BIO 3A LABORATORY
Animal Metabolism

Objectives

- Determine the raw oxygen consumption of mice at two different ambient temperatures.
- Calculate weight specific metabolic rate adjusted to STPD for a homeotherm
- Measure metabolic rate in a heterotherm, the goldfish, at different ambient temperatures

Introduction

In the reactions known as cellular respiration, oxygen is always reduced; it is the ultimate proton (H^+) acceptor in this process:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \qquad (1)$$

Sometimes this occurs almost immediately (aerobic metabolism); occasionally it is delayed (anaerobic metabolism). Temperature can have a profound effect on the rate of metabolic reactions. In general, metabolic reactions are governed by the Q_{10} rule. As temperature is increased by 10 degrees C the rate of the reaction will double. Your earlier labs involving proteins showed us the temperature sensitivity of enzyme-mediated reactions. However, in animals with variable body temperatures (heterotherms), several forms of an enzyme may exist (isoforms or isozymes). Each of these forms may demonstrate maximum activity at a different temperature. Thus, the organism may be able to survive over a range of ambient (and body) temperatures. Another group of animals puts a lot of energy (literally) into maintaining a constant body temperature. By doing this, they can minimize the number of forms of an enzyme required; in addition, if these animals maintain a temperature slightly below the critical maximum (point of protein denaturation) they can maximize the reaction rates (Q_{10} rule). These organisms are called homeotherms.

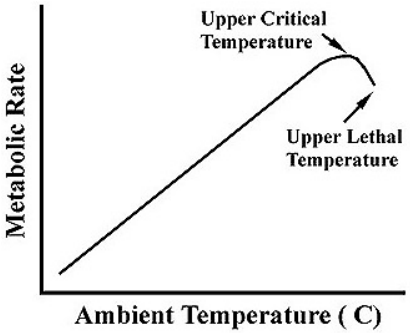

Figure 1. Heterotherm metabolism vs. temperature

Indirect measurement of metabolism in a heterotherm

Goldfish are heterothermic ectotherms. Their body temperature is virtually identical to the water in which they live. Small variations in Tb can result from the exploitation of the variable microhabitat in which they live. For instance, the area of water nearer the surface may be slightly warmer; water under plant leaves may be cooler. However, in general, their Tb will vary through the daily cycle. The temperature-related metabolic response of most ectotherms is linear over a range of reasonable environmental temperatures.

At extremely cold temperatures, ice crystals form, causing fatal cell rupture. At higher temperature a critical maximum temperature is generally reached, at which enzyme activities, and reaction rates, are maximized. Beyond this temperature protein denaturation results in decreased activity and ultimately death.

Procedure A. Indirect respirometry of goldfish metabolism

The gill structures of most fishes are cover by an operculum. The operculum works in concert with the mouth and buccal cavity in moving water over the gills. The mouth opens, the buccal cavity expands, the mouth closes, the operculum opens and the buccal cavity contracts. This forces water over gills where oxygen and carbon dioxide are exchanged. If the volume of water is constant during each of these cycles, then the rate of opercular pumping is proportional to the oxygen demand. Thus one could indirectly measure metabolic rate as opercular pumping rate.

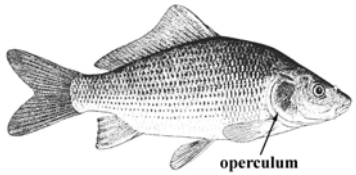

Figure 2. Operculum of a

In this experiment you will measure the opercular pumping rate of goldfish at three different temperatures.

1. Place a goldfish into approximately 200 mL of water in a 250 mL beaker. Place the beaker in the ice bath until the temperature has reach approximately 3 to 4 ˚C.
2. Remove the beaker gently and place it in a good viewing area. Extraneous stimulation will cause to fish to not settle down. Use one of the ice containers to shield the fish from extraneous stimuli. Count the opercular pumping for 2 min (120 sec). Repeat this measurement two more times. Record your data in Table 1. Counters are available if needed.
3. Place the beaker into a room temperature bath. When the temperature has reach the ambient, let the system equilibrate for ten minutes.
4. Remove the beaker gently and place it in a good viewing area. Again, extraneous stimulation will cause to fish to not settle down. Use one of the ice containers to shield the fish from extraneous stimuli. Count the opercular pumping for 2 min (120 sec). Repeat this measurement two more times. Record your data in Table 1.
5. Place the beaker into the 30 ˚C bath. When the temperature has reach bath temperature, let the system equilibrate for ten minutes.
6. Remove the beaker gently and place it in a good viewing area. Again, extraneous stimulation will cause to fish to not settle down. Use one of the ice containers to shield the fish from extraneous stimuli. Count the opercular pumping for 2 min (120 sec). Repeat this measurement two more times. Record your data in Table 1.
7. Once you have completed all of your measurements, record all of your measurements on the computer so that others can download the data.
8. Using your data set, create a data table in Excel, in this table you should take the average of the pumping rate at each temperature. Create a graph of opercular pumping rate vs. ambient temperature. Is there a significant difference between the average opercular pumping rate at the low temperature and the high temperature? (hint: use the **appropriate** t-test!).
9. Using the whole class data set (download this) for the goldfish experiment, is there a significant difference between the average opercular pumping rate at the low temperature and the high temperature? Construct a graph for this comparison also.

