Chapter 1

Cellular Respiration

Have you ever wondered how your body converts the food you eat into the energy you need to maintain your body systems, grow, as well as move around? Unfortunately, the cells in your body cannot use pizza or hamburgers, in their undigested form, as fuel. Thus, your body must break down the food you eat into simpler elements like amino acids, single sugars, and fatty acids. This breakdown is accomplished through a process called digestion.

Digestion begins at the moment the food enters your mouth. Your teeth tear and grind the food into smaller pieces, while a mixture of enzymes in your saliva begin to chemically break down your meal. Once you swallow, the food moves down your esophagus and into your stomach. Here, gastric juice, a mix of enzymes and hydrochloric acid is secreted into the stomach by cells in the stomach wall. The acid provides the correct pH for the function of the enzymes, which break down mainly proteins.

Fats and carbohydrates (sugars) are broken down in the small intestine. In addition, the digestion of proteins is completed in this structure, and the small molecules that result from all this chemical digestion are absorbed through the walls of the small intestine and enter the bloodstream. Thus, through digestion, the food you eat is turned into molecules that the cells of your body can use for fuel, mainly single sugars and fatty acids.

However, the conversion of the food you eat into energy your body can use for activity has only begun. Just like gasoline in a car, the fuel must be burned to release the energy for use, and the motor of the cell is the mitochondrion. Thus, the single sugars and fatty acids resulting from digestion are transported to the cell by the bloodstream, are absorbed through the cell membrane, and transported to the mitochondrion. Here they will be burned through a process known as cellular respiration.

Cellular Respiration

Burning fuel usually is a very wasteful process. A large amount of energy is given off all at once in the form of heat or light. This type of release is useful if you want to heat your house, but a cell cannot use heat or light energy to fuel its processes. Thus, cellular respiration is a type of controlled burning, where energy is released in a series of stepwise reactions catalyzed by enzymes. At each step, energy is released in small enough amounts to be captured and stored in molecules of adenosine triphosphate (ATP). ATP can be used by cells to fuel all their activities and, thus, can be viewed as the energy "currency" of cells.

Cellular respiration can be divided into three stages: glycolysis, the Krebs cycle, and the electron transport chain.

Glycolysis

Glycolysis occurs in the cytosol (cytoplasm) of the cell and converts glucose into 2 molecules of pyruvic acid. During this process, two molecules of ATP and two molecules of nicotinadmide adenine dinucleotide (NADH) are formed (See Diagram 1 on the next page). Although you do not need to know all the details of glycolysis, it is important to realize that there are many steps in the glycolytic process. Each of these steps produces an intermediate molecule and is catalyzed by a specific enzyme.

Diagram 1:Glycolysis

[MISSING FIGURE]

Interestingly, the first few reactions of the glycolytic pathway actually require energy. Thus, the cell must spend energy to start the reaction. In other words, the cell needs to "prime the pump," before energy can be released from glucose. However, the molecules of ATP expended in "priming the pump" will be reformed in subsequent steps of the process, and the cell will gain much more energy from the breakdown of glucose than it expends to get the reactions started.

Along with ATP and NADH, 2 molecules of pyruvic acid are also produced by glycolysis. The fates of the pyruvic acid molecules as well as those of the two molecules of NADH depend on the type of cell carrying out glycolysis and on whether the environment is aerobic (with oxygen) or anaerobic (without oxygen). In the presence of oxygen, the molecules of pyruvic acid are broken down in the Krebs cycle, and the 2 molecules of NADH are utilized in the electron transport chain. The combination of these processes release 36 molecules of ATP and is much more efficient than fermentation, the process that occurs if the cell lacks oxygen. Thus, the Krebs cycle and the electron transport chain, collectively known as the citric acid cycle, will be discussed first.

Krebs cycle

For simplicity, only one molecule of pyruvic acid will be tracked through the citric acid cycle. However, the breakdown of one molecule of glucose actually produces two molecules of pyruvic acid. Thus, the yields of ATP and NADH diagrammed below should be doubled.

