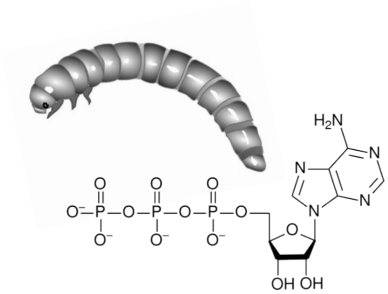
Lab 8
Cellular Respiration
(April 2014)



Section 1 – Review of Energy Molecules

[2] Welcome to this week's lab on cellular respiration. This lab continues the exploration of how organisms obtain and use energy. As you study this diagram you will see that photosynthesis and cellular respiration are basically opposite reactions. In photosynthesis, autotrophs capture light energy and store the energy in glucose. In cellular respiration, organisms break down glucose to release energy

[3] Before getting to respiration, let's review some concepts from last week. Look at Section 1 and see if you can answer the first two questions and then return to the program to see if you remembered correctly.

[4] How did you do? Did you remember that ATP carries energy in a chemical bond and NADPH carries energy in high energy electrons? If so great! If not, you can look back at Section 2 of Lab 7 for a brief review.

[5] Cellular respiration uses two new molecules to carry high energy electrons. These molecules are seen here. Look at them and see if you can recognize which of these molecules have picked up the high energy electrons.

[6] OK, did you figure it out? See if you did by answering this question.

[7] Did you remember that the negative charge of electrons is cancelled by the positive charge of hydrogen ions and thus conclude that the NADH and the $FADH_2$ are the molecules that contain the high energy electrons? With this information you can answer the last question in Section 1 and then return to the program.

Section 2 – Biochemical Pathways in Cellular Respiration

[8] Now we will continue our exploration of energy and cellular respiration. All organisms, including plants, break down organic molecules to obtain energy. This energy is then used to produce ATP. The ATP in turn is used to carry on cellular activities.

[9] Just like we saw with photosynthesis, there are several different chemical pathways and sub-reactions associated with how organisms release energy from organic molecules to produce ATP. The two main pathways are respiration and fermentation. List these pathways in the first part of Section 2.

[10] Last week we saw how plants produce glucose using the energy and electrons from ATP and NADPH. Do you remember where the energy is stored in glucose?

[11] Yes, the energy is stored in both chemical bonds and high energy electrons. The chemical reactions involved in extracting energy from glucose will use the energy released when the bonds in glucose are broken and the high energy electrons are released.

[12] Both fermentation and respiration start with glucose. Glucose is first broken down during a process called glycolysis. The product of this process is pyruvate. Notice one of the bonds in glucose is broken to release energy. The energy yield from this breakdown is 2 ATP. You can fill in this amount of ATP in the diagram in Section 2.

[13] Once glycolysis is complete there are two pathways that can be followed. If oxygen gas is ***not*** present, an organism's cells will undergo fermentation. This pathway doesn't yield any additional ATP but is important is setting the stage for additional glycolysis. We'll come back to this concept at the end of the lab. Fill in zero ATP in the diagram in Section 2 for fermentation.

[14] The other possible pathway after glycolysis is cellular respiration. This reaction requires oxygen gas and will yield an additional 30 or more ATP depending on the organism and the type of cells involved. Fill in this last energy yield in the diagram in Section 2 and then continue.

[15] Try answering this question.

[16] Now that you know that the presence or absence of oxygen gas will determine the pathway, let's see what happens when there is no oxygen available. You may want to jot down the answers to the next few questions in Section 2 as we go along.

[17] Again the actual pathway depends on the type of organism. Plants produce ethyl alcohol and carbon dioxide. This type of fermentation is used in the production of alcoholic beverages. It is also used in baking. Here you can see an example of how the carbon dioxide produced by yeast makes bread rise. The process of plant fermentation is diagrammed for you.

[18] Animals have a different fermentation pathway as you can see here. Animal muscle cells will undergo fermentation when the cell's demand for oxygen is greater than the body's ability to deliver oxygen to the cells. After vigorous exercise when lactic acid is produced, the lactic acid is transported to the liver where it is reconverted into pyruvate. Make sure you have completed Section 2 through the product of animal fermentation and then continue with the program.

[19] If oxygen gas is present an organism will complete a series of reactions that can be summarized in the overall process you see here. Copy this overall reaction for cellular, or aerobic respiration into your laboratory manual and then return to the program.

[20] Now see if you can identify the reactant molecule that provides the energy for the production of ATP. Write this answer in your laboratory manual and then return to the program.

