WARD'S

AP Biology Lab 5
Cellular Respiration
Lab Activity
Student Study Guide

BACKGROUND

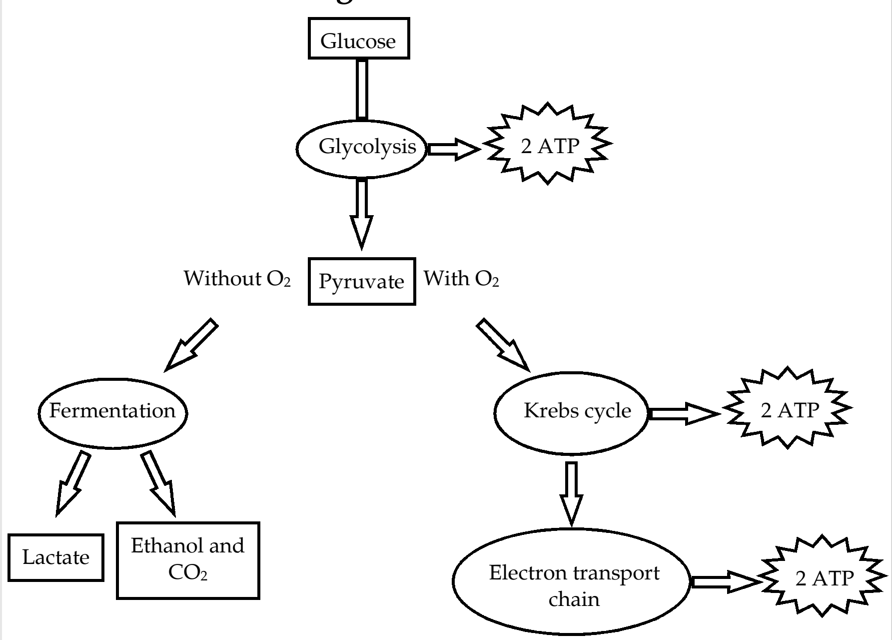
Aerobic respiration:
Oxygen is used in the respiratory breakdown of an organic substrate.

Anaerobic respiration:
Oxygen is not used in the respiratory breakdown of an organic substrate.

In order for our bodies to function, we need fuel. This fuel comes from the foods we eat in the form of proteins, carbohydrates, and fats. Of these organic compounds, it is the carbohydrate glucose that is the most preferred energy source for our bodies. However, in order for this energy to be useful to us, it must first be converted to ATP (adenosine triphosphate), the common carrier of chemical energy in the cell.

All body cells split glucose molecules to transfer the energy to ATP through a process called cellular respiration. This energy transfer occurs in two stages; glycolysis and aerobic respiration. In the first stage, a small amount of ATP is produced when glucose is broken down to pyruvate during glycolysis. In the second stage, the pyruvate either passes through aerobic respiration (producing a large amount of ATP) or anaerobic fermentation (producing a small amount of ATP). In the absence of oxygen, pyruvate is converted to either lactate or ethanol and carbon dioxide (Figure 1).

Figure 1



Anaerobic Respiration **Aerobic Respiration**

In eukaryotic cells, aerobic respiration occurs in the mitochondria but in prokaryotic cells this occurs in the cell membrane. ATP provides the energy used for synthetic reactions, active transport, and all cell processes.

The overall equation for the breakdown of glucose can be represented in the following way:

Glucose + Oxygen → Carbon Dioxide + Water + Energy (ATP)

DID YOU KNOW?
Glycolysis means "the splitting of glucose" and originates from the Greek words: *glykys* meaning "sweet" and *lysis* meaning "to dissolve".

The energy-rich carbohydrates that undergo cellular respiration in plants and animals are produced in the chloroplasts of plants and algal cells. Photosynthesis uses carbon dioxide, water, and light energy to produce oxygen and carbohydrates. Cellular respiration uses carbohydrates and oxygen to produce carbon dioxide, water, and energy for the activities of the cell. Cellular respiration, then, is essentially the reverse of photosynthesis. It is the link between the energy captured by plant cells and the energy utilized by both plant and animal cells; the means by which energy flows from the sun through living things.

Glucose Metabolism

Most cells use glucose as an energy source for producing ATP. The first stage of glucose metabolism is referred to as glycolysis, a process that occurs outside the mitochondria in the cytosol. Glycolysis is anaerobic, meaning that it does not require oxygen. Glucose is converted to two molecules of pyruvate with little breakdown; however, oxidation by removal of hydrogen atoms ($e^- + H^+$) does occur, and the energy of oxidation is used to generate two molecules of ATP. A small amount of NADH is also produced.

