Why Does Hydrogen Peroxide Bubble When You Pour It On A Wound?
Student Led Experimentation of Catalase Activity

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Science is a process. One that involves asking questions and seeking answers to those questions. In the high school biology classroom, most of the experiences that students have are that of being presented facts about living systems and asked to memorize them. Requiring students to memorize facts, without questioning those facts, or learning about the supportive data that lead to them is the antithesis of what science is about. Science is a different way of thinking that is not natural to many students. It is a critical way of looking at things that requires a skeptical point of view and a desire for proof. Although students are given facts to learn, very little time is spent on the enormous amount of work that scientists have done that have added to this body of knowledge. For example, in a typical biology class they learn about cells and consider the cell theory but they rarely delve into the painstaking work of the many scientists that led to the development of this theory. It is critical to understanding science that students are given proper background information as well as facts to learn.

In addition to the background information, they must be given practice in *how* scientists work(1). This is known as the scientific method. In fact, in most science texts the students are simply given the list of the steps of the scientific method and asked to memorize them. A typical list of the steps is as follows:

Make an observation
Identify a research question
Develop a hypothesis
Carry out a controlled experiment
Write a conclusion

My first year biology students, most of which are in the ninth grade, can recite these steps flawlessly. In many text books that I have come across, or in meetings with other teachers, the typical example of an experiment is the famous one regarding a plant. Place one plant in the dark and one in the light and see which one will grow better. To ensure a controlled experiment all other factors must be kept the same, such as type of plant, amount of soil, size of pot, and amount of water. I am fairly confident that any student over the age of 8 could predict the outcome of this experiment. Reciting these steps does not indicate an understanding of the working of science. It does not foster curiosity. It does not show students that there are many questions yet to be answered. How can we encourage students to develop a research question and design a method to answer it? It begins with giving the younger student practice with simple experiments where data is easily

collected, such as observing the behavior of an earthworm and writing it down. Writing the result of testing for a simple substance such as starch with a drop of iodine is another easy example of data collection. It is extremely important that students make the connection that gathering data is the fundamental part of science, and when they write down their observations, they are in fact *doing* science. Observing and recording data is what scientists have done for hundreds of years and are still actively doing today. Although many lab activities designed for students involve collecting data, very few that I have come across have helped students make the connection that data is the most important part of science. Without facts, science becomes fiction.

My school, William A. Hough High School, is a suburban high school that is located in Cornelius, North Carolina. The number of students attending the school is approximately 2000. We offer standard level, honors level, Career and Technical Education and Advanced Placement courses that serve a variety of students. Students in AP biology have a 90 minute class period 5 days per week.This curriculum unit is aimed toward 11th and 12th grade students who have already completed introductory courses in biology and in chemistry.

When we began the year with units on organic chemistry and cell biology, most of them could not give me information about atomic theory or cell theory beyond the basic facts of each. They did not have a good background in the science that led to the development of these theories. In addition to this weakness, I observed that the students were not confident in the laboratory; they needed a great deal of direction and help. Their critical thinking skills also needed attention. For example, in the beginning of the year I asked them to relate the chemical properties of water to its importance in living things and they were stumped. They understood the polar nature of the water molecule, but when it was expected of them to apply this knowledge to biological systems they could not make the connection. These students had already studied biology and chemistry and yet they could not synthesize information from several sources. Regarding their laboratory skills, they could use the equipment to gather data and draw satisfactory conclusions from that data if they were given a guiding question, but for the most part, they failed to show to me that they were able to analyze much of the information gathered in the lab and relate that back to the curriculum covered in the classroom. Lastly, they had absolutely no experience developing their own lab procedure. They were only capable of following a procedure that I provided for them.

UNIT OBJECTIVES AND RATIONALE

This unit "Student Led Experimentation of Catalase Activity" is designed for students in the 11th or 12th grade who are enrolled in an upper level biology course such as Advanced Placement or International Baccalaureate biology. The goal of this unit is to give students background information on the production of hydrogen peroxide by the cell, the importance of decomposing this product, information regarding protein production and enzymatic action, and rates of reactions. Many of the scientists that led to this body of knowledge will also be included in the lessons. They will also be given general instruction on two techniques for determining reaction rate in the laboratory. One of the techniques involves a pressure probe. A majority of students have had experience with these probes and the accompanying software in their earth science and introductory biology classes. The

second method of determining reaction rate involves a titration. Students have completed an introductory course in chemistry where acid base titration is covered in the laboratory. Students have experience using a burette and recognizing endpoints of titrations. They will use their background information and laboratory skills to design an experiment that measures the effect of one factor on the reaction rate of the decomposition of hydrogen peroxide. The students will then analyze their data and critique their experimental design(2). My rational for this unit is as follows: students have little experience synthesizing information that is across several topics. This unit will allow them to tie together many of the facts that they have learned regarding basic cell anatomy and physiology. A connection between ideas and the interrelatedness of the anatomy and physiology of cellular structures is key for students that are college bound and expected to think critically. The design of the experiment also encourages them to connect ideas. They will be allowed to design their own experiment, within classroom parameters, so that they can take ownership of their work *and practice real science*. They can explore an aspect of this topic that they find interesting rather than follow a scripted, cookbook lab that allows for zero creativity. It is my goal that with the unit students will also understand that a scientific hypothesis is not simply an "If…. then" statement, but rather a well researched explanation that can be tested. With a deep understanding of the cellular processes behind their experiment, they will come up with a design which includes a detailed procedure that allows for collection of data that may support the hypothesis that they have generated(Blooms). The idea that science is a not a simple process, but one of repeated trials and many errors will not be lost on these students.

