

Exercise, CO₂, and Respiration

Student Laboratory Kit

Introduction

Respiration consists of breathing and cellular respiration. *Breathing* is the intake of air (inhaling) and the letting out of carbon dioxide and water vapor (exhaling). *Cellular respiration* is a complex process by which energy is released from nutrient molecules.

Concepts

- Cellular respiration
- Respiration
- Biofeedback mechanisms

Background

Inhalation and exhalation involve multiple systems of the body. A complex set of signals from the nervous system to various muscles must coordinate to create the pressure necessary for air to enter and exit the lungs. Muscular contractions change the size of the chest cavity (its volume) and create a difference in air pressure between the chest cavity and the atmospheric air pressure outside the body. Pressure and volume have an inverse relationship—when one increases, the other decreases. When the pressure outside the body is greater than the pressure inside the lungs, air enters the lungs (see Figure 1A). After inhalation the pressure inside the lungs becomes greater than the pressure outside the body. This reversal of the pressure differential causes air to leave the lungs (see Figure 1B).

Oxygen (O₂) and carbon dioxide (CO₂) inside the lungs are exchanged by diffusion across a single layer of cells called *alveoli*. In the alveoli, oxygen is more concentrated than in the surrounding capillaries. Oxygen diffuses from the air across the cell membranes of the alveoli and into the blood in the capillaries. In contrast, CO₂ is more concentrated in the capillaries of the lungs than in the alveoli. Therefore, CO₂ diffuses out of the capillaries into the alveoli, where it can be exhaled by the lungs.

Hemoglobin is the major transporter of oxygen and carbon dioxide in the blood. Hemoglobin releases oxygen and picks up carbon dioxide as the blood flows through the body. Oxygen is required by cells for the complex set of biochemical reactions that must take place to release the energy from nutrients absorbed by the digestive system. Specifically, oxygen is required by the mitochondria inside cells to make adenosine triphosphate or ATP by way of the oxidation of glucose. The production of ATP is called *cellular respiration*. Carbon dioxide produced by the mitochondria as a waste product of ATP production must be transported out of the cell, to the blood, and back to the lungs for exhalation.

It is the production of this CO₂ byproduct by the mitochondria that regulates one's breathing rate. The CO₂ dissolves in the blood, forming carbonic acid (H₂CO₃). Carbonic acid then breaks down into bicarbonate (HCO₃⁻) and hydrogen ions (H⁺). The more hydrogen ions that are in the blood, the lower the pH of the blood will be. This is important to the biosensors in the body. Blood that is high in CO₂ has a lower pH than blood that is low in CO₂.

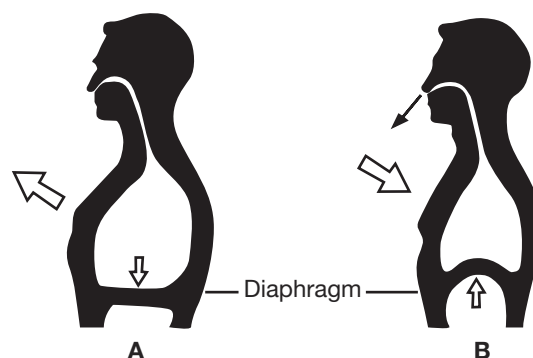


Figure 1. Respiration

- A. Inhalation**—with the diaphragm and chest muscles contracted, the rib cage is raised causing increased volume and decreased air pressure.
- B. Exhalation**—with the diaphragm and chest muscles relaxed, the rib cage is lowered, causing decreased volume and increased air pressure.

Biofeedback mechanisms in the body monitor the pH of the blood. If a drop in the blood pH is detected, nerve signals are sent to the breathing center in the brain. The breathing center sends signals to the muscles that control breathing—triggering faster, deeper breathing and increasing the removal of CO₂.

An acid–base indicator such as bromthymol blue (BTB) may be used to indicate the pH of blood and hence the relative amount of CO₂ dissolved in water. Bromthymol blue solution is blue when its pH greater than 7.6, green between 7.6 and 6.0, and yellow at a pH less than 6.0. By capturing exhaled air and bubbling it through a solution of BTB, the relative CO₂ concentration present may be determined visually based on the color and pH of the resulting solution.

