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Low Tech Oxygen Consumption of Terrestrial Animals

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Abstract

I use the Plexiglas Metabolism Apparatus sold by Ward's Natural Science and Carolina Biological in a junior/senior Animal Physiology class. The students determine the oxygen consumption of mice and leopard gecko to compare metabolic rates of an endotherm and an ectotherm. The students can then use this technique to design independent projects with variables such as temperature, mass, sexes, activity etc. The data collected by the students are very similar to literature values.

The Metabolic Apparatus is a Plexiglas tube 91/4"L x 4"W x 4"H closed with a rubber stopper with an inserted calibrated pipet. There is a place for drierite and soda lime on the bottom of the tube with a screen insert for the animal. The drierite decreases the water vapor and the soda lime absorbs the carbon dioxide. As the animal uses up oxygen the volume in the chamber decreases and the soap bubble in the pipet moves toward the chamber in proportion to the amount of o oxygen consumed.

The advantage of this system is that the students are in total control of this experiment and can see the changes while viewing the animals. The students calculate the volume of oxygen consumed under standard conditions, compare their data to literature values, and use appropriate statistical tests on their data. The following are some of the concepts explored: indirect calorimetry, closed respirometry, factors that affect metabolic rates, and endothermy versus ectothermy.

Student Handout

Objectives:

1. To learn to use closed respirometry to determinate oxygen concentration in air.
2. To design an investigation involving metabolism of terrestrial organisms using indirect calorimetry.
3. To compare data with published values.

Introduction

Metabolism can be defined as all of the chemical changes in an organism. This lab is only going to be concerned with energy metabolism, i.e., reactions involved in the production and utilization of energy. We will measure oxygen consumption to estimate energy metabolism because the rate of oxygen consumption and energy utilization are directly related under most circumstances. Can you think of any exceptions?

We will use indirect calorimetry to measure energy metabolism. One method of indirect calorimetry depends on determining the amount of oxygen consumed by an organism. The measurement of oxygen

consumption is a good estimate of energy metabolism because the rate of oxygen consumption and energy utilization is directly related under most circumstances. Closed respirometer set ups for terrestrial animals will be used in this laboratory. Because pressure in the closed systems is the same as atmospheric, we can detect decreases in the oxygen content of the closed respirometer by

Pressure x Volume = Number of moles of gas x R (gas constant) x T (absolute temp)

We will be able to detect volume change by following a fluid droplet in a pipet connected to the chamber with the organism. To insure that you are measuring only changes in oxygen and not carbon dioxide, add sodium calcium hydroxide (soda lime) to absorb the CO_2. Soda lime can burn skin --handle cautiously. Also, add some drierite to remove excess water vapor.

Before the lab period:

You need to consider what question you might investigate. You can work in groups of two or three. What are some of the hypotheses that you might investigate with this technique? What statistical tests are appropriate? What variables do you need to control if you can? Where will you find comparable published data? Ask your instructor before lab about what animals are available and other variables that you might investigate. You might want to compare the rates of oxygen consumption in an endothermal vertebrate--a mouse, with an ectothermal vertebrate--a lizard, at room temperature.

You need to prepare a lab plan with your hypotheses with data tables before you **begin this lab to help you make effective use of your time.**

Small Animal Metabolism Apparatus

The soda lime and drierite on the bottom of the chamber will remove CO_2 and water vapor from the air. The pressure in the metabolism chamber remains constant so any volume change represents oxygen consumed by the animal.

Procedure

1. Cover the bottom of the metabolism chamber with a thin layer of soda lime and drierite. CAUTION: Do not allow the experimental animal or student to make contact with this caustic chemical.
2. Accurately weigh a small mammal, e.g., a mouse, and place it in the cage of the metabolism chamber. (Hint: A spacing device such as a small jar placed in the cage will confine the animal, minimizing physical activity.) Close the chamber with rubber stopper with 5 mL pipet embedded in it.
3. Place a thermometer near the chamber (best if you can put into chamber). Allow the animal to remain in the chamber for about 15 minutes for temperature equilibration. Record the temperature.
4. Do not remove the calibrated tube (pipet) from rubber stopper. Using a pasture pipet, wet the inside of the 5-mlpipet with water. This will reduce the possibility of the soap bubble seal drying out and breaking during migration. The chamber is then sealed by applying a drop of bubble solution to the end of the pipet. Dish washing detergents or toy soap bubble solution are

adequate. Often the foam from shaking such a solution makes a satisfactory bubble. You may have to open the chamber to equalize the pressure before the bubble will remain in place.