Upon the completion of this unit students will:

1. Describe the metabolic pathway that creates hydrogen peroxide in cells.
2. Understand the importance of the decomposition of hydrogen peroxide in cells.
3. Describe the reaction of the decomposition of hydrogen peroxide.
4. Describe how intermolecular forces influence the structure of a protein molecule.
5. Describe how the enzyme catalase works in the decomposition of hydrogen peroxide.
6. List factors that may have an effect on enzymatic action and explain them.
7. Develop a hypothesis that tests the effect of a factor on enzyme action.
8. Design an experiment that tests their hypothesis.
9. Analyze data and evaluate their experimental design.
10. Write a formal lab report that reflects their work.
11. Appreciate the work of scientists that have led to the scientific body of knowledge.

OVERVIEWS AND STRATIGIES

This curriculum unit addresses the core standards for science since it is a unit which applies the concept of science as inquiry. As the unit progresses, students learn the history behind the topic that they are studying. This unit is student centered where they develop science process skills in the aim of learning how scientists work. They will be using their imaginations to devise hypotheses and give explanations in order to make sense of the data that they collect(1).

The background information is provided for the teacher and student. Teachers may choose to present this information in a variety of ways, including, but not limited to:
Brainstorming using key words of the topic;
Connecting the topic to what they have learned in biology and chemistry;
Assigning related reading assignments in texts or on websites;
Writing;
Planning;
Listening;
Viewing models;
Evaluating on- line sites for scientific information;
Working independently;
Working cooperatively.

Teaching the background information, laboratory activities and guidance for laboratory report writing will be included in the lesson plans of the unit.

This scientific investigation is a complex process that will take approximately 2 weeks. During the course of the unit students will be applying knowledge in an inquiry based assignment (3).

Cellular respiration and the production of hydrogen peroxide

Energy is life. Cellular respiration is defined as the process by which the energy in food molecules is made available for an organism to do work (4). The chemical energy trapped in the bonds of the food molecules cannot be used by the cell directly, but must be transferred to different molecules. These molecules are ATP or adenosine triphosphate. The ATP molecules are the currency for cellular work. It is during the process of cellular respiration that hydrogen peroxide is produced. Since the preferred fuel for cellular respiration is glucose, a six carbon simple sugar, we will use that molecule in the reaction.

Cellular respiration in all organisms begins in the cytoplasm of the cell with a reaction called Glycolysis. Glycolysis does not require any oxygen in the reaction and is therefore referred to as an anaerobic reaction. In glycolysis the glucose molecule is split into two molecules of Pyruvate. Pyruvate molecules contain 3 carbons each. During this reaction 2 molecules of ATP are generated. It is the Pyrvate however that will be used in the main phase of aerobic cellular respiration, the Citric Acid cycle. It is also known as the Krebs cycle, named after Sir Hans Krebs, a German-British Scientist who discovered its main components in the 1930s. When oxygen is available, the three-carbon pyruvate is converted into a molecule called acetylcoenzyme A (acetyl-Co-A). The acetyl-co-A is converted into a two carbon fragment; it is this two-carbon acetyl that diffuses into the mitochondria and enters the Krebs cycle. The purpose of the Krebs cycle is to generate high energy molecules which are then passed along to the next phase of aerobic respiration. The molecules contain electrons which are passed along a series of carrier molecules called the electron transport chain. The molecules are known as cytochromes and they resemble a series of steps. As the electrons are passed down the steps energy is released which is then used to generate ATP. At the

end of theses “steps” an oxygen atom is available as the final electron acceptor. When the oxygen atoms accept the electrons they are known as superoxides. An additional chemical reaction converts the superoxides into molecules of hydrogen peroxide. It is extremely important that the cell remove hydrogen peroxide since it has the ability to combine with proteins or with DNA and disrupt the normal functioning of these molecules. If enough of these vital molecules are damaged, cell death may occur. Fortunately, cells produce a molecule that helps to convert hydrogen peroxide into water and oxygen gas. This molecule is known as catalase. Water and oxygen are considered waste products of aerobic respiration but are not toxic to the cell and can easily diffuse out of the cell without causing molecular damage(4).

The decomposition of hydrogen peroxide

Hydrogen peroxide is a common household product that is used as an antiseptic. It will naturally decompose in its container into water and oxygen gas according to the following reaction.

$$H_2O_2 \rightarrow H_2O + O_2$$

This decomposition reaction is slow and is one of the reasons that hydrogen peroxide has a shelf life of many months.