Diagram 2: The Krebs Cycle

[MISSING FIGURE]

The Krebs Cycle occurs within the matrix of the mitochondrion. Thus, the pyruvic acid produced by glyolysis must enter this organelle so that it can be broken down. As you can see in Diagram 2, the first reaction of the Krebs cycle releases one molecule of carbon dioxide (CO_2) and produces another molecule of NADH. In addition, this reaction produces a molecule of acetyl-CoA by joining two of the carbon molecules of the pyruvic acid (remember one carbon atom was lost in the production of CO_2) to a compound called coenzyme A.

At this point, the reactions of the Kreb Cycle get a little confusing, but you need to remember that the Krebs Cycle is just that, a cycle. Thus, acetyl-CoA joins with the four carbon

compound that is shown as the third molecule in the circle portion of Diagram 2. Thus, a six-carbon molecule, citric acid, is produced. Citric acid is then broken down in the succeeding steps of the process, and 3 molecules of NADH, one molecule of ATP, and one molecule of $FADH_2$ are formed. In addition, the breakdown of citric acid also produces a four carbon compound, the same four carbon compound that binds with acetyl-CoA to form citric acid. Therefore, one of the substrates needed for the breakdown of pyruvic acid is reformed. However, for both glycolysis and the Krebs cycle to continue to function, the NADH, and $FADH_2$ formed in these processes must be returned to their original states, NAD+ and FAD+. If the NADH and FADH2 molecules did not lose their high energy electrons, they would not be available to pick up electrons when the cell tried to break down another molecule of glucose. Thus, there must be a way for the cell to take the high energy electrons from these molecules and convert those electrons into ATP as well as reform the NAD+ and FAD+ molecules that are required to keep the cycle going.

Electron Transport Chain

The process that converts the high energy electrons of the NADH and $FADH_2$ molecules into ATP and returns NAD+ and FAD+ molecules to glycolysis and the Krebs cycle is the electron transport chain. However, the conversion of high energy electrons into ATP is not as straightforward as one might think (see Diagram 3). Electron carriers embedded in the cristae of the mitochondria pick up the high energy electrons from NADH and $FADH_2$ molecules. These electrons are then passed down a chain of electron carriers, losing energy at every step. The energy that is lost is used to pump H+ ions across the membrane. Thus, hydrogen ions build up in the intermembrane space. These hydrogen ions then travel down their concentration gradient, and move back into the cristae. As the H+ ions move back across the membrane, a ATP synthase embedded in the membrane spins and forms ATP.

Diagram 3: Electron Transport Chain

[MISSING FIGURE]

As was the case with the NADH and $FADH_2$ molecules, the electron carriers of the electron transport chain cannot hold onto their high energy electrons indefinitely. If a carrier cannot get rid of an electron, it will not be able to pick up another electron, and the chain would cease to function. Thus, a final electron acceptor is required, and in this case, the acceptor is oxygen. Oxygen's role as the final electron acceptor explains the need for oxygen for the completion of oxidative respiration. In addition, as the oxygen picks up the electrons it also binds to hydrogen ions, and these reactions form one of the waste products of cellular respiration, water.

Fermentation

So, what happens when a cell doesn't have oxygen? As we learned above, oxygen serves as the final electron acceptor for the electron transport chain. Thus, without oxygen, the transport chain cannot function, and, because the Krebs cycle relies on the electron transport chain to provide it with molecules of NAD+ and FAD+, the Krebs cycle also doesn't function. However, although glycolysis also requires molecules of NAD+ to accept electrons, there is a way that this part of cellular respiration can function, and this process is termed fermentation.

