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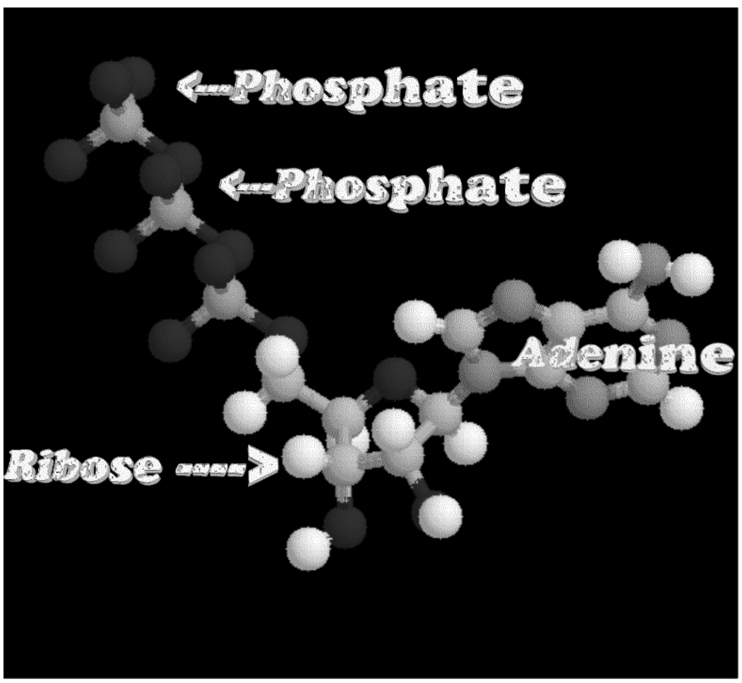
5:7 Respiration lab

Introduction

In biology, **respiration** refers to both the movement of air to and from our lungs and to the process by which our cells release the energy contained in food to do work. In all eukaryotic organisms, including fungi, plants and animals, energy in the form of ATP is released from the breakdown of glucose within the cells. The glucose reacts with oxygen in the reaction of respiration. The first stage of respiration is **glycolysis**, which occurs in the cytoplasm. The pyruvic acid that results from glycolysis is further broken down inside the mitochondria to carbon dioxide, in the Krebs cycle and Electron Transport chain. The energy from respiration is stored in the cell as ATP (Adenosine Tri-Phosphate).



Breathing allows us to acquire oxygen for respiration while disposing of the carbon dioxide generated as shown by the equation below.

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

glucose   oxygen   carbon dioxide   water   energy

Thus, if we want to measure the respiration of an organism we can measure the rate of oxygen consumption or the production of carbon dioxide or both. Note that the same number of molecules of oxygen is used, as the number of carbon dioxide molecules produced.

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In our experiment, we will measure the relative change in the rate of oxygen consumption between germinating and non-germinating peas at three different temperatures. In brief, the respirometer is a tube with a rubber stopper that has an opening for a graduated pipette tip. When the system is fully assembled and submerged in a water bath, the water enters the pipette tip until a point in which the pressure inside and outside of the respirometer is equal. In general, the system follows the *Ideal Gas Law* and the equation below.

$$PV = nRT.$$

Each component of the gas is represented by P (pressure), V (volume), n (number of molecules of gas), and T (temperature). R is the universal gas constant and is given by 8.31 J/(mol*K).

The Ideal Gas law states the following.

1) Gases move from regions of high pressure to low pressure.
2) When pressure and temperature are kept constant, the volume of gas is directly related to the number of molecules of gas.
3) When temperature and volume are kept constant, the pressure of gas is directly related to the number of molecules of gas.
4) When the number of molecules of gas and the temperature are kept constant, the pressure is inversely related to the volume.
5) When the temperature varies and the number of gas molecules is kept constant, the pressure or volume or both is directly related to the temperature.

When you assemble the respirometer, you should have potassium hydroxide (KOH) in the bottom of the respirometer, then a layer of cotton and then the peas. **Note: do not touch the potassium hydroxide with your bare hands. Always use a spatula to transfer the KOH.** Because the KOH absorbs gaseous CO_2 (that is produced during respiration) to produce a solid potassium carbonate (K_2CO_3), the setup will allow you to measure the **rate of oxygen consumption** during cellular respiration.

<u>Materials:</u>

Germinating Peas
Heat-treated Peas (Non-germinating peas)
Respirometers (Test tube, Rubber stopper, Graduated pipette tip)
KOH pellets
Water baths and ice
Thermometers
Beads

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<u>Procedure:</u>

Set up three water baths, with cold water (use some ice) in one, room temperature water in the second and warm water (not so hot that you cannot keep your fingers in it) in the third.

