis. Fermentation allows cells to make ATP in the absence of oxygen. Cells metabolizing glucose by fermentation harvest only about 5% of the available energy in glucose, however.

Most organisms use molecular oxygen in a process called cellular respiration. In this series of reactions, the glucose molecule is completely disassembled to yield CO_2 and H_2O. The process begins with glycolysis; the end product of glycolysis, pyruvate, enters the mitochondrion where it is further metabolized.

After entering the mitochondrion, the pyruvate loses a CO_2 molecule to form acetyl CoA, which enters a series of reactions known as the Kreb's cycle or citric acid cycle where it is completely oxidized to CO_2. The electrons released during this series of reactions are used to reduce NAD^+, and a related molecule FAD, to NADH and $FADH_2$, respectively. These electron carriers then transfer their electrons to the electron transport chain (ETC), a series of proteins embedded in the inner membrane of the mitochondrion. The passage of electrons through the ETC generates an H^+ gradient across the inner membrane which drives the synthesis of ATP by ATP synthetases embedded in the inner membrane. The last electron carrier protein in the ETC transfers the electrons to molecular oxygen to form water, with the addition of H^+. This transfer of electrons to oxygen returns the protein (cytochrome oxidase) to its oxidized state so that it can continue to accept electrons from the remainder of the ETC.

The process of cellular respiration can be summarized by the following equation:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + ATP$

Glucose Oxygen Carbon dioxide Water Energy

Complete respiration of one molecule of glucose results in a yield of 30-32 ATP molecules. Four of these ATP's are the product of substrate-level phosphorylation; the remaining ATP's are synthesized by the ATP synthetases using the energy of the H^+ gradient created by electrons passing down the ETC in a process called oxidative phosphorylation. The coupling of electron flow with ATP synthesis is described in the chemiosmotic model.

Overall, 16 times more ATP is produced by aerobic respiration than by anaerobic respiration. Even so, less than 50% (the actual number is about 39%) of the available energy in a glucose molecule is actually stored in ATP molecules. In contrast, fermentation and anaerobic respiration yield only two ATP's. This is equal to only about 2% of the available energy in glucose. Clearly aerobic respiration gives a bigger energy payback than anaerobic respiration.

In today's lab you will be carrying out a series of experiments that will demonstrate several aspects of respiration including the release of carbon dioxide as a product of respiration, the uptake of oxygen by organisms during aerobic respiration, and attempt to demonstrate that metabolic rate (the rate of oxygen usage per gram of body weight) is a function of animal size.

Safety Precautions:

1. Gloves must been worn while handling the chemicals used in the lab today. Gloves must be placed in the biohazard bucket when finished
2. The germinated, ungerminated and boiled germinated seeds must be placed in the appropriately labelled containers at the end of the experiments.
3. Phenol red solutions must be placed in the hazardous waste container.

Carbon Dioxide Production

Seeds contain stored food material in the form of some carbohydrate. When a seed germinates, the carbohydrate is broken down, liberating energy (ATP) needed for growth of the enclosed embryo into a seedling.

Two days ago, a set of dry bean seeds was soaked in water to start the germination process. Another set was not soaked. This experiment will compare carbon dioxide production between germinating bean seeds, germinating bean seeds that have been boiled, and ungerminated (dry) bean seeds.

Procedure:

1. Obtain three respiration flask setups. One of these flasks will already contain ungerminated bean seeds and have the rubber stopper with attached fixtures already inserted.
2. Fill one of the remaining flasks with germinated seeds to the same level as the flask of ungerminated seeds. Fill the remaining flask with boiled germinated seeds to the same level.
3. Fit the rubber stoppers securely into the two flasks you filled. Add enough water to each test tube to cover the ends of the glass tubes coming out of the respiration flasks.
4. Set the flasks aside for about 1½ hours while you complete the remaining experiments.
5. After about 1½ hours, replace the water in each test tube with phenol red solution. Phenol red is a pH indicator, that is, it changes color in response to changes in pH. The stock solution is red (pH neutral), but in the presence of an acid the solution turns yellow. When CO_2 is bubbled through water it forms a mild acid called carbonic acid
6. Pour water through the thistle funnel into each flask to force the gases through the glass tubing and into the phenol red solution.
7. Record your results in table 1 and use these results to answer the questions below.