The reactions of glycolysis under anaerobic (no oxygen) conditions are similar to those under aerobic conditions (Diagram 4). Glucose is broken down into 2 molecules of pyruvic acid, and two molecules of ATP and two molecules of NADH are formed. However, because the electron transport chain is not available to take the high energy electrons of NADH and return NAD+ molecules to glycolysis, the cell must find another way to reform these necessary molecules. Thus, the only available electron acceptor is the pyruvic acid. When pyruvic acid accepts the electrons, it is turned into lactic acid. The lactic acid is a waste product of fermentation and, if not removed, can cause problems for the cells that produce it.

Diagram 4: Fermentation

[MISSING FIGURE]

The addition of lactic acid to the internal environment of the cell will cause its pH to drop. If the production of acid continues, and it is not removed by diffusion into the blood, the cell's systems, especially enzymes, which are sensitive to pH will shut down. However, cells vary in their ability to deal with the production of lactic acid. There are some cells that are able to maintain their internal pH, even though acid is being added. This ability is brought about by the presence of substances called buffers. In comparison, some cells have no ability to deal with the build-up of lactic acid and quickly die if this end product is not removed.

Studying Cellular Respiration

The reactions of glycolysis, the Krebs cycle, and the electron transport chain are very complex, and you may be wondering how we can think about studying these processes in the laboratory. There a large number of ways that one can study cellular respiration. For example, a researcher could measure the amount of oxygen a particular organism consumed over a certain time period. This measurement would reveal how quickly the organism needed to produce ATP to fuel its cellular processes. Similarly, you could also measure the amount of oxygen the organism consumed during a specific activity, like running. However, these studies only provide information about how a particular organism utilizes the entire process of cellular respiration. These methods cannot be used to study the different stages of respiration in isolation.

To study each of the stages in detail, you can look at the activities of specific enzymes. As we learned above, each step of cellular respiration is catalyzed by an enzyme. Thus, by studying the activity of an enzyme involved in glycolysis, the Krebs cycle, or the electron transport chain, we can learn about each of these stages. For example, glycerophosphate dehydrogenase (GPD) is an enzyme involved in glycolysis. However, because the stages of cellular respiration are linked, the level of an enzyme involved in glycolysis may be related to the levels of the enzymes involved in the Krebs cycle and the electron transport chain. Again, we are having trouble separating the different stages of cellular respiration.

To isolate each stage of respiration, we need a tissue that has cells that have specialized in one of the stages of cellular respiration. Only a few tissues with this type of specialization exist, and skeletal muscle is one of them. Skeletal muscle is the tissue that is involved in voluntary movements. For example, you can use your biceps muscle to lift things with your arm, and there are skeletal muscles throughout your body (Diagram 5).

Diagram 5: Skeletal muscles

[MISSING FIGURE]

Muscles as a model system

NADH activity

Skeletal muscle tissue is composed of cells, which are also called muscle fibers (Diagram 5). Within a given muscle, like your biceps, there are fibers that utilize the whole process of cellular respiration to produce the ATP necessary to fuel muscle contraction. We can study these muscle fibers by examining the activity of an enzyme involved in the electron transport chain, nicatinamide adenine dinucleotide dehydrogenase (NADH-D). By staining for the activity of this enzyme, we can determine which fibers utilize this pathway, because they will have a large amount of this enzyme. Fibers that utilize only glycolysis to produce ATP will not have this enzyme. Thus, we will have fibers that will stain for the presence of this enzyme and fibers that will not.

Diagram 6: Muscle fibers

[MISSING FIGURE]

To visualize the activity of this enzyme, we will first add substrate, NADH, to the muscle tissue. The electron acceptor, NADH-D, will pick up the high energy electrons from this substrate and pass them down the electron chain. However, because the electrons are invisible, we need to add something else to the mixture to allow us to see the reaction that is occurring. Thus, we will add an electron acceptor, nitro-BT. When nitro-BT accepts the electrons from the transport chain, it turns blue. Thus, we will be able to see the activity of the enzyme as a blue stain.

