

Respiration

Objectives

1. To describe oxidation and reduction in terms of electron and H^+ transfer.
2. To distinguish anaerobic from aerobic cellular respiration in terms of ATP, oxygen, and chemiosmosis.
3. To demonstrate that carbon dioxide is a product of cell respiration.
4. To determine the effect of boiling on the aerobic respiration of bean seeds and explain the result in terms of enzyme activity.
5. To measure the rate of oxygen consumption in germinating bean seeds.
6. To determine the metabolic rates for several small animals and relate this to body size and lifestyle.

Introduction

All organisms, whether plant or animal, bacteria, protists or fungi, carry out cellular respiration. During respiration organic food molecules are oxidized and these exergonic oxidation reactions are coupled with the synthesis of ATP, an endergonic reaction. The ATP is then used to drive the metabolic reactions necessary to maintain the organism's physical integrity and to support all its other activities.

The cytoplasm of all cells contains the enzymes needed in the ancient central pathway of glycolysis, in which glucose is oxidized to pyruvate in the absence of oxygen. The energy released in this process is used to generate ATP directly by substrate level phosphorylation, in which phosphate groups are transferred directly from organic substrates to ADP.

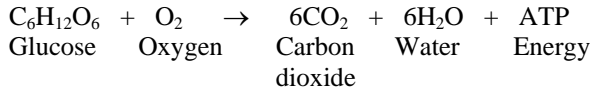
To obtain energy from glucose, hydrogen atoms are removed from the glucose molecule as it is metabolized. These hydrogen atoms can be removed only by hydrogen (electron) carriers, such as NAD^+ . Cells contain a finite amount of NAD^+ and since each NAD^+ combines with only two hydrogens (electrons), there must be a mechanism for removing the hydrogens (electrons) from $NADH$ so that glycolysis can continue.

In many organisms, respiration can occur under anaerobic conditions where no oxygen is present. Many bacteria, yeast, and animals ferment glucose, producing lactate or ethanol. During fermentation reactions, hydrogens are removed from glucose, passed to the electron carrier NAD^+ (forming $NADH$), and then on to pyruvic acid (the end product of glycolysis), converting it to lactate or ethanol. Concurrently, the $NADH$ is oxidized to NAD^+ , reconstituting the NAD^+ pool required for glycolysis. Fermentation allows cells to make ATP in the absence of oxygen. Cells metabolizing glucose by fermentation harvest only about 5% of the available energy in glucose, however.

Most organisms use molecular oxygen in a process called cellular respiration. In this series of reactions, the glucose molecule is completely disassembled to yield CO_2 and H_2O . The process begins with glycolysis; the end product of glycolysis, pyruvate, enters the mitochondrion where it is further metabolized.

After entering the mitochondrion, the pyruvate loses a CO_2 molecule to form acetyl CoA, which enters a series of reactions known as the Krebs's cycle or citric acid cycle where it is completely oxidized to CO_2 . The electrons released during this series of reactions are used to reduce NAD^+ , and a related molecule FAD, to $NADH$ and $FADH$, respectively. These electron carriers then transfer their electrons to the electron transport chain (ETC), a series of proteins embedded in the inner membrane of the mitochondrion. The passage of electrons through the ETC generates an H^+ gradient across the inner membrane which drives the synthesis of ATP by ATP synthetases embedded in the inner membrane. The last electron carrier protein in the ETC transfers the electrons to molecular oxygen to form water, with the addition of H^+ . This transfer of electrons to oxygen returns the protein (cytochrome oxidase) to its oxidized state so that it can continue to accept electrons from the remainder of the ETC.

The process of cellular respiration can be summarized by the following equation:



Complete respiration of one molecule of glucose results in a yield of 36-38 ATP molecules. Four of these ATP's are the product of substrate-level phosphorylation; the remaining ATP's are synthesized by the ATP synthetases using the energy of the H⁺ gradient created by electrons passing down the ETC in a process called oxidative phosphorylation. The coupling of electron flow with ATP synthesis is described in the chemiosmotic model.

Overall, 18 times more ATP is produced by aerobic respiration than by anaerobic respiration. Even so, less than 50% (the actual number is about 39%) of the available energy in a glucose molecule is actually stored in ATP molecules. In contrast, fermentation and anaerobic respiration yield only two ATP's. This is equal to only about 2% of the available energy in glucose. Clearly aerobic respiration gives a bigger energy payback than anerobic respiration.

In today's lab you will be carrying out a series of experiments that will demonstrate several aspects of respiration including the release of carbon dioxide as a product of respiration, the uptake of oxygen by organisms during aerobic respiration, and attempt to demonstrate that metabolic rate (the rate of oxygen usage per gram of body weight) is a function of animal size.

Carbon Dioxide Production

Seeds contain stored food material in the form of some carbohydrate. When a seed germinates, the carbohydrate is broken down, liberating energy (ATP) needed for growth of the enclosed embryo into a seedling.

Two days ago, a set of dry bean seeds was soaked in water to start the germination process. Another set was not soaked. This experiment will compare carbon dioxide production between germinating bean seeds, germinating bean seeds that have been boiled, and ungerminated (dry) bean seeds.

