h. Optional: If your instructor asks that you add food coloring to the water in the pipet, now is the time. Hang a drop of food coloring from the bottle and allow capillary action to pull the drop into the pipet. Place a paper towel beneath the tip to catch any excess food coloring.

4. Place the set of respirometers (1, 2, and 3) in the water bath with their pipet tips resting on the lip of the tray, as shown in Figure 3. Wait 5 minutes before proceeding. This is to allow time for the respirometers to reach thermal equilibrium with the water. If any of the respirometers begins to fill with water, you have a leak and must start over.

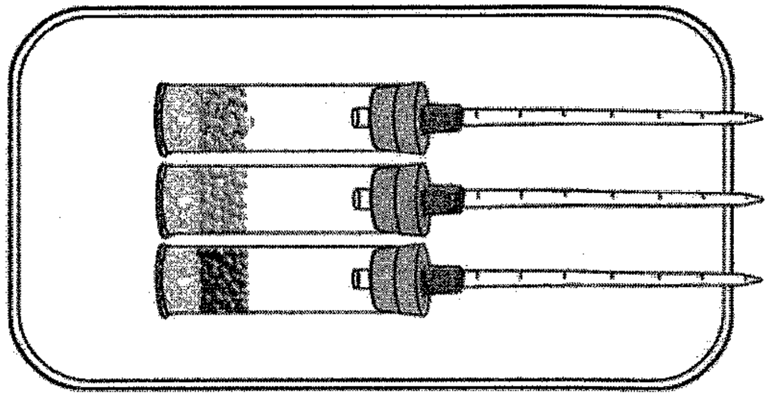

Figure 3. Respirometers in the water bath

5. After the equilibration period, immerse all the respirometers (including the pipet tips) in the water bath. Position the respirometers so that you can read the scale on each of the pipets. (The paper in the tray underneath the pipets makes them easier to read.) Do not put anything into or take anything out of the water bath until you have taken all the necessary readings.
6. Allow the respirometers to equilibrate for another 5 minutes.
7. Observe the initial volume reading on the scale to the nearest 0.01 mL. Record the data in Table 1 for Time 0. Also, observe and record the temperature of the water. Repeat your observations for all three samples. Every 5 minutes for 20 minutes, record the temperature of the water and take readings of the volume of air in each of the three pipets. Record your data in Table 1 and then perform the calculations necessary to complete the table.

Table 1: Respiration of Peas at Room Temperature

		Respirometer 1 Germinating Peas			Respirometer 2 Dry Peas + Beads			Respirometer 3 Beads Only	
°C	Time (Min)	*V* of Pipet	Δ*V*	Corrected Δ*V*	*V* of Pipet	Δ*V*	Corrected Δ*V*	*V* of Pipet	Δ*V*
24	0		–	–		–	–		–
24	5								
24	10								
24	15								
23	20								

$\Delta V = V$ at Time 0 – V at time of current reading
Corrected $\Delta V = \Delta V$ (for Respirometer 1 or Respirometer 2) – ΔV of Respirometer 3

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Guided Activity

Materials

tray of room-temperature water	3 rubber stoppers
25 germinating pea seeds	15% KOH solution (shared)
25 dry pea seeds	dropping pipet
beads	forceps
3 respirometers	paper
50-mL graduated tube	stopwatch, timer, or clock with a second hand
3 absorbent cotton balls	thermometer
nonabsorbent cotton	

Procedure

1. Set up the water baths. The water bath will buffer the respirometers against temperature change during the experiment.
   a. Place a sheet of paper in the bottom of the water bath. This makes the graduated pipet easier to read.
   b. Place a thermometer in each tray. Observe the thermometer to make sure the temperature of the water bath is stable.
2. Prepare the peas and beads.
   a. For Respirometer 1, put 25 mL of water in your 50-mL graduated tube. Drop in 25 germinating peas. Determine the volume of water that has been displaced, which is equivalent to the volume of peas. Record the volume of the germinating peas. Remove these peas and place them on a paper towel.
   b. For Respirometer 2, refill the graduated tube to 25 mL by adding water. Drop 25 dry, dormant peas into the tube. Next, add enough beads to equal the volume of the germinating peas. Remove the dormant peas and beads, and place them on a paper towel.
   c. For Respirometer 3, refill the graduated tube to 25 mL with water. Add enough beads to equal the volume of the germinating peas. Remove these beads and place them on a paper towel.
3. Prepare samples.
   a. Place an absorbent cotton ball in the bottom of each respirometer vial.
   b. Use a dropping pipet to saturate the cotton with 2 mL of 15% KOH solution. (Caution: Avoid skin contact with KOH. Be certain that the respirometer vials are dry on the inside. Do not get KOH on the sides of the respirometer.)
   c. Place a small wad of dry, nonabsorbent cotton on top of the KOH-soaked absorbent cotton. The nonabsorbent cotton will prevent the KOH solution from contacting the peas. It is important that the amount of cotton and KOH solution be the same for all three respirometers.
   d. Place 25 germinating peas in the vial of Respirometer 1.
   e. Place 25 dry peas and beads in the vial of Respirometer 2.
   f. Place the equivalent volume of beads in the vial of Respirometer 3.
   g. Insert a stopper fitted with a calibrated pipet into each respirometer vial. The stopper must fit tightly. If the respirometers leak during the experiment, you will have to start over.