Acid buffering capacity

To study the muscle fibers that utilize only the glycolytic pathway to produce ATP, we will utilize a slightly different method. In the discussion above, we learned that cells that use only glycolysis produce lactic acid in a process called fermentation. Thus, a muscle that has a large number of fibers that utilize only glycolysis will experience a large influx of lactic acid that could potentially damage its enzymes. To prevent this damage, these muscles have proteins that are able to buffer the acid. For example, one protein that is thought to buffer the production of acid in muscle is carnosine (Davey, 1960).

How do these proteins prevent a change in pH when acid is being produced in the cell? Or, put more generally, how do buffers work? Buffers are usually composed of a weak acid and

its related base, composed of a salt. The concentrations of these elements in the cell are large compared to the concentration of the base or acid being added to the system. If an acid is added, some of the base of the buffer is converted into the weak acid. Thus, acid is still formed, but because the acid that is formed is weak, there is only a small change in pH. Similarly, if base is added, the weak acid component of the buffer gives its protons to the added base, converting it into the base of the buffer. Again, base is formed, but because the formed base is weak, there is little change in pH.

Diagram 7: Acids and Bases

[MISSING FIGURE]

We are going to measure the ability of muscles to buffer the addition of lactic acid. To do so, we will grind up muscle samples in a salt solution. We will then add a set volume of sodium hydroxide (NaOH) to the muscle and measure the change in pH. Although we are interested in the muscle's ability to buffer the addition of an acid, lactic acid, other researchers have determined that adding hydrochloric acid (HCl) to muscle produces artifacts. These artifacts prevent any accurate measurement of the buffering capacities of a particular muscle. However, systems that can buffer acid also have the ability to buffer base, as we learned above, and it has been demonstrated that the addition of NaOH to muscle tissue does not cause artifacts. Thus, we will use NaOH in our assay.

Once we have measured the buffering capacities of different types of muscle, we will be able to identify the muscle samples that can maintain their pH despite the addition of the base. These muscle samples will have high buffering capacities, and, thus, large numbers of fibers that rely on glycolysis for their ATP production.

How would you expect the acid buffering ability of a muscle to compare to its NADH-D activity?

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_____

References

Castellini, M.A., G.N. Somero. 1981. Buffering capacity of vertebrate muscle: correlations with potentials for anaerobic function. Journal of Comparative Physiology B 143: 191-198.

Davey, D. 1960. The significance of carnosine and anserine in striated skeletal muscles. Archives of Biochemistry and Biophysics 89: 303-308.

Miller, K.R., Levine, J. 2002. Biology. Upper Saddle River: Pearson Education, Inc. 1114 p.

Novikoff, A.B., Shin, W., Drucher, J. 1961. Mitochondrial localization of oxidative enzymes: staining results with two tetrazolium salts. Journal of Biophysics, Biochemistry, and Cytology 9: 47-61.

Protocol 1. Nicotinamide adenine dinuclueotide dehydrogenase (NADH-D) activity in muscle fibers

Purpose: To learn a technique that demonstrates nicotinamide adenine dinucleotide dehydrogenase (NADH-D) activity in muscle cells and observe the levels of activity in different muscle fiber-types

Equipment:

- coplin jar
- scale
- weigh dishes or paper
- spatula
- two small beakers
- large plastic tub
- glass staining dishes
- compound microscope
- digital image capturing system (optional)

Materials:

- microscope slides with frozen cross-sections (10 μm thickness) of muscle tissue
- Trizma buffer
- Nicotinamide adenine dinucleotide
- nitro-BT
- distilled water
- glycerogel
- coverslips

Procedure:

1. Set up a warm water bath by placing glass staining dishes into the bottom of the large tub.

2. Fill the tub with warm water and allow the water to run during the entire experiment.

3. Label slides with your group name, muscle type, and test. Label a coplin jar with your group name.

4. Measure out 10 mg of NADH and 20 mg of nitro-BT. Place these chemicals in a small beaker and add 20 ml of Trizma buffer. Gently swirl to mix.