Procedure:

1. Obtain three respiration flask setups. One of these flasks will already contain ungerminated bean seeds and have the rubber stopper with attached fixtures already inserted.
2. Fill one of the remaining flasks with germinated seeds to the same level as the flask of ungerminated seeds. Fill the remaining flask with boiled germinated seeds to the same level.
3. Fit the rubber stoppers securely into the two flasks you filled. Add enough water to each test tube to cover the ends of the glass tubes coming out of the respiration flasks.
4. Set the flasks aside for about 1½ hours while you complete the remaining experiments.
5. After about 1½ hours, replace the water in each test tube with phenol red solution. Phenol red is a pH indicator, that is, it changes color in response to changes in pH. The stock solution is red (pH neutral), but in the presence of an acid the solution turns yellow. When CO₂ is bubbled through water it forms a mild acid called carbonic acid
6. Pour water through the thistle funnel into each flask to force the gases through the glass tubing and into the phenol red solution.
7. Record your results in table 1 and use these results to answer the questions below.

Table 1.CO₂ Production

| | Germinating bean seeds | Boiled germinating bean seeds | Ungerminated bean seeds |
|-------------------------|------------------------|-------------------------------|-------------------------|
| Color | | | |
| CO ₂ Present | | | |

1. Which set of seeds was undergoing respiration?

2. What happened during boiling that caused the results you found? (**Hint: Think about the effect of boiling on the enzymes**)

Oxygen Consumption

The equation for cellular respiration indicates that oxygen is consumed and carbon dioxide is given off. The uptake of oxygen is evidence that cellular respiration is occurring. It is possible to remove the released carbon dioxide by absorbing it with soda lime so that any change in volume in a closed system is due to the oxygen being consumed. The apparatus used in this experiment is called a volumeter, that is, a way of measuring changes in volume.

One set of pea seeds has been soaked in water for the past 48 hours to initiate germination. In this experiment you will measure the respiratory rate of germinating and ungerminated seeds as determined by oxygen consumption.

Procedure:

1. Obtain a volumeter set up as in figure 2.
2. Remove the test tubes from the volumeter. With a china marker, number the tubes and then fill as follows:

Tube 1: 20 germinating (soaked) pea seeds

Tube 2: 20 ungerminated (dry) pea seeds plus enough glass beads to bring the total volume equal to that of Tube I.

Tube 3: Enough glass beads to equal the volume of 1. This tube serves as a thermobarometer and is used to correct experimental reading to account for in temperature and barometric pressure taking place during the experiment.

3. Pack cotton loosely into each tube to a thickness of about 1.5 cm above the peas/beads.
4. Add a layer of soda lime about $\frac{1}{2}$ - $\frac{3}{4}$ of an inch in depth over the cotton wool.
5. Insert the stopper-syringe assembly in place.
6. Add a small drop of marker fluid to each side arm pipette by touching the dropper to the end of each; the drop should be taken up by capillary action. Adjust the position of the drop so that it is between the 0.8 and 0.9 cm marks on the scale by gently withdrawing the plunger on the syringe.
7. Make sure that each side arm is parallel to the bench top and wait 5 minutes before collecting data.
8. At time=0, record the position of the drop in each pipette in table 2 and every 5 minutes for the next 60 minutes. (If the drop moves rapidly, that is, respiration is rapid, reset the drop by gently pushing in the syringe plunger. Add the new readings to the old.)
9. To determine total change in volume of gas within each, subtract each subsequent reading from the first (time=0).
10. At the end of the experiment, correct for any volume changes caused by changes in temperature or barometric pressure by using the reading obtained from the thermobarometer. If the thermobarometric marker moves toward the test tube (decrease in volume), subtract the volume change from the last total oxygen consumption measurement of the pea-containing test tubes. If the marker droplet moves away from the test tube (increase in volume), add the volume change to the last total oxygen consumption over time.

Graph your results on the provided graph scale, using a "+" for data points of germinating peas and a "-" for dry peas.

Table 2. O₂ Consumption

| Time (min) | Germinating bean seeds | | | Ungerminated bean seeds | | | Glass beads (control) | |
|------------|------------------------|-------|------------|-------------------------|-------|------------|-----------------------|-------|
| | Reading | Total | Adjustment | Reading | Total | Adjustment | Reading | Total |
| 0 | | | | | | | | |
| 5 | | | | | | | | |
| 10 | | | | | | | | |
| 15 | | | | | | | | |
| 20 | | | | | | | | |
| 25 | | | | | | | | |
| 30 | | | | | | | | |

Answer the following questions.

1. How do the respiratory rates for germinating and non-germinating seeds compare?
2. How do you account for this difference?
3. It takes about 820ml of oxygen to completely oxidize 1g of glucose. How much glucose are your germinating beans consuming during one hour?

Determining Metabolic Rate

The amount of oxygen used or carbon dioxide produced by the respiration of animals is one measure of all the organic processes going on within the animal. The term *metabolism* is sometimes used to describe this sum total of all life processes.

There are many factors that influence the metabolic rate of an animal. Some of these are physical activity, diet, general state of health, and hormones such as those secreted by the thyroid gland and the adrenal cortex. The metabolic rate of an animal is also influence by whether the animal is warm-blooded or cold-blooded. Warm-blooded animals (homeotherms), which have the ability to maintain a relatively constant internal temperature, have a fairly uniform rate of metabolism. In the investigation here, you will measure the metabolism of several animals.

Procedure:

NOTE: SODA LIME (NaOH) WILL HARM ANIMALS-DO NOT LET THE ANIMALS TOUCH THE SODA LIME!!!

1. Prepare the container: Cover the bottom of the plastic chamber with a thin layer of soda lime (NaOH) (4 to 6 tablespoons full).
2. Weigh the empty wire cage.
3. Place the animal in the wire cage.
4. Weigh the animal in the cage.