DID YOU KNOW?
Diabetes is a disease in which your body is unable to properly use and store glucose. Glucose backs up in the bloodstream, causing your blood glucose or "sugar" to rise too high.

The fate of pyruvate, the end product of glycolysis, depends on the availability of oxygen. It can either be involved in fermentation or incorporated into the Krebs Cycle. Under anaerobic conditions, fermentation occurs within the cytosol and pyruvate accepts the two molecules of hydrogen that were removed from glucose during glycolysis. Fermentation yields only two ATP molecules altogether.

Pyruvate can be reduced to lactate; the process is known as lactate fermentation. The lactate is used by many bacteria and some animal cells. Pyruvate reduced to alcohol with the release of carbon dioxide or the process of alcoholic fermentation, is utilized by yeast and most plant cells.

Cellular Respiration

If oxygen is available to the cell, cellular respiration occurs. This is an aerobic process that produces much more energy than fermentation. In this case, pyruvate is not reduced, but instead enters mitochondria and is completely oxidized to carbon dioxide and water. The removal of hydrogen atoms ($e^- + H^+$) is accompanied by the release of carbon dioxide, which diffuses out of the mitochondria and the cell; the hydrogen atoms are sent to an electron transport system located in the mitochondrial cristae, which are folds of the inner membrane.

As electrons are passed from one carrier to the next in the cristae, energy is made available that is eventually used to generate ATP molecules. Oxygen, the final acceptor for these electrons, is reduced to water. Most of the ATP production in cells comes from this electron transport system.

DID YOU KNOW?
Oxidation-reduction reactions are reactions that involve the transfer of electrons. When a substance accepts electrons, it is said to be reduced. When a substance releases electrons, it is said to be oxidized.

If glycolysis is followed by cellular respiration, then the overall reaction for glucose breakdown to carbon dioxide and water can be represented as:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 36\ ATP + 6CO_2 + 6H_2O$$

The complete oxidation of glucose by cellular respiration yields a total of 36 ATP molecules, most of which are produced by the electron transport system. It is possible to calculate the ATP yield for the complete breakdown of glucose to carbon dioxide and water.

Oxidative Respiration

In the presence of oxygen, the pyruvate from glycolysis enters a mitochondrion, where a series of reactions called oxidative respiration occurs. This is the transition reaction between glycolysis and the Krebs Cycle. The electrons made available are passed to the electron transport system by either NAD^+ or FAD. The reactions of oxidative respiration drive the production of large amounts of ATP.

In order to prepare for the ATP-producing part of oxidative respiration, the pyruvate molecules from glycolysis are first converted from three- to two-carbon fragments. As this occurs, a carbon dioxide molecule and a hydride ion with high-energy electrons are removed from each pyruvate molecule. The carbon byproduct leaves the mitochondrion and then the cell. The donation of hydride ions to an NAD^+ molecule forms NADH, which is later used in oxidative respiration. The remaining two-carbon fragment, an acetyl group, is attached to coenzyme A to form a compound called acetyl-CoA. Acetyl-CoA is funneled into fat synthesis if the cell's supply of ATP is plentiful, allowing high-energy electrons to be stored for later needs. If the cell needs ATP immediately, however, acetyl-CoA is directed to the next phase of oxidative respiration, which is the Krebs Cycle.

DID YOU KNOW?
Hans Adolf Krebs, in 1953, received the Nobel Prize for Physiology or Medicine for the discovery in living organisms of the series of chemical reactions known as the tricarboxylic acid cycle, or the Krebs cycle.

Krebs Cycle

The Krebs Cycle is a repeating series of reactions that produces ATP, electron carriers, and carbon dioxide. The cycle begins when the two-carbon fragment of acetyl-CoA is attached to a four-carbon molecule found in mitochondria. This forms a six-carbon compound called citrate; it is for this reason the Krebs Cycle is also known as the citric acid cycle. During the cycle, oxidation occurs, and most of the hydrogen atoms are donated to NAD^+, except for one instance where the hydrogen atoms are donated to FAD, another coenzyme of oxidation and reduction in cells. Some of the energy of oxidation is used immediately to form ATP by substrate-level phosphorylation, as in glycolysis. Since the Krebs Cycle occurs once for acetyl-CoA, it occurs twice for each glucose molecule.