Table 1. CO_2 Production

	Germinating bean seeds	Boiled germinating bean seeds	Ungerminated bean seeds
Color			
CO_2 Present			

1. Which set of seeds was undergoing respiration?

2. What happened during boiling that caused the results you found? (**Hint: Think about the effect of boiling on the enzymes)**

Oxygen Uptake by Germinating Beans

The equation for cellular respiration indicates that oxygen is consumed and carbon dioxide is given off. The uptake of oxygen is evidence that cellular respiration is occurring. The uptake of oxygen and the parallel release of carbon dioxide can be monitored using gas sensors and plotted as a function of time. In this experiment you will examine respiration rates for both ungerminated and germinated kidney bean seeds.

Procedure:

1. Line the bottom of the chamber with two layers of paper towel.
2. Count out 200 ungerminated seeds and add these to the chamber.
3. Insert the two gas sensor probes into the lid of the chamber (if not already done) and place the lid securely on the chamber.
4. Connect the O_2 probe to port #1 and the CO_2 probe to port #2. The data logger will automatically recognize each probe and open a reading window for each.
5. Tap on the O_2 window and change the units to ppm (parts per million). Do the same for the CO_2 probe.
6. Change the data collection parameters to one sample every 5 seconds for 600 seconds, if not already set.
7. Wait five minutes for the readings to stabilize then begin data collection by tapping the green arrow at the bottom of the screen.
8. Tap the “Graph” tab and select “Autoscale once”. The data logger will now automatically adjust the plot scale during the 10 minutes of data collection, stopping collection at the end of 10 minutes.
9. Tap on the “Analyze” tab from the top menu followed by “Curve Fit”, select the O_2 graph and “Linear Regression”. Record the value for m (the slope of the regression line and therefore the rate of O_2 uptake) in the appropriate box in the table below. Repeat for the CO_2 graph.
10. Store your data at the end of the run by tapping on the filing cabinet icon. The graph plots will be automatically reset for the next run once the data have been saved.

11. Remove the ungerminated seeds, placing them in the appropriate container. CO_2 is heavier than air - invert the chamber to remove any residual gas.
12. Lightly dampen the paper towel in the chamber and add 200 germinated seeds.
13. Repeat steps 7-9 for the germinated seeds.
14. Remove the germinated seeds, placing them in the appropriate container, and clean and dry the chamber. CO_2 is heavier than air - invert the chamber to remove any residual gas.

You must print your results from the bean experiments before continuing on to the next part of the lab. Failure to do so may result in the loss of your data.

15. Tap on the "Run 2" icon and select "All Runs". The graphs for the ungerminated and germinated seeds will now be displayed on a common set of axes ready for printing.
16. Confirm that your data logger is connected to the HP laser printer in the lab (tap on the wi-fi icon in the bottom right-hand corner to see the available connections and the current connection).
17. To print the currently displayed graph, tap on the "File" tab in the top menu, select "Print" then "Graph". You will have an opportunity to add a label to your graph before it is printed. Make sure to print copies for every member of your group.

Table 2. O_2 uptake and CO_2 output of ungerminated and germinated bean seeds

Beans	O_2 Rate of consumption (ppm/sec)	CO_2 Rate of respiration (ppm/sec)
Ungerminated seeds		
Germinated seeds		

Answer the following questions using the data from the table and your knowledge of respiration.

1. Do you have evidence that cell respiration occurred in beans? Explain.

2. How do the respiratory rates for germinating and non-germinating seeds compare?

3. How do you account for this difference?

4. The gas sensors measure gas concentration in units of parts per million, or ppm. In gaseous mixtures, 1 part per million refers to 1 part by volume in 1 million volume units of the whole. A concentration of 100 ppm for O_2 would simply mean that there are 100 L of O_2 gas for every 1,000,000 L of air (or 0.1 mL of O_2 per 1 L of air). Multiply the rate of O_2 uptake for the germinating seeds by 60 to convert this to ppm/minute. If it takes about 820ml of oxygen to completely oxidize 1g of glucose, how much glucose are your germinating beans consuming during one hour? The volume of the chamber is 2000ml or 2 liters.

