Cricket Respiration Lab

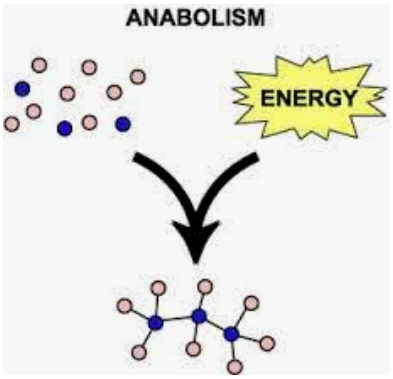
Purpose:

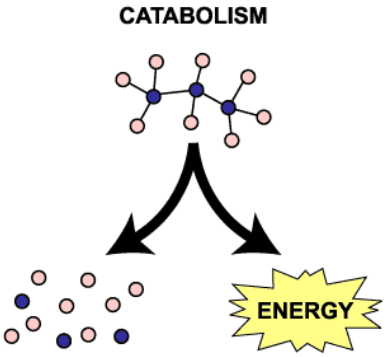
To observe how temperature affects the rate of cellular respiration in cells

Background Reading: (Keep this for your notes)

Animal cells use **cellular respiration** to generate energy. This happens when cells combine O_2 and glucose to make ATP (adenosine triphosphate), CO_2 and water. The sum of these reactions happening in all your cells at once is called your **metabolism**. Metabolism in essence is when there is an exchange of energy between the cells through a series of chemical reactions.

There are two types of metabolism that occurs in the cell: **anabolism**, which is to build up and **catabolism**, which is to break down.
In anabolism, this is when the cells *build up smaller molecules into larger ones.* This process requires energy called *endergonic process*.
In catabolism, this is when the cells *break down larger molecules into smaller ones.* This process gives off energy in the process called *exergonic process*.



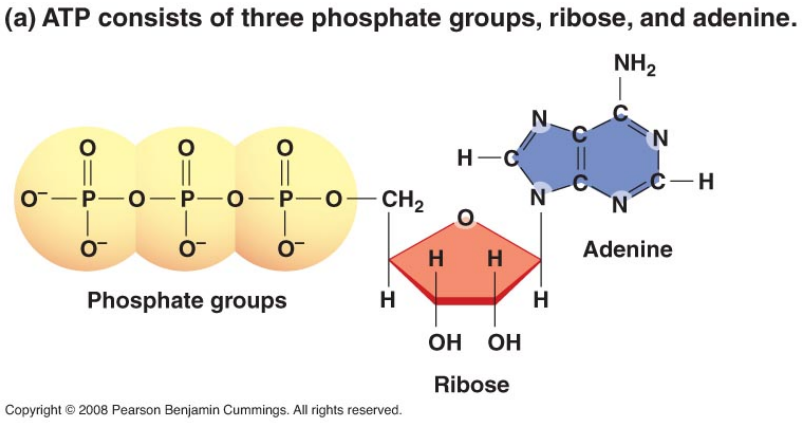


Why do animals respire and how does it generate ATP?

Energy is required for cells to function. In cells, energy is stored as adenosine triphosphate, which is a molecule used by a series of reactions. When the ATP molecule is broken down, there is a high amounts of energy released.

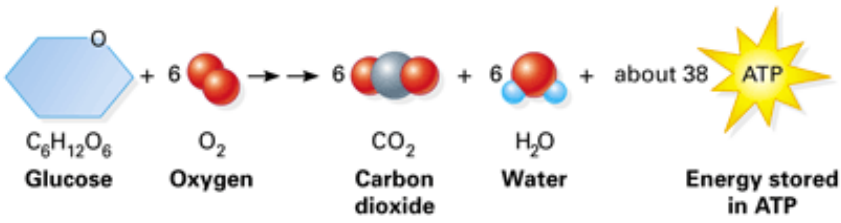
Structure of ATP:

Contains 1 adeonsine molecule which consists of a ribose and adenine linked together with 3 additional phosphate groups



When animals breathe or respire, there is a stepwise reaction using glucose as energy to generate ATP. During this process, Oxygen is required for the reactions to occur and as a result, CO_2 is expelled out as a waste product.