[21] You should have identified glucose as the reactant that provides the energy for the production of ATP. Look again at the reaction and determine the relationship between the number of oxygen molecules used and the number of carbon dioxide molecules produced. This relationship will be important in the experiment you will perform later. Fill in the answer to this question in your laboratory manual.

[22] Did you say 6? Great! Now take another look at the equation and determine how many glucose molecules are used for every six oxygen molecules used. When you have answered the question correctly, continue with the program.

[23] Now here is another opportunity to see how much you remember from last week's lab. See if you can finish up Section 2 by answering the questions about the Laws of Thermodynamics and then return to the program.

[24] Here is another question for you to try.
[25] The First Law of Thermodynamics tells us that energy can be transformed or transferred. While photosynthesis was a transformation of light energy to chemical energy, cellular respiration is a transfer of chemical energy in glucose to chemical energy in ATP.

[26] The Second Law of Thermodynamics tells us that energy transformations and transfers are not 100% efficient and that some energy is dispersed as heat. Therefore all of the energy in glucose is not transferred into ATP but some is "lost" as heat. Be sure your answers are correct to the last questions of Section 2 and then return to the program.

Section 3 – Volumeters and Measuring Air Pressure

[27] The experiment you are going to perform this week will allow you to measure a meal worm's rate of cellular respiration. Look again at the overall equation. This is a balanced equation and if you know the number of one molecule used, you also know the number of other molecules used or produced just as you figured out the relationship between the number of glucose and oxygen molecules used and the number of carbon dioxide molecules produced.

[28] We can determine the metabolic rate, which is the use of energy, if we can measure the amount of any of the molecules either used or produced during the reaction. For instance we could measure either the oxygen or glucose consumed, or the carbon dioxide produced.

[29] Try this question.

[30] How about another one?

[31] Since it is the easiest to do, you will determine the metabolic rate of the mealworms by measuring the rate of oxygen consumption. To do this you will be using an apparatus called a volumeter with the items you see here. Look at the volumeter diagram in your laboratory manual in Section 3 as I explain how this apparatus works.

[32] The volumeter apparatus has two parts you will need to obtain - the reaction vessel with its central well and the sidearm apparatus with the rubber stopper and syringe. You need to exercise care when using the volumeters.

[33] The reaction vessel has a central well. You will be placing a small piece of filter paper in the well and then dropping KOH onto the paper. You must be sure that the KOH goes into the well and nowhere else.

[34] The sidearm apparatus has a thin glass tube that is easily broken, so handle it carefully. If it does break, just contact the lab instructor or the lab technician for a new one. You will be placing the sidearm apparatus onto the reaction vessel.

[35] Once you have placed the stopper into the reaction vessel you will be putting some red solution into the sidearm. The syringe will allow you to adjust the position of the red solution.

[36] When the sidearm apparatus is in place and the red solution is in the sidearm, the system is sealed. All entries are closed off so no molecules can enter or leave the volumeter and it is considered a closed system.

[37] Since the system is closed or airtight, only changes in the internal pressure can cause the red solution to move.

[38] OK, try this question.
[39] While performing the experiment you will be working with two volumeters. One will have mealworms and is the experimental volumeter. The other volumeter is called the control and will have no worms.

[40] Both volumeters will have KOH which is potassium hydroxide in the reaction vessel well. Potassium hydroxide absorbs carbon dioxide. Why do we need to have the potassium hydroxide?

[41] Before we go on here is a question.

[42] And here is a follow up question.

[43] Another thing to think about is why we need two volumeters. Why do we need a control? What is its role in the experiment? To answer these questions, we need to know a little more about what happens to the volumeters.

[44] The control volumeter will **monitor** changes in the internal pressure of both volumeters caused by external conditions. Let's see what changes can occur.

[45] The most likely cause for a change in internal pressure would be a change in room temperature. When the temperature increases, the heat from the room is transferred to the air molecules in the reaction vessel. As the molecules move faster with the increased energy the volume of the air molecules expands and the red solution moves out.

[46] Here is the beginning and end of the previous animation. As you study these diagrams you can see the starting point of the red solution in the top diagram. As the room temperature increased, the air volume inside the volumeter increased and the red solution moved out as depicted in the lower diagram.

[47] If the temperature drops, heat is lost from the reaction vessel, the molecules move more slowly, and the volume contracts. When this happens the red solution moves in.