The Metabolic Rate of Earthworms

The amount of oxygen used or carbon dioxide produced by the respiration of animals is one measure of all the organic processes going on within the animal. The term *metabolism* is sometimes used to describe this sum total of all life processes.

There are many factors that influence the metabolic rate of an animal. Some of these are physical activity, diet, general state of health, and hormones such as those secreted by the thyroid gland and the adrenal cortex. The metabolic rate of an animal is also influenced by whether the animal is warm-blooded or cold-blooded. Warm-blooded animals (homeotherms), which have the ability to maintain a relatively constant internal temperature, have a fairly uniform rate of metabolism. Cold-blooded animals (poikilotherms), whose internal body temperature is determined by their surroundings, have a metabolism that is determined by their surroundings.

Even apparently simple animals such as earthworms are capable of significant rates of respiration. These animals are members of a group called annelid, or segmented, worms. While simple in appearance, these worms have many of the same organ systems as more complex animals, including a complete digestive tract, a nervous system (including a simple "brain"), a closed circulatory system (with five hearts!), and excretory system. In this investigation, you will determine the metabolic rate of earthworms using a volumeter setup similar to the one you used for the bean seeds above.

Procedure:

1. Line the bottom of the chamber with a double layer of lightly dampened paper towels.
2. Obtain 15 nightcrawlers and rinse them gently with water to remove any dirt. Record the weight of the worms in the table below then place them in the chamber.
3. Insert the two gas sensor probes into the lid of the chamber (if not already done) and place the lid securely on the chamber.
4. Connect the O_2 probe to port #1 and the CO_2 probe to port #2. The data logger will automatically recognize each probe and open a reading window for each.
5. Tap on the O_2 window and change the units to ppm (parts per million). Do the same for the CO_2 probe.
6. Change the data collection parameters to one sample every 5 seconds for 600 seconds, if not already set.
7. Wait five minutes for the readings to stabilize then begin data collection by tapping the green arrow at the bottom of the screen.

8. Tap the "Graph" tab and select "Autoscale once". The data logger will now automatically adjust the plot scale during the 10 minutes of data collection, stopping collection at the end of 10 minutes.
9. Tap on the "Analyze" tab from the top menu followed by "Curve Fit", select the O_2 graph and "Linear Regression". Record the value for m (the slope of the regression line and therefore the rate of O_2 uptake) in the appropriate box in the table below. Repeat for the CO_2 graph.
10. Return the earthworms to their container and clean and dry the chamber.
11. Confirm that your data logger is connected to the HP laser printer in the lab (tap on the wi-fi icon in the bottom right-hand corner to see the available connections and the current connection).
12. To print the currently displayed graph, tap on the "File" tab in the top menu, select "Print" then "Graph". You will have an opportunity to add a label to your graph before it is printed. Make sure to print copies for every member of your group.

Table 3. Metabolic rate of earthworms

	Total weight of earthworm's in grams	Rate of O_2 uptake (ppm/sec)	Rate of respiration (ml O_2/min)	Metabolic rate (ml O_2/min/g body weight)
Earthworms				

Refer to the previous experiment for the technique for converting ppm/sec to ml/min and complete the calculation of the metabolic rate for the earthworms.

Answer the following questions using the data from the table and your knowledge of respiration.

1. This experiment was carried out at room temperature (about 20^{o}C). If the same experiment was carried out at a higher or lower temperature, what change (if any) would you expect to observe in metabolic rate?

2. Most animals that we are familiar with carryout gas exchange by respiration (breathing in and out). Earthworms must also carryout gas exchange. How do you think this is carried out? Is there anything you can observe about the earthworms that supports your idea?