LAB 12:
Cellular Respiration

I. Objectives:

Upon completion of this topic you should know:

- The role of glucose and ATP in the powering of cellular reactions
- How germination affects aerobic respiration in bean seeds
- How temperature affects aerobic respiration bean seeds
- How aerobic respiration differs from fermentation

II. Safety Considerations:

- The 15% KOH is caustic, so avoid contact with it.

III. Introduction:

All cells must acquire and then use energy to carry out their necessary functions (moving, growing, dividing, etc). Cells acquire their energy by either using sunlight to make the high-energy molecule glucose, or by consuming organisms that have glucose (or other high-energy molecules). Regardless of how the glucose is obtained, all organisms (even plants) must then break down glucose in order to harness the stored energy and do something useful. It may be surprising that no cellular process is powered directly by the breakdown of glucose. Glucose stores so much energy that if it were all released at once, it could damage the cell. In much the same way your car burns its gasoline a small amount at a time (instead of in one big fiery explosion), cells harvest the chemical energy of glucose a small amount at a time over several steps. Collectively, the set of chemical reactions used to harvest the chemical energy of glucose as it is broken down into carbon dioxide (CO_2) and water is called **cellular respiration.** Through cellular respiration, the chemical energy in glucose is used to create a large number of high-energy molecules of adenosine triphosphate--**ATP**.

ATP is an extremely abundant molecule in the cell and is used directly to power a large number of energy-consuming activities of the cell. Because of this, it is sometimes referred to as the "universal energy currency" of cells. ATP is a relatively simple molecule that is formed by putting a third phosphate group onto a molecule of adenosine diphosphate (ADP). The addition of the third phosphate requires energy, which is then stored in the bond once the bond is formed (see Figure 1). Enzymes needing energy to power a reaction break the phosphate bond of ATP to liberate and use the chemical energy it stores.

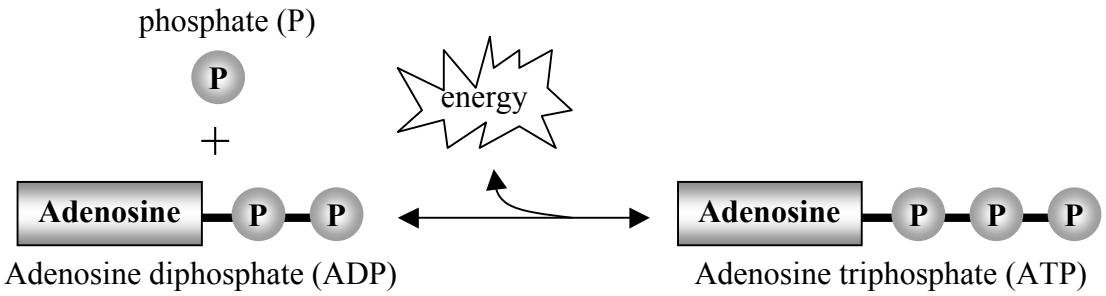


Figure 1. ATP Synthesis
ATP synthesis requires energy, while its breakdown to ADP and phosphate gives off energy. The reaction is reversible.

The purpose of cellular respiration is to harvest the chemical energy stored in glucose to synthesize ATP from ADP and phosphate. For most cells, cellular respiration can be divided into two general steps; glycolysis and oxidative phosphorylation. **Glycolysis** is the set of chemical reactions that starts the breakdown of glucose. These reactions take place in the cytosol and do not require oxygen (O_2) and are therefore **anaerobic**. If oxygen is available, the pyruvate produced in glycolysis will be used in **oxidative phosphorylation** in the next set of chemical reactions. These reactions occur in the mitochondria of the cell and continue the process of glucose breakdown. Oxidative phosphorylation produces much more ATP than glycolysis, but it requires O_2. Because it requires O_2, oxidative phosphorylation is called **aerobic** ("with air") **respiration**. Figure 2 illustrates the steps of cellular respiration.

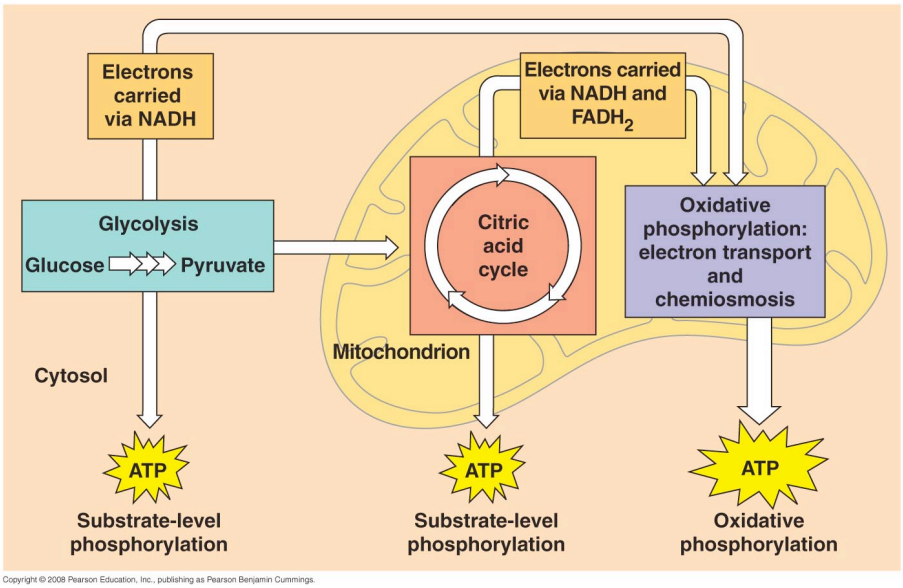


Figure 2. Overview of Cellular Respiration. (From Campbell et al., 2009)

