# AP Lab #6 - Cellular Respiration

16/10/14

## Introduction

Cellular respiration is a process in which the energy in carbon compounds is harvested to make ATP, which powers many vital cellular processes. In eukaryotic cells this process occurs in the mitochondria.

This is the equation for respiration:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{energy}$$

This means that sugars (specifically glucose) and oxygen yield carbon dioxide, water, and energy in the form of heat and ATP. So, if glucose is the energy source, one molecule of carbon dioxide is produced for every molecule of oxygen.

Students will learn how to measure the rate of cellular respiration by using a respirometer system, which measures relative volume as oxygen is consumed by germinating plant seeds. As mentioned, carbon dioxide usually replaces each molecule of oxygen, however, in this procedure the produced $CO_2$ will be removed by potassium hydroxide, forming potassium bicarbonate ($K_2CO_3$), which is a solid. This is the reaction:

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

In this way, the overall gas volume in the respirometer will decrease as oxygen is consumed in cellular respiration, which is how the rate can be determined. The rate of respiration may be affected by temperature, amount of available nutrients, and sufficient oxygen.

In this experiment, students will learn how to use a respirometer system and explore the effect of certain environmental variables on the rate of cellular respiration. The objective is to investigate the relationship between cell structure and function, strategies for capture, storage, and use of free energy, and the physical laws of properties of gases.

## Hypothesis

If the process of constructing the microrespirometer is completed, a control is set up, and the position of the manometer fluid in the capillary tubes is recorded, the rate of cellular respiration can be measured from the data.

## Materials

The materials used include:

- germinating/hangerminating seeds
- safety goggles, aprons, gloves
- 1ml plastic tuberculin syringes
- thin-stem plastic dropping pipettes
- 40 µL plastic capillary tubes
- hot glue gun, (non) absorbent cotton
- 3 or 4 quarter inch metal washers
- thermometer
- centimeter rulers
- glass marking pens
- constant-temp. water bath
- manometer fluid
- 15% solution of KOH

## Procedure

### Constructing a Microrespirometer

1. Plug in the glue gun so it warms up.
2. Take a tuberculin syringe and make sure the plunger is pushed in all the way. (Make two syringes)
3. Carefully insert a 40μL capillary tube into the syringe where the needle would be. Insert it as far as the plunger tip and no farther.
4. Hold the tube straight up and add a small amount of hot glue around its base (where it meets the syringe) to seal the capillary to the syringe.
5. Keep it straight until glue cools. Ensure airtight seal.
6. After it cooled, pull back on the plunger and make sure the glue has not plugged the capillary. If it has, start over.

### Preparing the Microrespirometer

1. Draw a small amount of manometer fluid into the full length of the capillary. Then eject the fluid again.
2. Carefully insert a small plug of absorbent cotton into the barrel of the microrespirometer, all the way into the 0ml or cc mark.
3. Add a small drop of KOH to the cotton inside. Do not add too much! CAUTION: WEAR GLOVES AND GOGGLES
4. Add small plug of nonabsorbent cotton on top of absorbent cotton.
5. Slowly reinsert syringe plunger. CAUTION: POINT CAPILLARY INTO CONTAINER.
6. Push the plunger until it reaches the cotton.
7. Remove the plunger to add seeds.
8. Add 0.5ml of germinating seeds. Push plunger in to 1.0ml mark.
9. Place 3-4 washers around barrel of microrespirometers.
10. Place microrespirometers in water bath (about 20°C).
11. The temp. of water bath must be maintained. Adjust level of water so the capillary sticks out, while the barrel is submerged.
12. Make sure the top end of the capillary is open, not sealed.

### Setting Up Your Control

1. Add 0.5ml beads to other microrespirometer. Reinsert plunger to 1.0ml mark.
2. Place 3-4 washers around the barrel.
3. Place control into water bath next to other syringe.
4. Adjust water level so capillaries stick out, while barrel is submerged. Make sure top of capillary is not sealed.
5. Do not touch or move the respirometers after they have reached equilibrium.

### Collecting Data

1. Prepare a table for recording data. You will need to record data for experimental and control respirometers.
2. Place both into water bath. Wait 5 minutes for temperature to equalize.
3. Use a pipette to add a small drop of manometer fluid to the tip of each capillary. The drop should be sucked down into the tube. (Use plunger on control until manometer is about halfway down capillary.)