[48] Once again, here is the beginning and end of the previous animation. As you study these diagrams you can see the starting point of the red solution in the top diagram. As the room temperature decreased, the air volume in the volumeter decreased and the red solution moved in as seen in the lower diagram.

[49] Let's see if you understood this by answering this question.

[50] Another less likely reason the pressure inside the volumeter will vary is if the atmospheric pressure changes. This is a little more complicated so you don't need to worry about what changes occur, only that the control volumeter will monitor these changes as well.

[51] Now see if you can answer the questions that follow the diagrams of the volumeters in Section 3. Return to the program after you have filled in the table for the control volumeter and changes in room temperature.

[52] How did you do? Did you remember that the volumeters are considered closed systems because no air molecules can enter or leave?

[53] Did you remember that potassium hydroxide absorbs carbon dioxide?

[54] Did you remember that the control volumeter monitors changes in the room conditions and either a change in temperature or atmospheric pressure will cause the bubble to go in or out?

[55] Lastly did you fill in the table correctly? If not, correct it now and then return to the program.

[56] OK, we have our control volumeter to tell us if there are external causes for changes in the internal pressure. We also have our experimental volumeter to measure the amount of oxygen gas the worms use. The reason we need both the control and experimental volumeters is because the experimental volumeter is also affected by external factors. We need to account for both environment changes and the oxygen gas used by the worms.

[57] Because we are collecting data from the two volumeters, we will need to calculate an adjusted measurement. Study the adjusted measurement table in your lab manual. The first example shows you a case where there was no room pressure change as indicated by the control volumeter. In this case it's easy to see the amount the experimental volumeter's red solution moved was due to oxygen consumption by the worms.

[58] Now what happens if the red solution in both volumeters moves? Well it depends on which way they move. In the second example, both the control and experimental volumeters' red solutions have moved

in. One centimeter movement was due to an air pressure change. This is true for both volumeters. Therefore to determine oxygen consumption you will subtract to get the centimeters the solution moved due to oxygen consumption.

[59] In the third example, the control moves out and the experimental moves in. Can you see how the adjusted measusrement of 2.5 centimeters is determined?

[60] Did you understand why you had to add the two measurements? With no worms the red solution moved out a half a centimeter. Because the experimental volumeter's red solution also moves due to the temperature change the worms use the half centimeter "out" plus the two centimeters "in" or a total of two and a half centimeters of oxygen consumption.

[61] Last example. This time both red solutions move out and once again we subtract.

[62] Try this sample problem to see if you understand.

[63] How about another one?

[64] If you understand this concept you should be able to see why something went wrong in the experiment if the solution in the control goes in and the experimental goes out, or if the control goes in and the experimental doesn't go in as far, or if the control goes out and the experimental goes out further.

[65] There can be a number of reasons the experiment does not work as expected, but the most common is an air leak in the system or extra "bubbles" of red solution in the sidearm. Therefore before you start your experiment and while setting up the volumeters you should check the sidearms for excess red solution and be sure you seat the sidearm stoppers firmly in the reaction vessels.

[66] Because it is very important to understand how the volumeters should work see if you can answer this question.

Section 4 – Measuring the Rate of Cellular Respiration

[67] It's time to get to that experiment! Two cautions before you start. First, please use the "soft" forceps to transfer the mealworms to and from the experimental volumeter. Also be sure you do not get any potassium hydroxide on the mealworms.

[68] Second, place your volumeters close together in your booth. They are very sensitive to temperature changes and if you have one closer to you or your computer the external temperature may not be the same for both volumeters.

[69] Now complete your experiment by following the directions in Section 4 and any additional instructions at the demonstration table. Once you have completed the experiment, have the instructor check and initial your results and then return to the program.

[70] I hope all of your worms made it safely back into their dish and that you have returned your volumeter equipment to the proper locations. Now that you have all your data, you can calculate how much oxygen your worms consumed. Just follow the calculations and once again have the instructor check and initial your results. Return when you have done this.

[71] Answer this question about experiments.

[72] Not all worms consume oxygen at the same rate so your results can be quite different from those obtained by other students. You can use this information shown here to see how your worms compare to worms of the past. Were your worms sleeping or running a marathon? Now finish up Section 4 by answering the last two questions and then return to the program.

Section 5 – The Cellular Organelle of Aerobic Respiration

[73] What were the worms doing with the oxygen they took in? In order to see what happened we need to start back at the beginning with glucose. Once again glucose contains the energy organisms use to manufacture ATP.

[74] As I mentioned at the beginning of the lab, the process of getting the energy out of glucose starts with glycolysis. This reaction takes place in the cytoplasm of a cell.