5. Use a stopwatch to determine the time in seconds required for the soap bubble to traverse a measured distance along the pipet. Practice this technique until the measured time intervals appear consistent. No more than six trial runs should be required. If inconsistencies persist after six trials, look for the following sources of error: (a) leaks in the system, (b) insufficient or saturated soda lime. (c) dirty or blocked pipet, or (d) failure to sufficiently wet the interior of the pipet.

Calculate the volume of oxygen consumed per minute (VO_2) by dividing the total number of ml consumed during three consistent runs by the total length of time required to complete the measurements. The following will serve to illustrate:

Table 1. Time for animal to consume 4 mL of oxygen.

Measurement number	mL moved	Time required in seconds
1	4	105
2	4	95
3	4	100

Example of Calculations of Oxygen consumption in mL/min

- Total number of seconds for 3 trials was 300 seconds
- Convert to minutes by dividing total number of seconds by 60 seconds/min

  300 seconds/60sec/min = 5 min
- Divide the total number of mL (12) by the number of minutes

  Milliliters of oxygen consumed/minute = 12mL/5min = 2.4 ml/min

6. Remove the animal from the metabolism chamber as soon as the necessary measurements of oxygen volume have been obtained. To remove the mouse from the cage, one laboratory partner should rotate the cage slowly while the other partner maintains tension on the base of the animal's tail (do not twist the tail). The mouse will release its hold on the cage when it is upside down. It then can be easily taken from the cage. DO NOT HOLD LIZARDS BY TAILS. Try gently tapping the cage instead.
7. In comparing the results of experiments performed under different environmental conditions, you can use some correction factors. All observed gas volumes (oxygen) should be corrected to the volume that would have been observed under conditions of standard temperature (0^oC) and pressure (760 mm Hg) or V_{STP}. The following formula may be used for this purpose:

$$V_{STP} = V_{obs} \times B.P/760. \times 273/(T^oC + 273)$$

V_{STP} = volume in milliliters at Standard Temperature and Pressure
V_{obs} = observed volume in milliliters
B.P. = barometric pressure in mm Hg

Example of calculations to determine the volume (V) ml of oxygen consumed by the animal under standard conditions. These conditions are illustrated as follows:

Given: V_{obs} = 2.4 mL/minute
B. P. = 755 mm Hg
T^oC = 27^o

Therefore: V_{STP} = 2.4mL/min x 755/760 x 273/300
V_{STP} = 2.2 mL of oxygen/minute

8. Determine the oxygen consumption per unit mass of animal. Divide the standardized volume of oxygen per unit time consumed by the mass of the organism.
9. Compare your rate with published data for the same organism. Make sure to note the size of the animal and the temperature.

In your lab notebook, include all of your data and calculations. Summarize your results, make a figure if appropriate. In your discussion, use your data to support or deny your null hypothesis and compare your data to literature values. Comparing your data to previously published data will help to establish that your techniques were valid. Remember to write a brief summary of the physiological mechanism and implications of your investigation.

Possible sources of further information.

Prosser, C. L. 1973. Comparative Animal Physiology 3rd ed. W. B. Saunders, Philadelphia.
→ This has a table of oxygen consumption values.

Materials

Living organisms: mice, lizards or other organisms that will fit in chamber

Apparatus for group of 10 students

- Balance for mice and lizards
- Thermometers
- 5 metabolism chambers
- Stopwatches
- Barometer
  Carolina Biological
  Small Animal Basal Metabolism Studies Kit HT-68-2000 $133.75
  Ward's Natural Science
  Metabolism Apparatus 14 W 4235 $115.00

Supplies

- Bubble solution
- Cotton
- Vaseline
- Soda lime (Wards 970 W 6906 500 gram bottle $10.95)
- Drierite (Ward's 37 W 1575 500 g bottle $34.25)

Respirometers for larger or smaller animals.

To measure oxygen consumption in larger animals, you can construct closed respirometers from institution sized mayonnaise jars with opening that fit a rubber stopper. You need to be able to separate the animal from the drierite and KOH. I have put chemicals in cloth bags and then put a wire mesh floor over bag.

To measure the oxygen consumption of a terrestrial pill bug, you can use a test tube with drierite and KOH under some type of open mesh barrier and a stopper with three way stop cock with a plastic syringe that you can put a soap bubble in. The three way stop cock will allow you to keep the air flowing to animal during an acclimation period.

Possible investigations and some of their problems.