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4. Manometer fluid will move toward chamber as oxygen is consumed.
5. Record starting position of each plug by marking its position on the capillary with a marker. Mark the bottom edge of the plug. These are the Time 0 marks.
6. Begin timing.
7. Mark the position of the manometer fluid at 5 minute intervals. Mark bottom edge of fluid plug.
8. At 25 minutes, remove respirometers from water.
9. Use cm ruler to measure distance from initial mark (Time 0) to each 5-minute interval.
10. Record measurements in correct column of table.
11. Calculate change in fluid position for each time interval. Subtract fluid position at beginning of interval from fluid position at the end. Record.
12. Repeat calculations for the control.
13. Using the values from the control, correct for any changes in volume that may be attributed to changes in temperature and air pressure.
14. Construct a graph using the data table. How should the data be plotted? Which variable should be on the x-axis? Y-axis?
15. Determine the rate of respiration for the germinating seeds.

Data (Results)

Germinating Peas (Tube 1)

| Time | Water Temp. | Total Distance (from top of tube) | Position change/interval |
|---|---|---|---|
| 0 min | 25°C | 4.5 cm | — |
| 5 min | 25°C | 5.5 cm | 1 cm |
| 10 min | 25°C | 6 cm | 0.5 cm |
| 15 min | 25°C | 7 cm | 1 cm |
| 20 min | 25°C | 7.5 cm | 0.5 cm |
| 25 min | 25°C | 7.8 cm | 0.3 cm |

Non-germinating Peas (Tube 2)

| Time | Water Temp. | Total Distance Moved | Position change/interval |
|---|---|---|---|
| 0 min | 25°C | 3.3 cm | — |
| 5 min | 25°C | 3.5 cm | 0.2 cm |
| 10 min | 25°C | 3.6 cm | 0.1 cm |
| 15 min | 25°C | 4 cm | 0.4 cm |
| 20 min | 25°C | 4.6 cm | 0.6 cm |
| 25 min | 25°C | 4.9 cm | 0.3 cm |

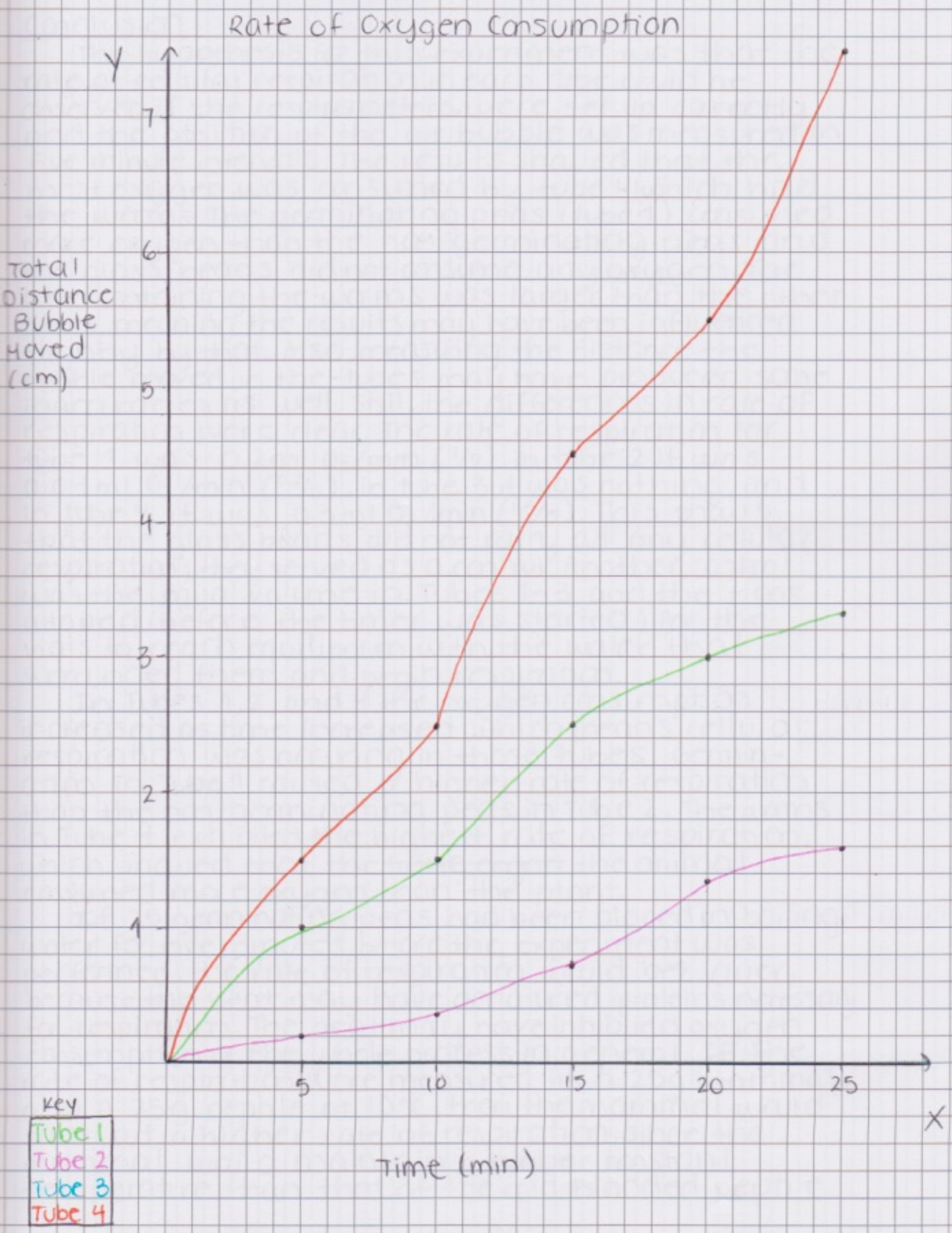
Glass Beads (Tube 3)