Experiment Overview

The purpose of this laboratory activity is to determine the amount of carbon dioxide in exhaled air when the body is at rest compared to the amount present after moderate exercise.

Pre-Lab Questions

1. Create a diagram which indicates the relative pressure differences inside the body versus outside the body during both inhalation and exhalation.
2. If the pH of blood were tested immediately after it exited the lungs and then again just prior to entering the lungs, which sample would be expected to have a lower pH value? Explain.

Materials

| | |
|--|---------------------------|
| Bromthymol blue solution (BTB), 100 mL | Ruler |
| Balloon, 12-inch | Stopwatch or timer |
| Cups, clear plastic, 2 | Straw |
| Graduated cylinder, 100-mL | String, 18-inch pieces, 2 |
| Marker | Tape |

Safety Precautions

Although latex is not considered hazardous, it may be an allergen. Wear safety glasses or goggles whenever working with chemicals, heat or glassware in the laboratory. Do not share balloons. Wash hands thoroughly with soap and water before leaving the laboratory. Please follow all laboratory safety guidelines.

Procedure

1. Read the procedure completely before beginning the activity.
2. Using the 100-mL graduated cylinder, measure and add 50 mL of bromthymol blue solution into each of the two cups.
3. Inflate and deflate the balloon once to stretch the latex.
4. Insert the straw into the neck of the balloon.
5. Securely tape the straw to the balloon—ensure that no air can escape around the straw.
6. One partner should take a deep breath and exhale completely into the straw with *one* full breath of air. Empty the lungs as much as possible. Pinch off the straw and balloon. Do not let any air escape the balloon. *Note:* The largest volume of air that can be inhaled and exhaled is called the *vital capacity*. The volume of a normal breath is called the *tidal volume*.
7. Without allowing any air to escape, wrap the string around the widest part of the balloon.
8. Mark the string to show the width of the balloon. Using a ruler, measure the circumference of the balloon in millimeters, and record the result in the data table.
9. Insert the free end of the straw into the bottom of one cup containing the BTB solution.

10. *Slowly* release the exhaled air from the balloon so that it bubbles through the BTB solution.
11. As soon as the BTB solution turns yellow or yellow-green, pinch off the straw and balloon to keep any additional air from escaping.
12. Wrap the same string around the widest part of the balloon.
13. Mark the string to the final circumference of the balloon. Allow the balloon to deflate.
14. Using a ruler, measure the final circumference marked on the string in millimeters and record the result in the data table on the Respiration Worksheet. Note that the values obtained in steps 8 and 14 are both before exercising.
15. The same partner should run in place for one full minute.
16. Repeat steps 6–9 using the second piece of string and the second cup of BTB solution. Record the initial circumference of the balloon “after exercising” in the data table.
17. Slowly release the air from the balloon into the second cup until the BTB solution turns exactly the same shade of yellow or yellow-green as the solution in step 11. This is very important to ensure accurate comparisons can be made of the CO₂ in exhaled air before and after exercising.
18. Pinch off the straw and balloon to keep any additional air from escaping. *Note:* Save both cups with solution to complete question 3 of the *Post-Lab Questions*.
19. Wrap the same string around the widest part of the balloon.
20. Mark the string again to measure the final circumference of the balloon after exercising. Allow the balloon to deflate.
21. Use a ruler to measure the final circumference marked on the balloon. Record the result in the data table on the Respiration Worksheet.

Disposal

Consult your instructor for appropriate disposal procedures.

Exercise, CO₂, and Respiration Worksheet

Data Table

| | |
|---|----|
| Initial circumference before exercising | mm |
| Final circumference before exercising | mm |
| Change in circumference (initial – final) | mm |
| Initial circumference after exercising | mm |
| Final circumference after exercising | mm |
| Change in circumference (initial – final) | mm |

Post-Lab Questions

1. Using both the before- and after-exercising results, calculate the change in circumference of the balloon to determine how much air was needed to cause the BTB color change. Which situation caused the larger change in circumference of the balloon—before or after exercising?
2. Which exhaled breath (before or after exercising) contained a higher concentration of carbon dioxide? Explain.
3. Compare the color of your two cups of BTB solution to those of your classmates. Did everyone choose the same color for an endpoint?
4. Why was it important to stop adding exhaled air after exercise when the BTB color matched that of your initial sample?
5. Compare your circumference results to those of your classmates. Did everyone get the same result? Describe some of the factors that would influence the results.
6. Athletes train to increase their breathing efficiency. Would you expect trained athletes to have a higher or lower concentration of carbon dioxide in their exhaled breaths? Explain.