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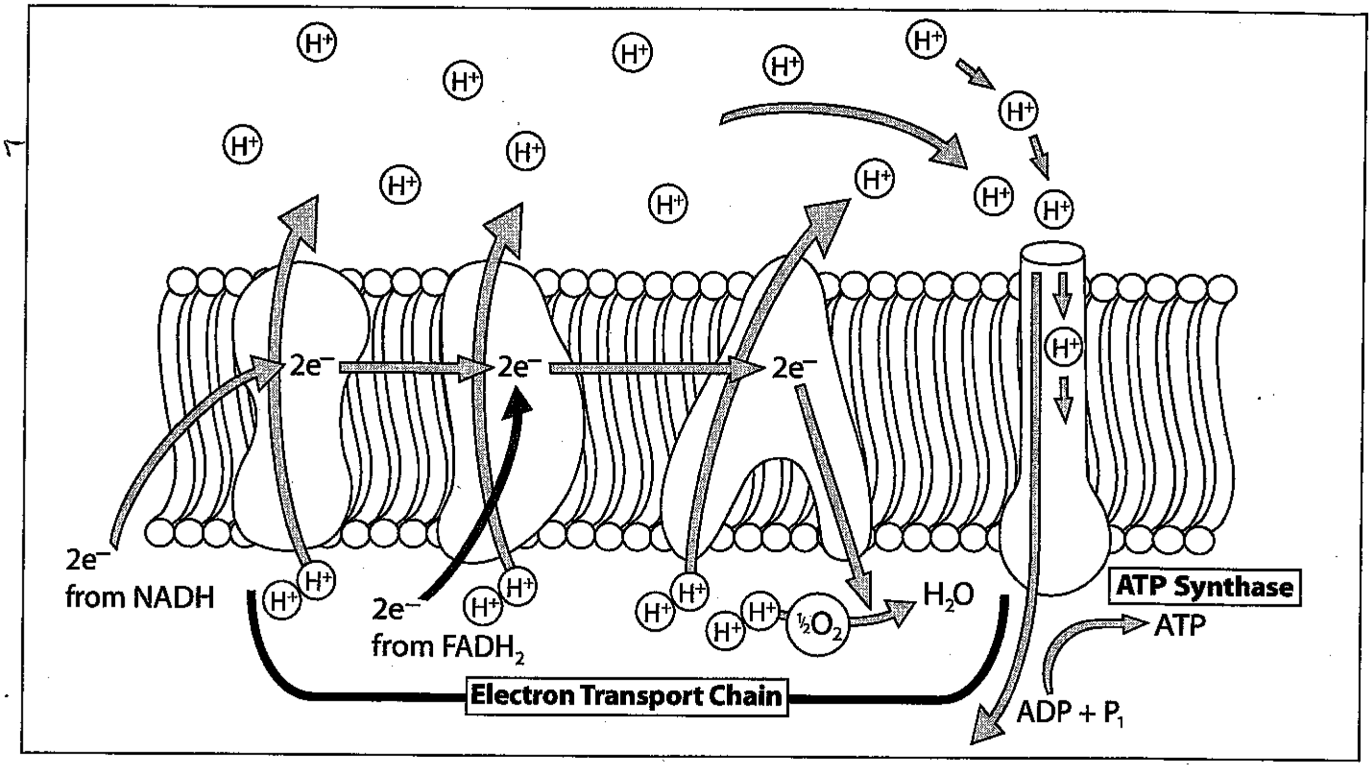

Figure 2. Electron transport chain and ATP synthase

at the end of the electron transport chain, it enables more electrons to be passed through. If oxygen is not available to pick up electrons, no more electrons can enter the system from NADH and $FADH_2$. Thus, in the absence of oxygen, the electron transport and Krebs cycle processes cease to function.