5. Your instructor will prepare a control solution, containing 20 mg of NBT and 20 ml of Trizma buffer. A slide with your muscle tissue on it will be run in this control solution.

6. Gently place your slide into the coplin jar with the sections facing to the right.

7. Pour the solution into the coplin jar, letting it run down the side of the jar.

8. Put the coplin jar onto the glass staining dishes in the warm water bath.

9. Place glycerogel into the warm water bath.

10. Pour out the NADH solution and rinse 4x in distilled water.

11. Place a drop of glycerogel on top of the muscle section.

12. Place a coverslip over the drop.

13. Allow glycerogel to harden for approximately 10 minutes.

14. View the test slide under 20x power. Muscle fibers with high activity will be blue. Those with low activity will be white.

15. In your field of view, count the number of fibers that are blue and record in table 1. Then, count the number of fibers that are white and also record in the table.

16. Repeat step 14 until you count 50 fibers total (blue + white).

Table 1: Numbers of muscle fibers with high (blue) and low (white) NADH activities.

View #	High (Blue)	Low (White)
1		
2		
3		
4		
5		
Totals		

17. Compute the percentage of muscle fibers with high NADH activity in your cross-section by dividing the number of blue fibers by the total number of fibers you counted (blue + white).

_____

18. Compare the test slide to the control slide. Are there any differences?

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19. Compare your results with those of others in the class. Are there differences in your values?

20. Now that you have collected some data, how many fibers would you count to calculate the percentage of high NADH activity fibers in the **entire muscle**?

21. How did you determine this figure?

22. How would you collect this data? Would you have the entire class contribute or would you only let one person or one small group of people collect the data?

23. Why?

Interactive Research: Muscles with Different NADH-D activities

Once you have mastered the staining technique for NADH-D activity, you can study the activity of this enzyme in different muscles. For example, you could compare the activity of NADH-D in different muscles in the same animal. What might you expect if you looked at the leg muscles of a cat in comparison to its back muscles? You could also compare the same muscle in different animals. Do you think a sprinter would have the same number of high NADH-D activity muscle fibers in his or her leg muscles in comparison to a long distance runner?

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There are many questions that you could pose and answer using the technique you learned in this lab. For example, you could compare the muscles of terrestrial mammals with those of marine mammals. Marine mammals like whales, dolphins, and porpoises dive to deep depths and hold their breaths while they dive. What would you hypothesize about the NADH activity of their locomotor muscles in comparison to those of terrestrial mammals?

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What other muscles would you like to compare using the NADH staining protocol? What would be your hypothesis? How would you test your hypothesis?

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Protocol 2: Buffering capacity of vertebrate muscle

Purpose: To learn a technique that assesses the ability of muscle to prevent a pH decrease when acid is added, a measurement that correlates with a potential for anaerobic function.

Equipment:
- Blender
- Scissors
- 250 ml beakers
- 50 ml Erlenmeyer flasks
- Stir bars
- Stir plate
- pH meter
- Graduated cylinders
- Graduated pipets
- Razor blades

Materials:
- 0.9% NaCl
- Frozen muscle tissue – 3.0 g
- 1.0M HCl
- 0.2N NaOH

Procedure:

1. Record type of muscle tissue that you are using. _____
2. Weigh muscle and record its weight in grams. _____
3. Pour 60 ml of 0.9% NaCl into blender.
4. Add muscle.
5. Homogenize for approximately 1 minute, until there are no large pieces of muscle in the mixture.
6. Place a stir bar into a 50 ml Erlenmeyer flask.
7. Pour 10 ml of homogenate into the flask.
8. Place the Erlenmeyer on the stir plate.
9. Turn on the stir plate.
10. Calibrate pH meter, if you are instructed to do so.

11. Measure the initial pH of the homogenate and record in the table below.

12. Add 0.25 ml of 0.2N NaOH to homogenate. Record pH in the table below.

13. Repeat step 13 five times.

Table 2: Milliliters of base (NaOH) added to _____ muscle homogenate and recorded change in pH.