The basic overview of cellular respiration (remember photosynthesis? Well this is the reverse version), is using glucose with oxygen to give carbon dioxide, water and energy. See below:



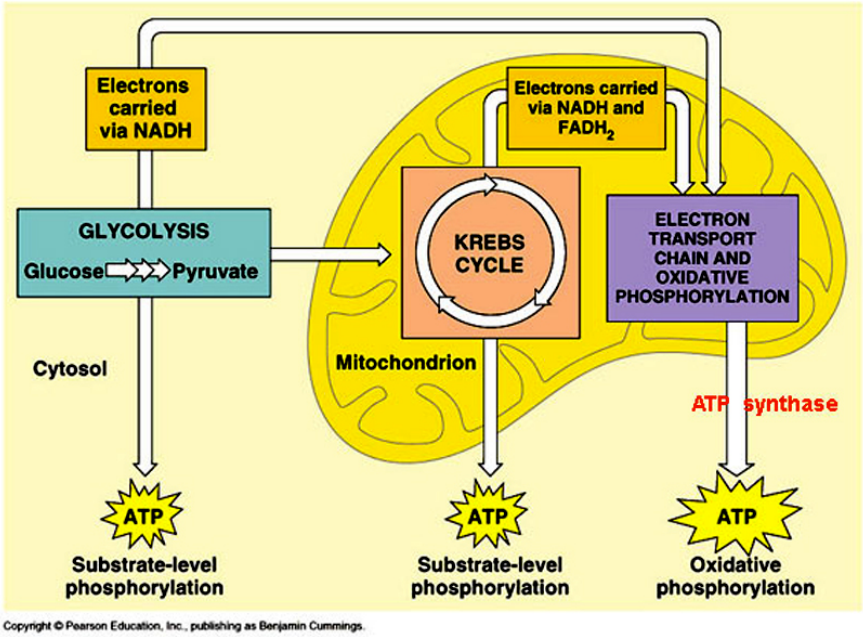
The question is now, what is happening in these steps to generate 38 molecules of ATP from 1 molecule of glucose?

The three main processes:

1. Glycolysis
2. Citric Acid Cycle (Kreb's cycle)
3. Electron transport chain

Don't worry, you do not need to know all the minute details of these 3 cycles. You will however learn all these cycles in 2nd year Biochemistry course.

Remember how we say that the mitochondria is the "power house" of the cell? Well, here is the reason. 2 of the 3 main cellular respiration processes occur in the mitochondria, and it is also the location where a lot of ATP are generated.



Glycolysis

This processes begins all cellular respiration and occurs in the cytoplasm.
This phase does not need oxygen to occur.
When this process starts, one glucose molecule is broken into another called *pyruvate*. The pyruvate molecule enters the mitochondrion and enters the **Kreb's cycle**.

Kreb's cycle

Another cycle occurs where more ATP is produced. This is also where CO_2 released.

The last cycle is called the Electron Transport Chain (ETC).
The ETC produces the most number of ATP during cellular respiration. It uses a series of chemical reactions to move electrons via the interaction with other molecules such as $FADH_2$ and NADH.

As a summary,

Glycolysis occurs in the cytoplasm and generates 2ATP
Kreb's cycle or Citric acid cycle occurs in the mitochondria and generates 2 ATP
ETC generates about 32-34 ATP and occurs in the inner membrane of the mitochondria.

Resulting a total of 36-38 ATP

How this relates to the lab?

How fast does metabolism happen? It depends on the temperature of the cells, since reactions happen slowly at cold temperatures and quickly at warm temperatures. In this lab we will measure the rate of metabolism in crickets at different temperatures. Since animals use up O_2 for metabolism, we can measure the metabolic rate of the crickets by measuring how fast they use up (or consumes) O_2.

Hypothesis:

What will happen to the rate of O_2 consumption as temperature increases?