Teacher's Notes

Exercise, CO₂, and Respiration

Materials Included in Kit (for 15 groups of students)

| | |
|---|---|
| Ammonia water, 50 mL | Cup, clear, 10-ounce, 30 |
| Bromthymol blue solution, BTB, 0.04%, 25 mL | Straws, plastic, ¼" o.d., package of 50 |
| Balloons, 12-inch, mixed colors, 30 | String, thin, 330-m ball |

Additional Materials Needed (for each lab group)

| | |
|----------------------------|--------------------|
| Graduated cylinder, 100-mL | Stopwatch or timer |
| Marker | Tape |
| Ruler | |

Additional Material Needed (for Pre-Lab Preparation)

| | |
|-------------|----------|
| Beaker, 2-L | Scissors |
| DI water | |

Pre-Lab Preparation

1. Cut string into 18-inch lengths. Two pieces of string are needed for each pair of students.
2. Prepare a basic (blue) BTB solution:
 - a. Add 25 mL of 0.04% BTB solution to 1475 mL DI water.
 - b. Add enough ammonia water to turn the BTB solution bright blue. Typically <1 mL is needed.

Safety Precautions

Although latex balloons are considered nonhazardous, they may cause an allergic reaction. Ammonia water is slightly toxic by ingestion and inhalation. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Do not share balloons or straws. Remind students to wash their hands thoroughly with soap and water before leaving the laboratory. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. The leftover bromthymol blue may be regenerated and reused or disposed of down the drain with an excess of water according to Flinn Suggested Disposal Method #26b. The leftover ammonia water may be disposed of down the drain with an excess of water according to Flinn Suggested Disposal Method #26b. The balloons and straws may be disposed of in the regular trash. Do not reuse the balloons.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12

- Evidence, models, and explanation
- Constancy, change, and measurement

Content Standards: Grades 5–8

- Content Standard A: Science as Inquiry
- Content Standard B: Physical Science, properties and changes of properties in matter
- Content Standard C: Life Science, regulation and behavior

Teacher's Notes *continued*

Content Standards: Grades 9–12

Content Standard A: Science as Inquiry

Content Standard B: Physical Science, chemical reactions

Content Standard C: Life Science, energy, and organization in living systems

Lab Hints

- Enough materials are provided in this kit for 30 students working in pairs, or for 15 groups of students. Both parts of this laboratory activity can reasonably be completed in one 50-minute class period. The pre-laboratory assignment may be completed before coming to lab, and the data calculations and *Post-Lab Questions* may be completed the day after the lab.
- Demonstrate how the bromthymol blue solution is a pH indicator by adding drops of dilute acid or base to a dilute solution of bromthymol blue.
- Regenerate the blue color of the bromthymol blue solution by adding ammonia to the solution a drop at a time. The solution may be reused. Please test solution before performing the experiment.
- Do not reuse the balloons. Each student must use a fresh balloon to eliminate any biohazards.
- For both partners to complete the activity, simply regenerate the BTB solution and supply the team with a second balloon. Use a different color marker on the string.

Teaching Tips

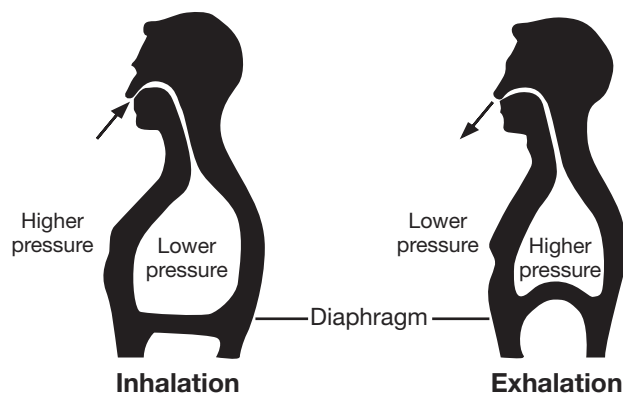
- Explain that the carbon dioxide produced during respiration enters the organism as food.
- Extend the activity by having students measure their tidal volume and vital capacity using the string to measure the circumference of the inflated balloon. In order to calculate the volume of the balloon use the equation

$$\text{Volume} = 1/6 \times 1/\pi^2 \times \text{Circumference}^3$$

- Extend the activity by walking students through glycolysis, aerobic respiration, and the electron transport chain.
- Determine each student's lung capacity using lung bags (Flinn Catalog No. AB1242).