| Time | Water Temp. | Total Distance | Position change/interval |
|---|---|---|---|
| 0 min | 25°C | 2 cm | — |
| 5 min | 25°C | 2 cm | — |
| 10 min | 25°C | 2 cm | — |
| 15 min | 25°C | 2 cm | — |
| 20 min | 25°C | 2 cm | — |
| 25 min | 25°C | 2 cm | — |

Data (continued)

## Worms (Tube 4)

| Time | Water Temp. | Total Distance | Position change/interval |
|---|---|---|---|
| 0min | 25°C | 6.5cm | — |
| 5min | 25°C | 8cm | 1.5cm |
| 10min | 25°C | 9cm | 1cm |
| 15min | 25°C | 11cm | 2cm |
| 20min | 25°C | 12cm | 1cm |
| 25min | 25°C | 14cm | 2cm |



## Conclusion

### Error Analysis

Several factors may have influenced the accuracy of the results. Not maintaining a constant temperature could have affected the results, as well as moving the vials after beginning the experiment. Additionally, allowing the KOH to come into contact with the peas or worms would probably also have produced inaccurate results. Having different amounts of cotton in the tubes could have also caused an error in the results.

### Conclusion

The hypothesis for this experiment was that the rate of cellular respiration in each tube could be observed if the respirometers were set up correctly and the position of the air bubble was measured in five minute intervals. The results showed that the most oxygen was consumed by tube 4 which had the worms. The germinating peas (tube 1) consumed more oxygen than the non-germinating peas and the glass beads did not consume any oxygen. The tube containing the worms was larger than the other tubes, meaning the results may have been influenced slightly by that. Also, measuring the distance the bubble moved in the tubes may have produced some inaccuracies as well. Still, the differences in rate of respiration were clear. The rate of respiration for tube 1 was 0.2ml $O_2$/min ($^1/_5$), in tube 2 it was 0.04 ml $O_2$/min ($^{0.2}/_5$), in tube 3 it was nothing, and in tube 4 it was 0.3 ml $O_2$/min ($^{1.5}/_5$). This shows that the glass beads did not carry out any cellular respiration; they served as a control. Another control was the equal volume in Tubes 1-3 and the time allowed (before the timer was started) for the vials to reach equilibrium with the water that surrounded them and begin respiration.

In Tubes 1, 2, and 4 the oxygen consumption increased as time increased, which means cellular respiration was occuring in those tubes. Germination in Tube 1 caused a higher rate of respiration than the non-germinating peas in Tube 2. The worms in Tube 4 exhibited the highest rate of respiration, which showed that the ~~living organ~~ the animal consumed more oxygen than the plant.

If 25 germinating peas had been placed in boiling water for five minutes before the experiment was performed, the rate of respiration would be lower, because the heat may have denatured proteins necessary for respiration. The heat may have inhibited oxygen consumption or the whole process in general. If the rate of respiration were measured in a 25g mammal and a 25g reptile at 10°C, then the mammal would carry out a higher rate of respiration, since the mammal would maintain a higher constant temperature than that of the cold blooded reptile.

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If the rate of respiration in the mammal and the reptile were measured at 22°C, the oxygen consumption ~~of both animals would most likely increase, since the outside temperature rose.~~ of the mammal and the reptile would not be as different, since the body temperature of the mammal would be closer to the room temperature, causing lower energy requirement to maintain body temperature, and therefore less oxygen consumption. The reptile would still respire less at 22°C than the mammal. Furthermore, it may have been more difficult to use living green plants, since photosynthesis would occur as well and measuring oxygen consumption would be more complicated due to the balance between cellular respiration and photosynthesis in plants. If the temperature or pressure were to change during the experiment, the volume of oxygen consumption would change as well. The volume would change in direct proportion to the pressure or temperature, due to the $PV = nRT$ formula. If the pressure and temperature remain constant, the volume of gas within the tube would most likely decrease.