Using a Respirometer

You will use a respirometer to measure the rate of respiration of germinating and dormant pea seeds. The respirometer is composed of a vial that contains the peas and a volume of air; the mouth of the vial is sealed with a rubber stopper with a pipet inserted into the hole. During the experiment, the respirometer is submerged in water. If the peas respire, they will use oxygen and release carbon dioxide. Because 1 mole of carbon dioxide is released for each mole of oxygen consumed, there is no change in the volume of gas in the respirometer. (Avogadro's Law: At constant temperature and pressure, 1 mole of any gas has the same volume as 1 mole of any other gas.) You will alter this equilibrium by placing a solution of potassium hydroxide (KOH) in the vial. Potassium hydroxide reacts with carbon dioxide to form potassium carbonate, which is a solid. The following reaction occurs:

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

Because the carbon dioxide produced is removed by reaction with potassium hydroxide, as oxygen is used by cellular respiration, the volume of gas in the respirometer will decrease. As the volume of gas decreases, water moves into the submerged pipet. You will use this decrease of volume, as read from the scale printed on the pipet, as a measure of the rate of respiration.

Pre-laboratory Questions

1. During respiration, a seed metabolizes sugars. What is the source of the sugar metabolized by the seed?

2. What variables do you think affect the respiration rate of seeds? Brainstorm within your group to generate a list.

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Glycolysis

Glycolysis is a 10-step series of reactions that splits a 6-carbon glucose molecule into two 3-carbon molecules called pyruvate. The process occurs in the cytoplasm of a cell. As glucose is broken down, four ATPs and two NADH molecules are produced. Because two ATP molecules are required to start the reaction, there is a net gain of two ATP.

If oxygen is present, cells may undergo aerobic cellular respiration to produce more energy by further breaking down the derivatives of glucose. All higher organisms and many microorganisms have the necessary enzymes to perform aerobic cellular respiration.

Process	Starting Material	Net Energy Output
Glycolysis	1 glucose	2 NADH, 2ATP
Acetyl-CoA synthesis and Krebs Cycle	2 pyruvate	8 NADH, 2 $FADH_2$, 2 ATP
Electron Transport Chain	10 NADH, 2 $FADH_2$	32 ATP

Acetyl-CoA synthesis and Krebs Cycle

If oxygen is present, the pyruvate made during glycolysis enters the mitochondria of the cell, where it is initially converted into a molecule called acetyl-CoA. As this happens, one molecule of carbon dioxide (CO_2) and one molecule of NADH are produced. The acetyl-CoA is then broken down through a series of six reactions referred to as the Krebs cycle or the citric acid cycle. For each acetyl-CoA broken down, two CO_2 molecules are produced, and some of the energy released from its bonds is stored in ATP. Some of the energy also passes as electrons to the electron carrier molecules NAD+ and FAD, yielding NADH and $FADH_2$. The electrons held in these electron carriers can be used to make additional ATP through the electron transport chain.

Electron Transport Chain

The NADH and $FADH_2$ produced during glycolysis and the Krebs cycle are used to generate more ATP in the electron transport chain. NADH and $FADH_2$ molecules are called electron carriers because they transport electrons and associated hydrogen atoms to a series of membrane-embedded proteins called the electron transport chain. Here, the hydrogens and electrons are stripped from the electron carriers, leaving NAD+ from NADH and FADH from $FADH_2$. These molecules constantly cycle between forms within the cell.

As the electrons from the NADH and $FADH_2$ are passed between the proteins of the electron transport chain, energy is released and used to shuttle the hydrogen ions into the intermembrane space of the mitochondria. As the ions accumulate, they form a gradient across a membrane. This gradient creates a type of pressure that causes hydrogen ions to rush through a channel in the membrane formed by a protein called ATP synthase. ATP synthase functions somewhat like a windmill; the rush of hydrogen ions spins a component of ATP synthase, which drives production of ATP. In other words, ATP synthase adds another phosphate group to ADP. In eukaryotic cells, the electrons from each NADH yield three ATP molecules, and each $FADH_2$ yields two ATP molecules. The electron transport chain produces 16 ATP molecules for each pyruvate molecule. Remember that there are two pyruvate molecules from every glucose molecule that enters the system. Thus, from aerobic respiration of each glucose molecule, approximately 36 ATP molecules are made.