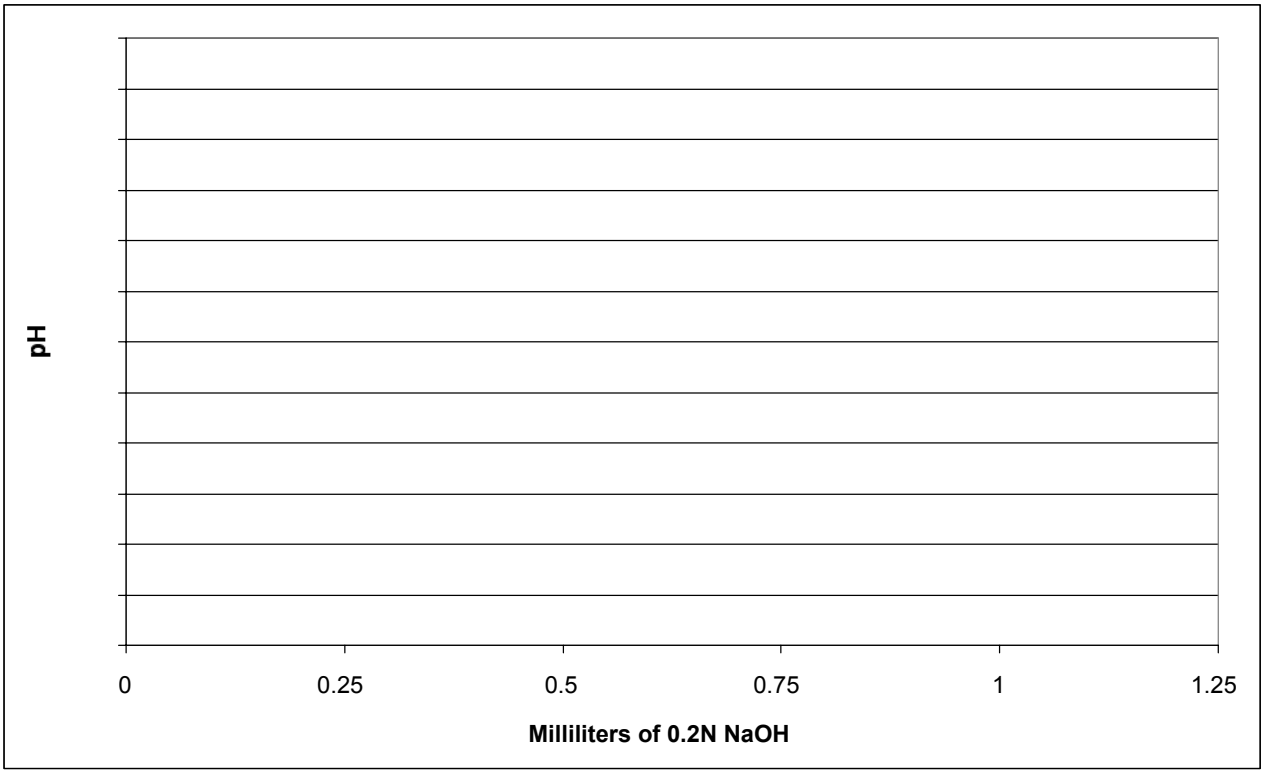
Milliliters of NaOH	pH
0	
0.25	
0.50	
0.75	
1.00	
1.25	

14. Repeat steps 7 through 13 for the second and third homogenates. Record your results in the table below.

Table 3: Milliliters of base (NaOH) added to _____ muscle homogenate and recorded change in pH.

Milliliters of NaOH	pH (#2)	pH (#3)
0		
0.25		
0.50		
0.75		
1.00		
1.25		

15. Graph the results of your experiment. You can include the results for each of the three homogenates on the same graph.