Measurement of oxygen consumption in a homeotherm

Mice are homeothermic endotherms. They maintain relatively constant body temperature (T_b) throughout the day. Thus, it should be expected that mice would increase their metabolic rate to maintain constant body temperature if the ambient temperature (T_a) begins to fall.

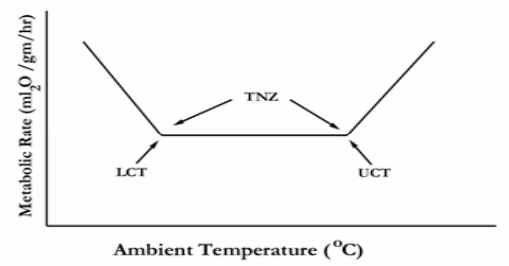

Figure 3. Typical Endothermic Metabolic Response to Ambient

In Figure 3 you can see the temperature-related metabolic response seen in most endotherms. TNZ refers to the thermoneutral zone, or the range of temperatures over which the animal's metabolic rate remains fairly constant. At these temperatures, the animal's metabolic rate is as low as it can be during the waking state. The lower critical temperature (LCT) is the point where metabolic rate increases to assist in maintaining body temperature in the face of the decreasing temperature. UCT, or upper critical temperature, is the point where energy is utilized to loose excess heat. The slope of the oxygen consumption rates above and below the critical temperatures, and the width of the TNZ are related to the type of animal, its size and its evolutionary history. For instance, the arctic fox, an animal well adapted for the life at cold temperatures, has UCT of only 0 °C. Even more startling is their TNZ, which extends far below -10 °C. Humans, on the other hand, evolved in a warm climate, witnessed by their narrow TNZ, high critical temperatures (Figure 4).

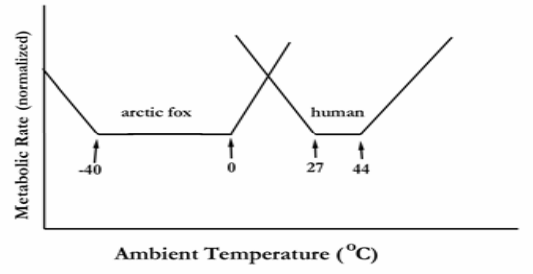

Figure 4. Thermoneutral Zones of Humans and Arctic

Procedure B. Mouse metabolism at ambient and cold temperatures

In this procedure we will measure the oxygen consumption of the common laboratory mouse (*Mus musculus*) at two temperatures, room or ambient (T_a) and a reduced temperature near 1 °C. The procedure is illustrated in Figure 5. NOTE: Each group will run one mouse at only one temperature. The mice will be placed in a respirometer containing soda lime (a CO_2 absorbent and drying agent) and equilibrated at temperature for 10 minutes. At this time, the respirometer is sealed and 10 cc of room air is added. The time for the manometer to return to the initial level is recorded. The respirometer is opened to room air for 1 minute to allow fresh air into the chamber, and then the determination is made again. Three determinations of oxygen consumption will be made at each temperature. Raw data should be recorded on the data sheet shown in Table 2. Mouse weight and respirometer temperature must be recorded at the beginning and end of a temperature session.

The computation of oxygen consumption is a straightforward algebraic manipulation of the raw numbers. Weight specific metabolic rate (MR) should be expressed as mL O_2/gm/hr. This may be calculated as for each run:

$$MR = \frac{10cc}{t} x \frac{60\,\text{sec}}{\text{min}} x \frac{60\,\text{min}}{hr} x \frac{1}{wt} \qquad (2)$$

where 10cc is the volume injected into the respirometer, *t* is the time in seconds to return to the initial manometer reading after the 10 cc injection and *wt* is the weight of the mouse in grams. You should set up an Excel spreadsheet to complete your calculations. You should calculate MR for each determination.

You must now convert your gas volumes to standard pressure and temperature, for dry air (STPD: 0 °C, 760 mm Hg). The soda lime dried the air in the chamber, so you won't need to deal with water vapor pressure. However, you will need to apply the temperature-modified form of Boyle's law to adjust the volumes to STP:

$$\frac{P_1 V_1}{T_1} = \frac{P_2 V_2}{T_2} \qquad (3)$$

Where P_B is the room barometric pressure (in mm Hg), V_1 is the gas volume (i.e. the MR), T_1 is the temperature of the respirometer in °K, P_2 is standard pressure (760 mm Hg), T_2 is 273 °K, and V_2 is the adjusted volume (STP).