1) What is the temperature of the room temperature bath? ___________________________

2) What is the temperature of the warm water bath? ________________________________

3) What is the temperature of the cold water bath? _________________________________

Fill a 100 ml graduated cylinder with 50 ml of water. Gently place 25 germinating peas into the cylinder and record the displacement of water. Dry the peas on a paper towel. These peas will be placed in the first tube and be labeled respirometer 1.

4) What is the volume of 25 germinating peas? _____________________________________

Refill a 100 ml graduated cylinder with 50 ml of water. Place 25 heat-treated (non-germinating) peas in the graduated cylinder, and then gently add glass beads until the ***same*** volume of water from step 2 is displaced. Remove both peas and beads and dry on a paper towel. These peas and beads will go in a second tube and be labeled as respirometer 2.

Create a second and third set of respirometers by repeating steps 2 and 3. These respirometers will be labeled respirometer 3, 4, 5, and 6, respectively.

For all six test tubes, place an equal amount of KOH pellets in the bottom of the tube. Then place a small wad of cotton over the KOH.

Add the peas, or peas and beads to each respirometer.

Place the rubber stopper with the graduated pipette tip snuggly onto the tube. **Ensure a tight fit.**

Submerge respirometers 1 and 2 into the room temperature bath and allow your system to equilibrate for 5 minutes. Make sure that the peas + beads are completely covered by the water but the pipette tip rests on the edge of water bath container. Submerging the respirometer reduces the temperature changes in the tube.

Submerge respirometers 3 and 4 into the warm water bath for 5 minutes. Again check to see if the peas + beads are completely covered by the water with the pipette tip on the edge of the container.

Repeat this for respirometers 5 and 6 with a cold water bath and let the system equilibrate for 5 minutes.

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After the 5 minutes, submerge the entire system (i.e. pipette tip) into the water. The water will move into the pipette tip until a point when the internal and external pressure is equivalent. (Note: If your respirometer has a leak, the water will continue moving into the pipette instead of maintaining its position.)

Adjust the pipette tip so that you can read the marks without having to move the respirometers after the experiment starts.

Let the respirometers equilibrate for another 3 minutes and then record the position of the water. This is your initial or time 0 reading.

Record the position of the water every 5 minutes for the next 20 minutes in the tables provided below.

Table 1: Oxygen consumption of room temperature-treated peas

	Distance traveled by the water in milliliters (ml)				
Respirometer	0 minutes (initial reading)	5 minutes	10 minutes	15 minutes	20 minutes
1. Peas alone	0				
2. Peas + beads	0				

Table 2: Oxygen consumption of warm water-treated peas

	Distance traveled by the water in milliliters (ml)				
Respirometer	0 minutes (initial reading)	5 minutes	10 minutes	15 minutes	20 minutes
1. Peas alone	0				
2. Peas + beads	0				

Table 3: Oxygen consumption of cold water-treated peas

	Distance traveled by the water in milliliters (ml)				
Respirometer	0 minutes (initial reading)	5 minutes	10 minutes	15 minutes	20 minutes
1. Peas alone	0				
2. Peas + beads	0				

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Graphing

Presenting data as a graph or figure is very important in science. Graphs allow us to see trends in our data that would otherwise look unorganized. In this exercise, you will graph the data you collected with the pea experiment above. The Y-axis is the response variable and the X-axis is the independent variable; that is, the Y-variable changes in response to changes in the X-variable.

5) What variable that you've measured goes on the Y-axis? ___________________________

6) What variable goes on the X-axis? ________________________

When you graph these data, you should be able to calculate the rate of oxygen consumption from the slope of the line for each of the treatments. Remember that you are graphing the difference between the initial reading and the reading at time X. Time X refers to the time at 5 minutes, 10 minutes, etc. For example, if you wanted to know the distance the water moved after 20 minutes, then you would subtract the value for initial position of the water by the value recorded for position of the water at 20 minutes.

What should your graph look like? First, each axis should be labeled correctly with the proper units for each variable. Second, your graph should have a title that describes what is being presented so that anyone not familiar with your experiment can tell what is going on. Third, your figure should have a legend because you are plotting several lines onto one sheet of graph paper to observe how each line compares to the other.

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Created in !GrphPaper, on a StrongARM-powered, RISC-OS Workstation http://start.at/scirep

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Discussion Questions

7) In which direction does the water in the pipette tip move. Why do you think this is the case?

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8) What is the rate of oxygen consumption for each of the treatments?

Treatment	Rate of Oxygen Consumption
Room temperature, Peas alone	*medium*
Warm water, Peas alone	*fast*
Cold water, Peas alone	*slow*
Room temperature, heat-treated peas + beads	*Very slow or zero*
Warm water, heat-treated peas + beads	*Very slow or zero*
Cold water, heat-treated peas + beads	*Very slow or zero*

9) How did temperature influence the rate of oxygen consumption?

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10) This activity uses a number of controls. Identify at least three of the controls.

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11) If you had repeated this experiment with a water bath of 55°C, what results would you expect? Explain why.

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