16. Calculate the slope (change in pH/change in ml) of each of the three lines on your graph.

    Homogenate #1: slope = _____

    Homogenate #2: slope = _____

    Homogenate #3: slope = _____

17. Calculate the ml of base that would be needed to change the pH of each homogenate by 1 pH unit using the following formula: 1 ml/Slope.

    Homogenate #1: _____

    Homogenate #2: _____

    Homogenate #3: _____

18. Calculate the grams of muscle in each homogenate by dividing the total weight of the muscle sample you used by 60 ml and then multiply that value by 10 ml.

    _____ g in each homogenate.

19. Calculate buffering capacity for each of the homogenates (#1, 2, and 3) using the following formula:
    0.2μmoles/ml x (answer of step #20) x (1 g muscle/answer of step #21).

    Homogenate #1: _____

    Homogenate #2: _____

    Homogenate #3: _____

20. Calculate an average buffering capacity for your muscle sample.

    _____ slykes

21. Record the class data below.

Group #	Muscle type	Average acid buffering capacity (slykes)
1		
2		
3		
4		
5		
6		

25. How do the acid buffering capacities of the different muscle types compare?

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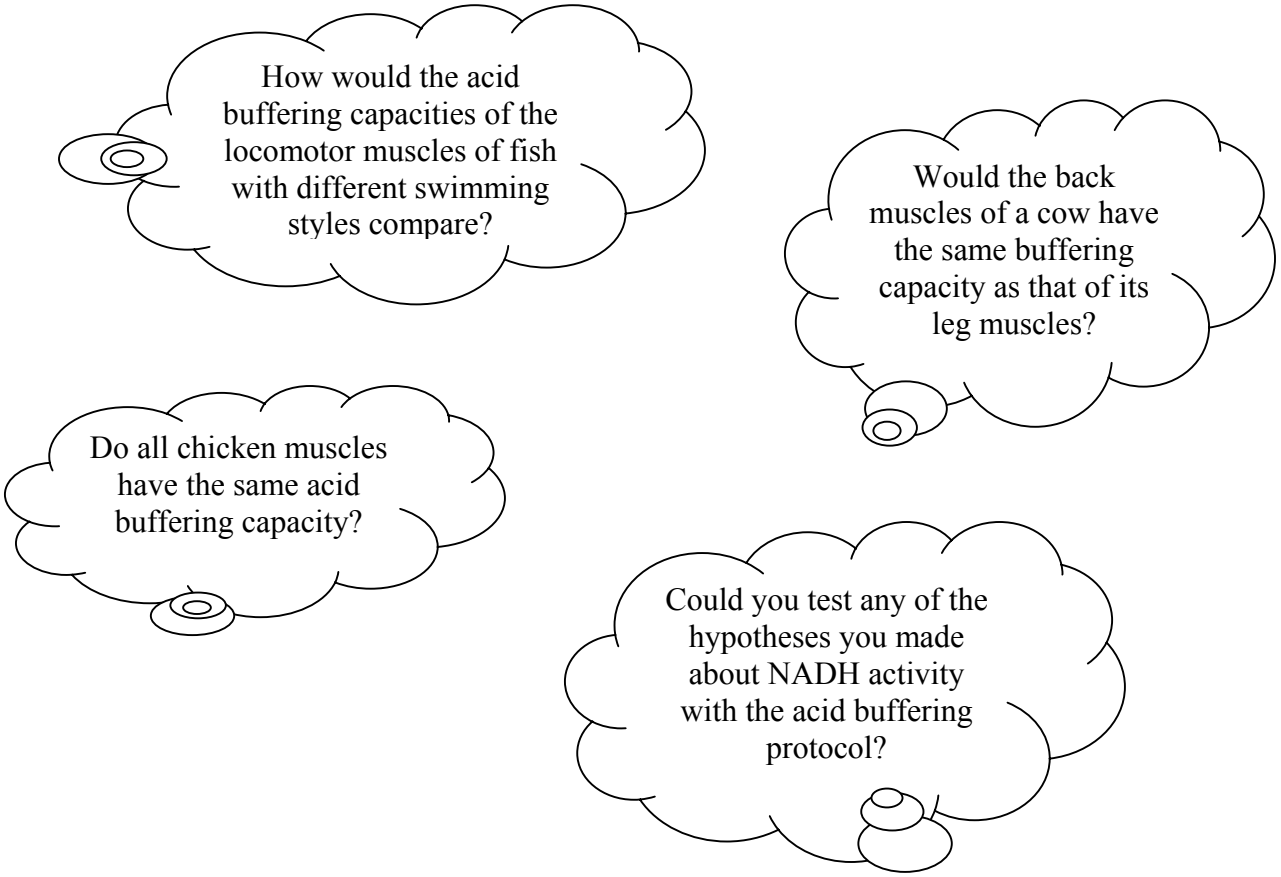
Interactive Research: Muscles with Different Acid Buffering Capacities