1. Compare the oxygen consumption of an endotherm, lab mouse, and an ectotherm, Leopard Gecko.

   The Leopard Geckos just fit in the chamber but do give readings although slowly. You may have to determine time for 1 ml of oxygen not 4 mL. Anoles take even longer because they are so small.

   Mice can be very active and the students will probably know that activity will increase oxygen consumption. I have put in glass jars or other spacers to confine the mice to smaller spaces.

2. Compare the oxygen consumption of same animals at different temperatures.

   Best if you have a walk in environmental chamber that you can pre cool or heat the apparatus before using. I have used goose neck lights in increase the temperature and ice to decrease the temperature.

   Some students realize that this would be a more physiologically relevant experiment if they put the animals at the test temperature about 24 hours before testing.

3. Compare male and females of the same species. This can be complicated by pregnant mice. The gender will also be a variable in investigations above.

4. Compare same species or same animal under fasting and non fasting conditions.

   I had one student do this with garter snakes. We wrapped the wire cage with a fine mesh to prevent the snake from escaping.

5. Compare different size classes of the same organism. This is hard because you normally do not have a very big range of sizes.

6. Compare different species of some type of animal. I had a student do this with snakes. The problem is that he could only get one specimen of each type of snake.

About the Author

Ruthannne Pitkin received a B.S. in Zoology from the University of Massachusetts, a M.S. in Biological Oceanography from the University of Washington, and a 5-College Ph.D. in Zoology from University of Massachusetts while in residence at Mt. Holyoke College. She is a Professor of Biology and teaches Principles of Biology II, Animal Physiology, Human Biology, and a variety of seminars.

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RELATIONSHIP BETWEEN METABOLISM AND OXYGEN CONSUMPTION

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PRINCIPLES OF CLOSED RESPIROMETRY

Closed Respirometers can be used to determine oxygen consumption because of Ideal Gas Law

Pressure X Volume = Number of moles of gas X R (gas constant) X T (absolute temperature)

The pressure inside and outside the chamber are both atmospheric, the gas constant remains the same, and we are going to use a constant temperature. Therefore, when the animal uses up oxygen in the chamber, the volume decreases which is directly proportional to the number of moles of gas. To control for carbon dioxide and water vapor, soda lime a CO_2 absorber, and drierite a water vapor absorber, are added to the chamber.

PROCEDURES

1. Cover the bottom of the metabolism chamber with a thin layer of soda lime and drierite. CAUTION: Do not allow the experimental animal or student to make contact with these chemicals.

2. Accurately weigh a small mammal, e.g., a mouse, and place it in the cage of the metabolism chamber. (Hint: A spacing device such as a small jar placed in the cage will confine the animal, minimizing physical activity.) Close the chamber with rubber stopper with 5 mL pipette embedded in it.

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7. In comparing the results of experiments performed under different environmental conditions, you can use some correction factors to get oxygen consumption at Standard Temperature and Pressure (STP). All observed gas volumes (oxygen) should be corrected to the volume that would have been observed under conditions of standard temperature (0°C) and pressure (760 mm Hg). The following formula may be used for this purpose:

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8. Determine the oxygen consumption per unit mass of animal.
Divide the standardized volume of oxygen per unit time consumed by the mass of the organism.

9. Compare your rate with published data for the same organism. Make sure to note the mass of the animal and the temperature.

Materials needed
- **Metabolism apparatus**
- **Drierite and Soda lime**
- **Soap Bubbles and stopwatch**

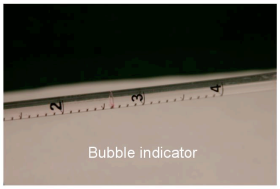

Table 2 Student oxygen consumption data compared with data from Prosser, C. L. 1973. Comparative Animal Physiology 3rd Ed.

Source	Organism	Mass grams	Temperature °C	mL/hr/g STP
Student	*Mus*	35.8	26.5	1.96
Student	*Mus*	39.7	27.5	2.19
Prosser *	House mouse	17	?	1.7
Student	*Rattus*	98.8	26.5	1.03
Student	*Rattus*	85.8	?	0.77
Prosser*	*Rattus*	280	?	0.88
Student	*Eublepha Iris macularis*	18.8	26.2	0.38
Student	*E. macularis*	19.1	24.5	0.20
Student	*E. macularis*	30.2	25	0.36
Student	*E. macularis*	40.9	25.5	0.36
Prosser*	*Gerrhonotius*	30	20 35	0.55 0.30