Electron Transport System

Due to the transition reaction and the Krebs Cycle, the carbons of glucose are completely oxidized to six molecules of CO_2. The hydrogen atoms ($e^- + H^+$) that were removed from the substrates of these metabolic pathways are donated by NADH and $FADH_2$ to the electron transport system. This system is a series of carriers and enzymes located on the cristae of mitochondria. It is also known as the cytochrome system, since some of the electron carriers are cytochromes. Each electron transport chain passes high-energy electrons to proton-pumping membrane channels. As electrons are passed from one carrier to the next, oxidation occurs, and the energy released is used to form ATP molecules. (ATP leaves a mitochondrion through other protein channels, entering the cytosol where it can be used by other reactions). This process is sometimes known as oxidative phosphorylation because oxygen receives the energy-spent electrons from the chain. The enzyme cytochrome oxidase splits and reduces molecular oxygen (O_2) to water:

$$\mathbf{{}^1/_2O_2 + 2e^- + 2H^+ \rightarrow H_2O}$$

The final acceptor for electrons during cellular respiration is oxygen.

DID YOU KNOW?
Substrate-level phosphorylation synthesizes ATP in one step without the use of intermediates, while oxidative phosphorylation has intermediates.

Substrate-Level Phosphorylation

For every glucose molecule, four ATP are formed directly by substrate-level phosphorylation — two during glycolysis and one during each of the two turns of the Krebs Cycle.

Oxidative Phosphorylation

For every glucose molecule, ten molecules of NADH and two molecules of $FADH_2$ take hydrogen atoms ($e^- + H^+$) to the electron transport system. For each molecule of NADH produced inside the mitochondria by the Krebs Cycle, three ATP are produced by the electron transport system, but for each $FADH_2$ there are only two ATP. This is because $FADH_2$ delivers its hydrogen to the transport system at a lower level, pumping only $4H^+$ instead of $6H^+$ into the intermembrane space per molecule of $FADH_2$.

A shuttle mechanism for the ATP yield of the NADH generated outside the mitochondria via glycolysis is used, since NADH cannot cross mitochondrial membranes. This shuttle, which consists of an organic molecule that can cross the outer membrane, allows electrons to be delivered to the electron transport system inside the mitochondria. The "shuttle" molecule crosses the outer membrane, accepts the electrons, and delivers them to an FAD molecule in the inner membrane. At this point, the FAD can produce two ATP molecules; therefore, the NADH produced in the cytosol results in the production of only two ATP rather than three.

DID YOU KNOW?
In 1662, Robert Boyle published what is known as Boyles Law: at a constant temperature, the volume of gas is inversely proportionate to the pressure.

Respiration applies to two distinct but interrelated processes: the active acquisition of oxygen by an organism and the release of energy by the breakdown of organic compounds by metabolic chemical oxidation within cells. Various factors, such as temperature change, will affect respiration, as well as the chemical oxidation of glucose. Oxygen consumption during respiration can be measured, with a respirometer, as a change in gas volume.

A number of physical laws related to gases are important to the understanding of how the respirometer works. The laws are summarized in the general gas law of PV = nRT.

P: pressure
V: volume
n: number of molecules
R: gas constant
T: temperature

DID YOU KNOW?
The fruit-ripening process may be greatly slowed down by using cold storage methods (refrigeration), which reduces the respiration rate of fruit.

This law summarizes the following important concepts about gases:

- Given a constant temperature and pressure, the volume of the gas is directly proportional to the number of molecules of the gas.
- Given a constant temperature and volume, the pressure of the gas changes in direct proportion to the number of molecules of gas present.
- Given a constant number of gas molecules and temperature, the pressure is inversely proportional to the volume.
- Given a change in temperature while the number of gas molecules remain constant, then either pressure or volume, or both, will change in direct proportion to the temperature.
- Gases and fluids flow from a high-pressure area to a low-pressure area.

Potassium hydroxide (KOH) can be used to remove the CO_2 produced during cellular respiration and form solid potassium carbonate (K_2CO_3) by the following reaction:

$$\mathbf{CO_2 + 2KOH \rightarrow K_2CO_3 + H_2O}$$

Therefore, the change in volume of gas in the respirometer will be directly related to the amount of oxygen consumed.

DID YOU KNOW?
A respirometer is an apparatus for measuring the amounts of gases used during respiration.