1. Make a list here of questions that you would be interested in investigating using the acid buffering protocol.

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Interactive Research Planning Sheet #1 (for exploratory level experiments)

Name _____
Date _____

1. What question have you chosen to investigate and why?

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2. Briefly describe a project you would like to do to address this question?

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3. What supplies will you need?

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4. How do you plan to schedule your project?

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5. Can you find reports by other students or professional scientists on this topic? If so, what can you learn from what has already been done?

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6. Meet with another student or group to discuss these plans using the **Experimental Design Peer Review Form**. Then describe any changes you've decided to make based on this discussion.

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Interactive Research Planning Sheet #2 (for rigorously designed experiments)

Name _____

Date _____

1. What question do you plan to investigate?

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2. Can you find reports by other students or professional scientists on this topic? If so, what can you learn from what has already been done?

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3. What is your **hypothesis** (the prediction of what you think will happen, stated in a way that can be tested by doing an experiment)? Why did you choose this prediction?

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4. What is your **independent variable** (the factor that you will change to make one treatment different from another)?

5. What is your **dependent variable**? (This is the factor you will measure to determine the results of the experiment – it is called "dependent" because the results depend on changes in the independent variable from one treatment to the next.)

6. What treatments do you plan? (Each level of your independent variable is a treatment. You should plan to change only the independent variable from one treatment to the next, keeping all other conditions constant.)

7. How many **replicates** will you have for each treatment? (These are muscle samples that are exposed to exactly the same conditions.)

8. What is your **control** (the untreated group that serves as a standard of comparison)?

9. What factors will you keep **constant** for all treatments (The constants in an experiment are all factors that do not change.)

10. What equipment and supplies will you need?

11. What will you measure, and how will you display your data? Sketch an empty data table here, with the appropriate headings (Think about what kind of table you will need to record the data from your experiment.)

12. On this graph, add labels for the x-axis and y-axis and sketch your expected results.

A Final Check: Evaluate Your Experimental Design

1. Does your planned experiment actually test your hypothesis?

2. Are you changing only one *variable* at a time? Which one?

3. Will your *control* be exposed to exactly the same conditions as your *treatments* (except for the *independent variable*)?

4. How many *replicates* will you have for each *treatment*?

_____

_____

5. Meet with another student or group to discuss these plans using the **Experimental Design Peer Review Form**. Then describe any changes you've decided to make based on this discussion.

_____

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Experimental Design Peer Review Form

Name _____

Name of Project Being Reviewed _____

Date _____

Is the research question clearly defined?

Very clear	☐	Comments about what you liked:
Mostly clear	☐	
Somewhat clear	☐	Suggestions for improvement:
Largely unclear	☐	

Are the procedures clearly described?

Very clear	☐	Comments about what you liked:
Mostly clear	☐	
Somewhat clear	☐	Suggestions for improvement:
Largely unclear	☐	

How well does this experiment address the research question?

Very clear	☐	Comments about what you liked:
Mostly clear	☐	
Somewhat clear	☐	Suggestions for improvement
Largely unclear	☐	