Raw data for all mice at all temperatures will be available on the class website. Using all of the data, please construct a graph similar to Figure 3, with metabolic rate (in mLs O_2/gm/hr, STPD) plotted against ambient temperature.

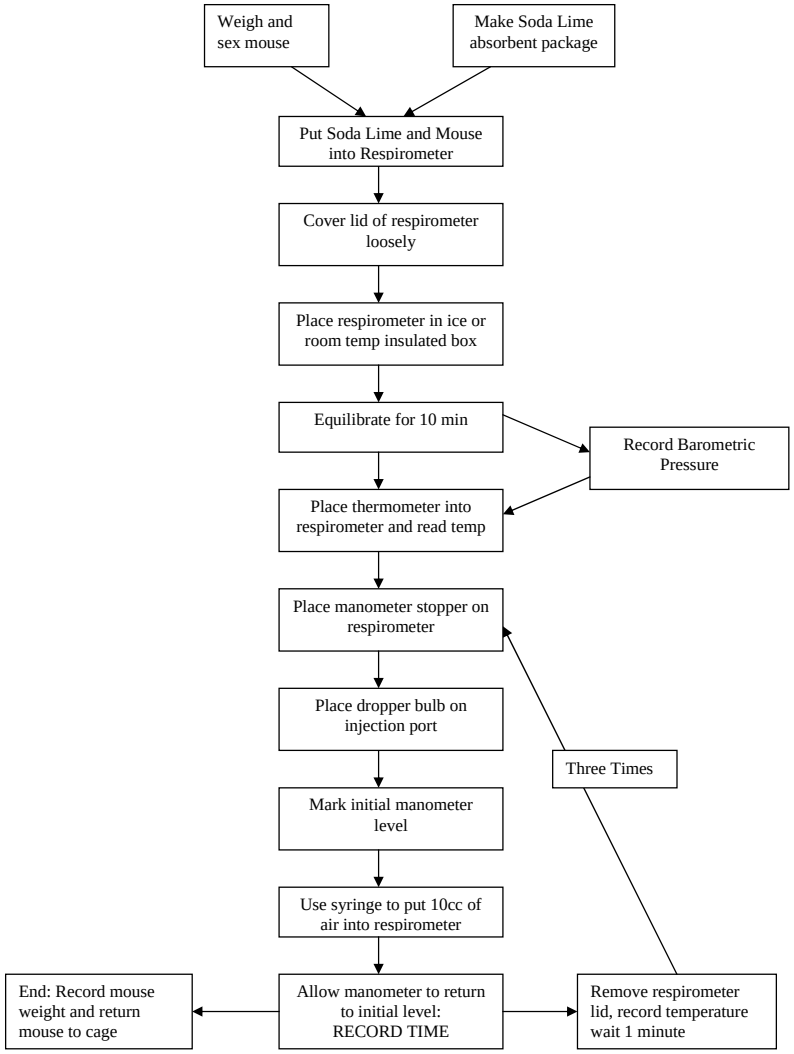

Figure 5. Flow Chart for Mouse Respirometry Measurements

After all data has been collected and recorded on Data Table 2, place your data on the computer at the front of the laboratory

Bio 3A Laboratory: Animal Metabolism **Name:**_____

1. What effect would you expect size to have on the opercular pumping rate? (explain your answer)

Table 1. Number of opercular contractions in 120 seconds at three different temperatures

	Low	**Room**	**High**
Measurement no.	**T =_____**	**T =_____**	**T =_____**
1			
2			
3			
Mean			

2. Using Excel, create a bar graph of average opercular pumping rate vs. ambient temperature for your data. Group the data as low, room and high temperature.

3. Using your data, is there a significant difference between the average opercular pumping rates at the lowest temperature and the highest temperature ? (hint: use the appropriate t-test!)

4. Using the whole class data set (download this) for the goldfish experiment, create a bar graph of average opercular pumping rate vs. ambient temperature. Group the data as low, room and high temperature. Include appropriate error bars on this graph. Is there a significant difference between the average opercular pumping rate at the lowest temperature and the highest temperature? (Show your analysis)

Table 2. Data sheet for mouse oxygen consumption

Mouse No. _____ Sex _____

Run No.	**T_a start**	**T_a end**	**Weight start**	**Weight end**	**P_B**	**Time**
1						
2						
3						

5. What effect would the animal's activity have on this measurement of oxygen consumption?

6. Raw data for all mice at all temperatures will be available on the class website. Using all of the data, please construct a graph (XY scatter plot) similar to the one shown in Figure 3, with metabolic rate (in mLs O_2/gm/hr, STPD) plotted against ambient temperature. Write a short paragraph comparing and contrasting your graph to Figure 3. You will have to manually draw in the trendlines for this figure.