[75] If oxygen is present organisms continue with cellular respiration and a specialized organelle, the mitochondrion, is necessary. You should be able to answer the first two questions in Section 5 and then come right back.

[76] Try another question.

[77] Here is a closer look at a mitochondrion. It is basically a folded membrane within another membrane. Different structures and areas within the mitochondrion are responsible for the different sub-reactions of cellular respiration.

[78] You should use the information in the next few program pages to label the mitochondrion in Section 5. First, the space between the inner and outer membranes is the intermembrane space.

[79] The inner folded membrane is called the cristae and is the site of electron transport.

[80] The area inside the cristae is the matrix and is the site of the Krebs cycle which is also referred to as the citric acid cycle. Be sure you have your mitochondrion labeled correctly and then continue.

Section 6 – Glycolysis: Beginning Cell Respiration

[81] As with photosynthesis, the cellular respiration pathway from glucose and oxygen to carbon dioxide and water is quite complex. For this lab, we will only consider the sub-reactions in general terms. You may get more detail in lecture.

[82] Let's start with a look at glycolysis. Got all this? Yeah, I don't want to deal with all of this either! I'll make the process a little less complicated if you'll just go to the next program page.

[83] Here is a simplified version of glycolysis. The term glycolysis translates to "glycol" which means sugar and "lysis" which means splitting. In Section 6 write down the reaction for glycolysis shown here and then return to the program.

[84] To understand what is happening, we'll look at just a few of the intermediate steps. Think about sugar – regular table sugar is a good example even though it's not quite the same as glucose. What happens to sugar over time? Pretty much nothing; it remains sugar. In other words the molecule is stable and doesn't break down by itself.

[85] Since we are going to trace the breakdown of glucose that has six carbon atoms to carbon dioxide that has one carbon atom it will be easier to follow the process if we just look at the carbon skeleton of the molecules produced along the way. Here you can see a linear molecule of glucose and right below the carbon skeleton with the oxygen and hydrogen atoms removed.

[86] To get the energy out of glucose the molecule must be made unstable. This requires energy and is the first step in glycolysis. The addition of energy from two molecules of ATP will destabilize the molecule of glucose and it will breakdown into two molecules of a three carbon intermediate over several steps.

[87] Once the three carbon intermediate is produced, it will breakdown into pyruvate over several steps and release enough energy to produce four molecules of ATP. High energy electrons will also be released and be picked up by NAD^+.

[88] OK, time for some bookkeeping. Since there are four ATP produced and two ATP used in this pathway, the net yield is two ATP molecules produced during glycolysis. We'll see what happens to the NADH molecules produced a little later. At this point you should be able to complete Section 6. A summary of glycolsis is show here to assist you.

[89] Let's summarize glycolysis with a few questions. Here is the first.

[90] Here's another question.

[91] Here is the next question.

[92] And now the last question.

Section 7 – The Krebs Cycle: Continuing Cell Respiration

[93] In Section 7 the next part of cellular respiration is summarized. Fill in the missing molecules and answer the question below the diagram and then return to the program.

[94] Let's look at each step a little more closely. First we have our three carbon pyruvate. There is still a lot of energy in each pyruvate molecule so to start getting the energy out each pyruvate molecule is broken down into a molecule of Acetyl CoA. This is now a two carbon compound and carbon dioxide has been given off. This will occur with both pyruvate molecules that were produced during glycolysis, but we will only follow one molecule.

[95] Also in the approach to the Krebs cycle some high energy electrons are given off and picked up by NAD^+ which will becomes NADH. These high energy electrons will enter the next sub-reaction and their energy will be used to produce ATP.

[96] When the Acetyl CoA enters the Krebs cycle proper it is broken down to carbon dioxide and the energy released is used to produce two ATP. The high energy electrons given off are picked up both NAD^+ and FAD. Again, the energy in the electrons will be used in the final sub-reaction to produce more ATP.

[97] Let's summarize this part of cellular respiration with a few questions.

[98] Now try another one.

[99] Here's one more.

[100] Last one for now.

Section 8 – Electron Transport: Completing Cell Respiration

[101] Speaking of the last sub-reaction let's move on to Section 8. This sub-reaction is called Electron Transport. This is where the high energy electrons stored in NADH and $FADH_2$ give up their energy to produce ATP. This process is very much like parts of the light reaction of photosynthesis. Study this diagram for a minute and then continue with the program.