Materials:

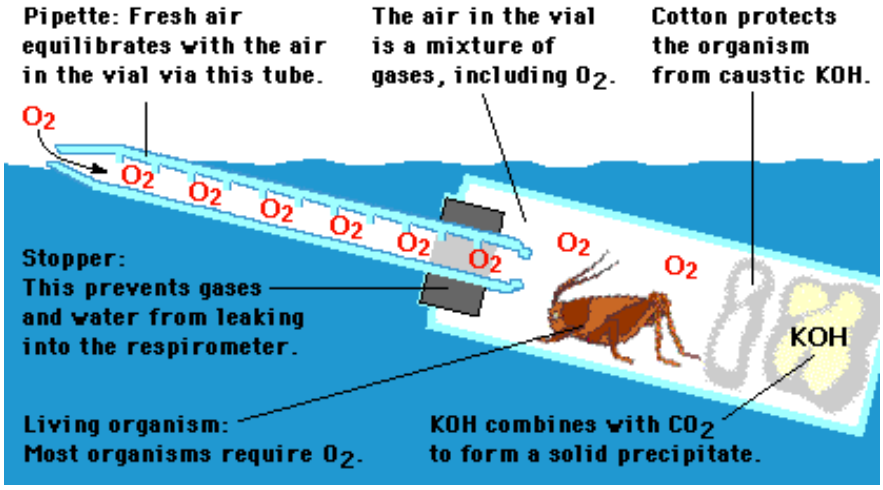
Large crickets	Graduated 2 mL pipettes	Water baths
Large test tubes	Cotton balls	Thermometers
Rubber stoppers w/1 hole	15% KOH solution	Ice

Procedure:

1. You will work in groups of 2-3.
2. Your group will need to create respirometers for your crickets, 2 water baths, and 2 thermometers.

Creating your respirometers

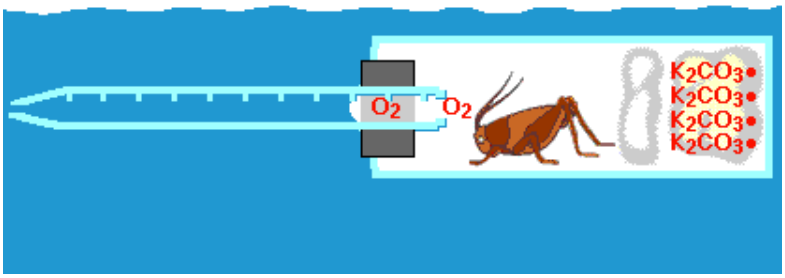
3. To create your respirometer, you are to use a large test tube, a rubber stopper with a single open hole, and a 2ml pipette.
4. Inside the respirometer, there is a cotton ball that has been soaked in the 15% KOH solution, and another cotton ball without the KOH solution.
5. Use hot glue to seal off the pipette with the rubber stopper to ensure water cannot enter.
6. The crickets will be placed inside the test tube similar to below:



http://www.phschool.com/science/biology_place/labbench/lab5/features.html

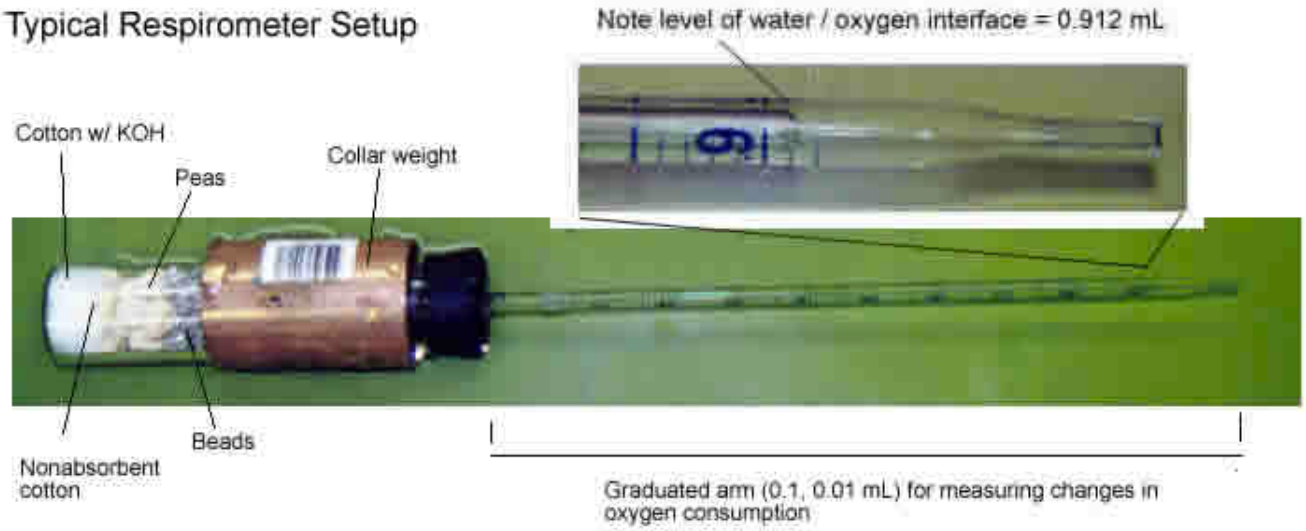
How does this work?

Once the respirometer and the tip of the pipette is submerged into water, water will enter the pipette until pressure is equalized.



As the crickets respire, they are breathing in Oxygen while breathing out CO_2, the KOH solution combines with the CO_2 to form a K_2CO_3 solid. This also causes the gas volume inside to decreases, which causes the water to come in the pipette.

The water volume of the pipette is how we can measure the rate of respiration!



Once your respirometers are all created

7. Create one water bath with warm water in a 500ml beaker and the other with cool water. Put ice into the second one until it is 2 inches deep.
8. Place one respirometer into each water bath, **standing up**. Don't lay them down yet! Let them sit upright in the water for 5 minutes. This is for them to reach equilibrium.
9. While you are waiting, put one thermometer into each water bath. Record the temperatures. (#1)
10. Now observe the crickets in each chamber. Are they moving around or holding still? Are they moving fast or slow? Record your observations. (#2)
11. Once 5 minutes are up, lay the respirometer down flat in the water so all the pieces are submerged. Make sure no water is leaking in.
12. Now take your first reading by finding the water level inside the pipette, and recording the number written on the pipette there. Do this for both respirometers. (#3)
13. Take a reading every 2 minutes. We will measure how much O_2 they used at the end.
14. While you are waiting, finish the questions below your data table.

15. After 20 minutes are up, read the water level and record. (#4)
16. Observe your crickets again. Now that they have been sitting in the water for a while, do you notice any difference between the cold and the warm cricket? Is one moving around more than the other one? Record your observations. (#6)

Analysis

1. Now let's calculate how fast each cricket used oxygen. For the cold cricket, subtract #4 from #3. This gives you how many milliliters of O_2 he used.
2. Then divide the number you got by 20. This is how many minutes the cricket consumed O_2. This gives you how many mL of O_2 the cricket used up per minute.
3. Repeat the same steps for the warm cricket and the worms
4. Compare your results and conclude which one used up more oxygen.

Pre-lab questions:

1. Provide the equation for cellular respiration
2. Describe the difference between anabolism and catabolism
3. What are the three processes involved in cellular respiration?
4. Compare between glycolysis, Kreb's cycle, and ETC in terms of location and amount of ATP generated.
5. What is the purpose of the KOH solution inside the test tube?
6. Describe how the water in the pipette enter as the crickets continue to respire?
7. Setup your laboratory notebook with Title, Introduction, Observation, Data, Calculations and Conclusion.
8. Analysis questions.
9. Create a data table for your experiment:
    a. You will need a data table
        i. for crickets in cold water bath for readings every 2 minutes for 20 minutes
        ii. for crickets in warm water bath for readings every 2 minutes for 20 minutes
        iii. for meal worms in cold water bath for readings every 2 minutes for 20 minutes
        iv. for meal worms in warm water bath for readings every 2 minutes for 20 minutes