Oxygen comes into play only during the final step of the electron transport chain. Remember that electrons are passed from protein to protein. When the electrons get to the end of the chain, they do not simply "fall off." The electrons, along with the hydrogens, are passed to oxygen, resulting in the formation of water (H_2O). For this reason, oxygen (O_2) is called the terminal electron carrier. As oxygen picks up electrons

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NAME ______________________________

DATE ______________________________

Carolina™ Cell Respiration for AP Biology

Background

You are probably familiar with photosynthesis, the metabolic process that plants use to harness energy from the sun. But how do plants acquire energy when they germinate underground, out of the reach of sunlight? They metabolize sugars much like humans do, through cellular respiration.

All cells need energy. Energy is contained in the molecular structure of organic compounds such as carbohydrates, proteins, and fats. Carbohydrates, also called sugars, are the primary source of cellular energy. When the bonds of a carbohydrate molecule are broken, in a series of small steps and with the help of specific enzymes, energy is released from the bonds. The energy, stored in a molecule called adenosine triphosphate (ATP), can then be used by the cell.

ATP is the chief energy source of cells. It stores energy in the structure of its three-phosphate tail. The removal of a phosphate from the ATP molecule releases energy that powers almost all metabolic processes. When a phosphate is removed, adenosine triphosphate becomes adenosine diphosphate (ADP). Through cell respiration, fermentation, and other metabolic processes, there is a constant cycling between ATP and ADP.

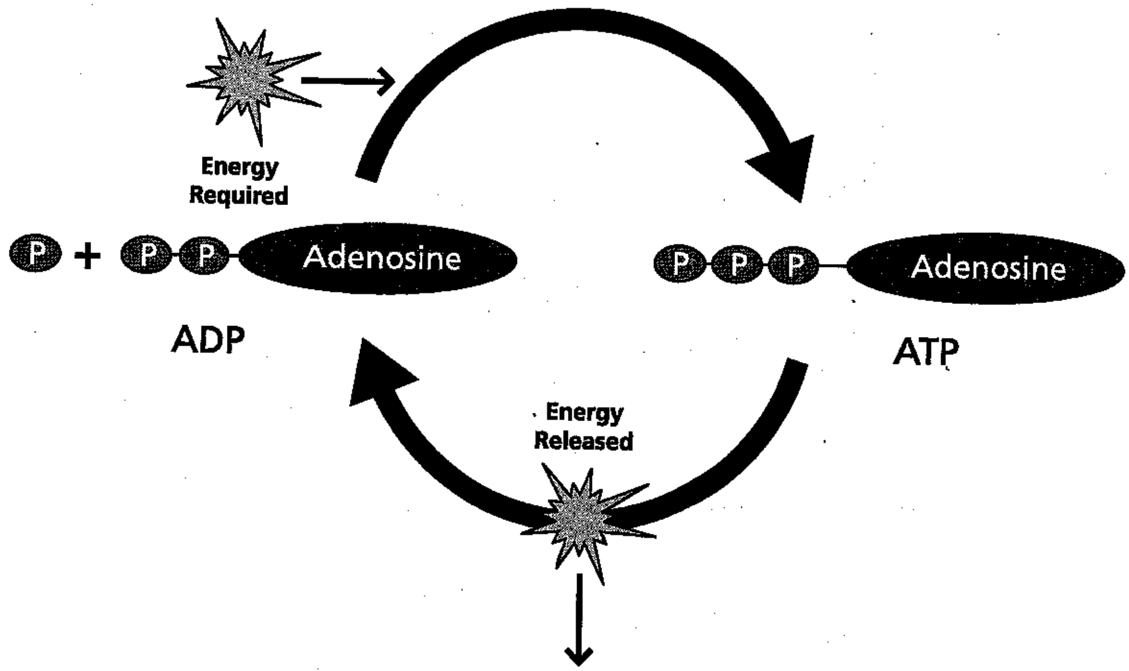

Figure 1. Cycling of ATP and ADP

In this lab, we will focus on aerobic cellular respiration. The series of reactions that occurs during aerobic cellular respiration is grouped into steps called glycolysis, Acetyl CoA synthesis, Krebs cycle, and electron transport chain. The breakdown of the sugar molecule is summarized by the following equation:

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

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