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.

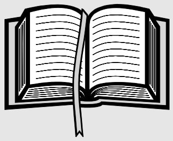
OBJECTIVES

- 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

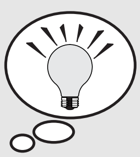
MATERIALS

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



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.

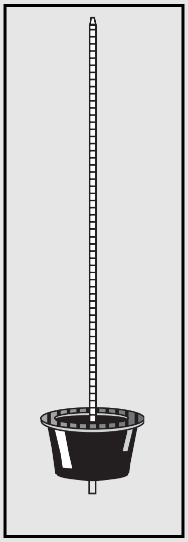
5. Refill the graduated cylinder with 50 ml water. Add glass beads until the water level is the same as that of the germinating peas. Remove the beads and place them on a paper towel; pat the beads dry and set aside.

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



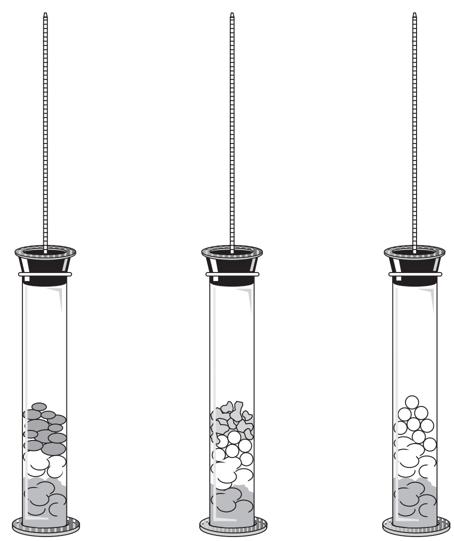
8. Without getting any liquid on the sides of the respirometers, use a pipet to add 1 ml 15% potassium hydroxide (KOH) to the cotton. Add a piece of non-absorbent rayon that is slightly smaller than that of the cottonball and place it on top of the KOH-soaked cotton.

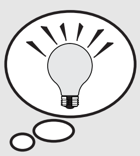
9. Using the first set of germinating peas, non-germinating peas and glass beads, and glass beads, place them in vials 1-3, respectively. Repreat this procedure using your second set of germinating peas, non-germinating peas and glass beads, and glass beads for vials 4-6.

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.

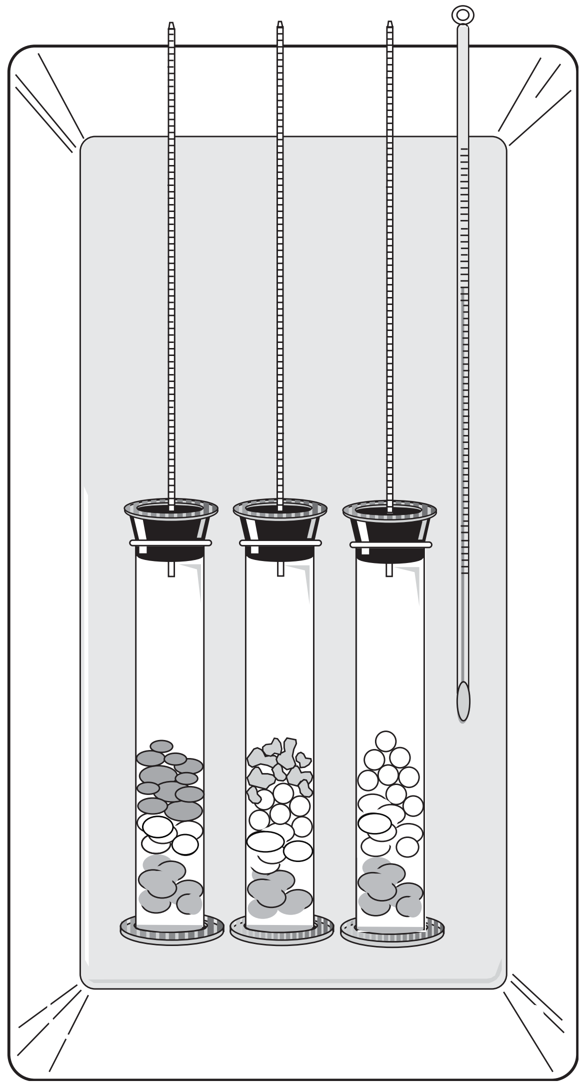
Figure 3





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.

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?
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.

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.

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.

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.