[102] As before, we'll take one step at a time. The first thing that happens is that NADH and $FADH_2$ enter the cristae and give up their high energy electrons to an electron transport chain. They also give up their hydrogen ions.

[103] The second step is where the high energy electrons are passed through a transport chain of molecules embedded in the cristae. As they pass from one molecule to another they give up some of their energy which is used to produce ATP.

[104] In the last part of the electron transport chain, oxygen gas, what you have been breathing in, picks up the now low energy electrons and hydrogen ions to produce water.

[105] OK, time to sum up again.

[106] Here is another question.

[107] See if you are following the energy flow.

[108] Last question for now.

[109] At this time you can complete Section 8. Here's the entire process of electron transport again to help you answer the questions. Return to the program when you have finished your electron transport summary.

Section 9 – Sub-reaction Summary

[110] Almost done! All that's left is to summarize what we have covered in this lab. In Section 9 there is a table listing the sub-reactions from glycolysis through electron transport. Let's look at glycolysis first. Notice that in the product column each product is aligned with its reactant. The first reactant listed is glucose, the starting molecule for glycolysis. The first product listed is pyruvate the end molecule of glycolysis. The reactants and products that follow are the molecules that transfer the energy released from or stored in chemical bonds and carried in the high energy electrons.

[111] While you are thinking about the reactants and products of glycolysis see if you can answer this question.

[112] OK, try another question.

[113] Look at the next two rows showing fermentation. Do you remember the end products for each type of fermentation? What happens to the NADH?

[114] Let's make sure you are on the right track.

[115] Fill in the products for ethyl alcohol fermentation and lactic acid fermentation and then continue with the program.

[116] Did you fill in the products for ethyl alcohol fermentation correctly? If not, correct them now and then continue.

[117] Did you fill in the products for lactic acid fermentation correctly? If not, correct them now and then continue.

[118] Why do you suppose fermentation occurs if there is no ATP produced from the energy in the electrons carried by NADH? Stop and think about what was necessary for glycolysis to occur. Look at the overall glycolysis reaction shown here and in the first row of your table. You need NAD^+ to pick up the high electrons given off during glycolysis. If there is no NAD^+, glycolysis will stop at the point where

ATP is used, but no ATP is produced. Therefore the function of fermentation is to regenerate NAD^+ so glycolysis can continue.

[119] Before you go on, answer this question to make sure you understand the significance of fermentation.

[120] The last three rows of the table complete cellular respiration. See if you can answer this question.

[121] Now that you have reviewed the reactions you can fill in the products for these reactions. If you need some hints, Sections 7 and 8 will help you. When you have finished filling in the products, return to the program.

[122] With the information shown here you can check your answers. Be sure they are correct before you continue.

[123] Let me just mention one thing before we go on. This will be important for those of you who are going to take microbiology. We've talked about glycolysis, fermentation, and cellular respiration. There is another pathway called anaerobic respiration carried out by some microbes. In anaerobic respiration no oxygen gas is used and the electrons are picked up by other substances.

[124] OK, just a few more things to do in this lab. First you need to calculate the net yield of ATP produced during glycolysis and fermentation. This a simple matter of adding up the ATP in the product column for the reactions involved and subtracting any ATP used in the reactant column. Record your answer in the space provided below the table in your laboratory manual and then return to the program.

[125] Now you need to do two last things for cellular respiration. First, place an asterisk next to the reactions that follow glycolysis in the cellular respiration pathway. Second, calculate the net yield of ATP production for cellular respiration. You can start by determining the ATP produced during electron transport. The ATP yields for each molecule of NADH or $FADH_2$ are given below the table. When you have completed these calculations, return to the program.

[126] How did you do? Did you get 2 ATP for glycolysis? How about cellular respiration? Did you remember to add the ATP from glycolysis and the Krebs cycle to those produced during electron transport? See if your total is correct by answering this question.

[127] Do you still remember where all of this began? Answer this question to see if you are correct.

Section 10 – Review of Cellular Respiration

[128] We've gone over a lot of information so be sure you understand everything by completing the review questions in Section 10. Your lecture instructor may collect this page so be sure to have the lab instructor sign the page. Return to the program when Section 10 is complete and signed off.

[129] To summarize this week's lab we can say that various organisms extract energy from organic molecules by carrying out either glycolysis and fermentation or glycolysis and cellular respiration. Also some microorganisms use glycolysis and anaerobic respiration. So now make sure you have cleaned up your booth and returned all of the equipment to the proper locations and you're done! We'll see you next week.