Answers to Pre-Lab Questions (*Student answers will vary.*)

1. Create a diagram which indicates the relative air pressure inside the body versus outside the body during inhalation and exhalation.



2. If the pH of blood were tested immediately after it exited the lungs and then again just prior to entering the lungs, which sample would be expected to have a lower pH value? Explain.

The pH of the blood just prior to entering the lungs will be lower than that of the blood exiting the lungs. There is more carbon dioxide and therefore more bicarbonate and hydrogen ions in the blood that has traveled through the body releasing oxygen and retrieving carbon dioxide from cells.

Teacher's Notes *continued*

Sample Data Table (*Examples from three people are given below. Student data will vary.*)

| | Person A | Person B | Person C |
|---|----------|----------|----------|
| Initial circumference before exercising | 366 mm | 337 mm | 333 mm |
| Final circumference before exercising | 337 mm | 305 mm | 311 mm |
| Change in circumference (initial – final) | 29 mm | 32 mm | 22 mm |
| Initial circumference after exercising | 353 mm | 354 mm | 343 mm |
| Final circumference after exercising | 332 mm | 346 mm | 332 mm |
| Change in circumference (initial – final) | 21 mm | 8 mm | 11 mm |

Answers to Post-Lab Questions (*Student answers will vary.*)

1. Using both the before- and after-exercising results, calculate the change in circumference of the balloon to determine how much air was needed to cause the BTB color change. Which situation caused the larger change in circumference of the balloon—before or after exercising?

In each case the balloon filled before exercising has a larger change in circumference.

2. Which exhaled breath (before or after exercising) contained a higher concentration of carbon dioxide? Explain.

The breath captured after exercising contains a higher concentration of carbon dioxide. The BTB solution required less exhaled air to be bubbled through it to obtain the same endpoint color. Therefore the balloon must have contained more CO₂ than the balloon prepared prior to exercising.

3. Compare the color of your two cups of BTB solution to those of your classmates. Did everyone choose the same color for an endpoint?

No, everyone's color is slightly different.

4. Why was it important to stop adding exhaled air after exercise when the BTB color matched that of your initial sample?

In order to test the amount of carbon dioxide in the exhaled air, the experiment could either measure the amount of exhaled air needed to create the same color change or measure the difference in color created by exactly the same amount of air. Without the instrumentation necessary to measure a difference in color, the only option is to hold the endpoint color constant and measure the difference in air volume necessary to create the same color. Since each person can choose a unique color endpoint, it is best to match the two colors.

5. Compare your circumference results to those of your classmates. Did everyone get the same result? Describe some of the factors that would influence the results.

Each person will have a different lung capacity. Lung size and lung health are two factors that affect a person's lung capacity. The variation in size may be especially great in high school and middle school as students grow and mature. Lung health may also vary greatly depending upon the student's overall fitness or damage to the lungs caused by viruses or disease.

6. Athletes train to increase their breathing efficiency. Would you expect trained athletes to have a higher or lower concentration of carbon dioxide in their exhaled breaths? Explain.

An athlete's exhaled breath should contain a higher concentration of carbon dioxide as a result of intense training.

Acknowledgment

Special thanks to Diane Sweeney, Punahou School, Honolulu, HI for sharing this activity with Flinn Scientific.

The Exercise, CO₂, and Respiration—Student Laboratory Kit is available from Flinn Scientific, Inc.

| Catalog No. | Description |
|-------------|--|
| FB1975 | Exercise, CO ₂ , and Respiration—Student Laboratory Kit |
| AB1242 | Lung Bag Activity |
| FB1442 | Lung Model—Student Laboratory Kit |

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.