IV. Things To Do:

We will utilize germinating bean seeds to examine the process of cellular respiration that is summarized in the chemical equation:

$$C_6H_{12}O_2 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 36\ ATP\ (ENERGY)$$

As the equation above indicates, there are two gases (oxygen and carbon dioxide) involved in cellular respiration. Note that for every molecule of oxygen that is consumed, one molecule of carbon dioxide is produced. Because of this relationship, the total volume of gas in a closed chamber containing bean seeds carrying out respiration would theoretically remain unchanged (although its composition would change). However, if the CO_2 could be removed as fast as it is evolved, the volume of gas in the chamber would decrease and this decrease would reflect the volume of oxygen consumed by the seeds. This latter principle is used in the following exercise to provide a method for measuring aerobic cellular respiration rate. You will add 15% KOH to the chambers you will set up as described below. The CO_2 that is produced in cellular respiration will react with the KOH to produce solid potassium carbonate (K_2CO_3) according to the equation:

$$CO_2 + 2\ KOH \rightarrow K_2CO_3 + H_2O$$

You will measure the resulting change in volume using your respirometers to determine respiration rates.

Procedure (Figures, Tables and Procedures from: Ward's, 2002)**:**

1. Set up an ice water bath 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. Place a Styrofoam pad under the ice water bath to insulate it from the benchtop.

2. Obtain six vials with steel washers on the bottoms. Number the vials 1 through 6 with a Sharpie or grease pencil.

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 for vial 1. 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. Repeat this with a new set of germinating peas for vial 4 and record the data below.

   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 nongerminating peas, and then add glass beads until the water level is the same as that of the germinating peas in vial 1. Remove the peas and beads and place them on a paper towel; pat the peas and beads dry and set aside. These will go in vial 2.

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 in vial 1. Remove the beads and place them on a paper towel; pat the beads dry and set aside. These will go in vial 3.

6. Repeat steps 4 and 5 with more nongerminating peas and beads, and beads – this time comparing the water levels with the germinating peas for vail 4. Set these aside set aside for vials 5-6.

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.

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.

10. Repeat this procedure using your second set of germinating peas, non-germinating peas and glass beads, and glass beads for vials 4-6.

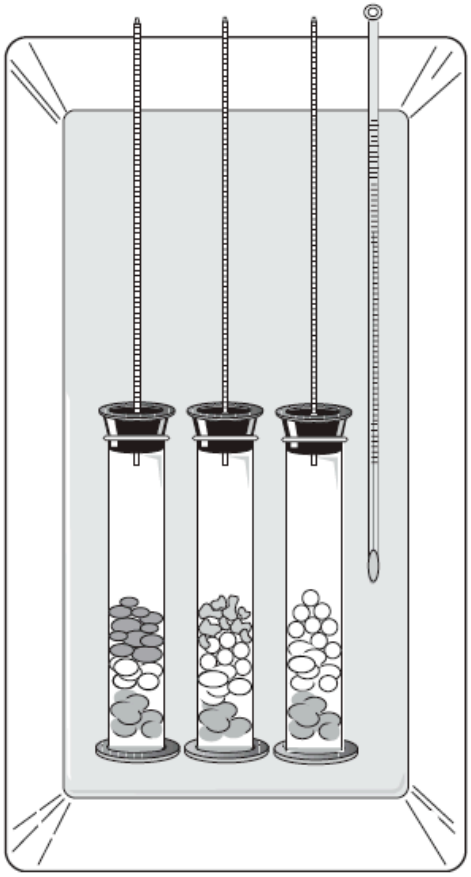
11. Insert the non-tapered end of a graduated pipet into the wide end of a stopper so that the tapered end of the pipet is furthest from the stopper and so that the pipet extends just beyond the bottom of the stopper.

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

Figure 3

Germinating peas | Non-germinating peas and beads | Beads

Figure 4



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

14. 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 water baths. Do not touch the respirometers once the experiment has started! Let the respirometer equilibrate for another 5 minutes before proceeding to step 15.

NOTE: 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 11 and 12 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.

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

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

17. 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 0 – pea seed reading at time X) – (initial bead reading at time 0 – bead reading a time X)

NOTE: 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.

18. 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 water baths. Plot the time in minutes.

Table 1. Respiration in bean seeds.

			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				—	—		—	—		—

References:

Campbell NA, Reece JB, Urry LA, Cain ML, Wasserman SA, Minorsky PV, Jackson RB. (2009) Biology. 8th ed. San Francisco (CA): Pearson, Benjamin Cummings.

Ward's AP Biology Lab 5. 2002 WARD'S Natural Science Establishment, Inc

Record the answers to the following questions in your lab notebook:

1. Which used the most oxygen, germinating seeds or non-germinating seeds? What is a reasonable explanation for this?
2. How did the respiration rate compare between germinating seeds in the ice bath and those at room temperature?
3. How can you account for any differences that you observe?

4. How do you think the respiration rate of a mouse or human would compare with bean seeds under each of these conditions? Why do you conclude this?
5. Were respiration and fermentation affected by temperature in the same way? What is a reasonable explanation for this?