One of the defining characteristics of chemical reactions is that they require a particular amount of energy to reach completion, i.e. to be converted from the reactants, which are written on the left side of the arrow, to the products, which are written on the right side of the arrow. This amount of energy is known as the activation energy (Ea).

The reactant is hydrogen peroxide and the products are water and oxygen gas. Activation energy can be thought of as analogous to the energy required to roll a ball up a hill so that it can roll down the opposite side. The hydrogen peroxide is the ball; when it gets pushed up the hill, the reaction occurs and changes it into new substances at the bottom of the hill, the oxygen and water.

A simple way that chemists can make a reaction happen faster, or increase its rate, is to heat up the reaction vessel. Heating gives the reactant molecules enough energy to overcome the activation energy and be converted to product. In living systems, there is not an option to raise temperatures in order to help metabolic reactions happen at a fast rate. Living systems depend on enzymes which are molecules that lower activation energy. They increase the rate at which the products are formed. Enzymes can increase the rate of metabolic reactions to more than a billion times their normal rate. Using our ball being pushed up a hill analogy, an enzyme reduces the elevation of the hill. It is easier, or takes less energy, to push a ball to the top of a short hill than it is to push a ball up a steep hill. The enzyme allows for an easier path for the reaction to go from reactants to products. An easier route makes the reaction happen faster (5).

The history of proteins and the structure of enzymes

Enzymes belong to a category of biological molecules known as proteins. The word "protein" comes from a word in the Greek language, prota. Prota means "of primary importance" (6). This name was given to proteins by Jons Jakob Erzelius in 1838. He used the name to represent large organic (chemical compounds that contain carbon atoms) compounds that had similar chemical formulas. In 1926, James B. Sumner made an important discovery. He was able to add to the knowledge about proteins when he was able to isolate and crystallize enzymes. In order to understand how an enzyme works, it is important to understand the structure of a protein. A protein's specific structure determines how it works. Therefore, since enzymes are proteins it is their structure that determines exactly how it functions in cells. A protein molecule is a huge polymer constructed from a set of 20 amino acids. This polymer is also known as a polypeptide. It was in 1955 that the first protein was sequenced. This was accomplished by Sir Fredrick Sanger. He determined the amino acid sequence of the protein insulin (6). The 20 different amino acids have properties that are essential for the uniqueness of each protein produced by the cell. In total the number of different proteins, which it is possible to produce from 20 amino acids is enormous. For example, for a 10 amino acid sequence it is possible to have 20^{10} different sequences, which is approximately equal to 10^{13} or 10 trillions of different structures(6). The sequence of amino acids in the polypeptide is known as the primary structure of the protein. The primary structure can be hundreds of amino acids long and is determined by inherited genetic information. All amino acids have a common carboxyl (COOH) and amino group (NH_2), but vary in a side chain known as an R group. To see a diagram of a general amino acid go to the following link: http://www.proteincrystallography.org/amino-acids/. The amino acids with non-polar side chains are hydrophobic, which means they avoid contact with water. The amino acids with polar side chains and those with electrically charged side chains are hydrophilic, which means they prefer contact with water. Amino acids that are electrically charged also have either acidic or basic properties. The nature of these amino acids, as they are joined in a polypeptide will interact with one another to give the unique three dimensional shape of the protein molecule. It is this three dimensional shape which determines exactly how an enzyme will function in living systems (7,5).

The primary structure of the protein is produced when the chemical reaction dehydration synthesis forms a bond between each amino acid and the next one in the chain. It is a covalent bond that forms between the amino group (NH_2) of one amino acid and the carboxyl group (COOH) of the subsequent amino acid. It is known as the peptide bond. A protein can range from hundreds to thousands of amino acids linked by peptide bonds. The primary structure of a protein is also known as a polypeptide.

Once the polypeptide is formed, the polypeptide will coil into an alpha helix or fold up into a beta pleated sheet. An alpha helix is a cork screw shape while a beta pleated sheet resembles a folded paper fan. This is the **secondary structure** and is caused by intermolecular forces known as hydrogen bonding between the amino acids in the chain. These interactions involve the backbone part of the polypeptide. Along the amino acid chain, the amino acids have partial charges, either positive or negative. A negatively charged oxygen atom of one amino acid will be attracted to the positively charged hydrogen atom of a neighboring amino acid. Although hydrogen bonds are weak,

they are repeated over and over along the length of the polypeptide and serve to give the secondary structure strength.

The **tertiary structure** of the protein is its precise three-dimensional shape. This results from the interactions between the R groups of the amino acids. There are several types of interactions. The hydrophobic interaction causes the amino acids with hydrophobic properties to clump up in the middle of the protein, thereby avoiding the watery environment of the cell. Once these nonpolar ends are close together, van der Waals interactions keep them together. (Van der Waals interactions are another type of intermolecular force which results from the weak attraction of partially charged ends of the amino acids or the nonpolar R groups in the amino acid structure). Hydrogen bonds also act between polar ends of the amino acids; there are ionic bonds that form between positively and negatively charged ends of the amino acids. Although these intermolecular forces are weak, there are hundreds of them and together they provide enough strength to stabilize the three dimensional shape of the molecule. Lastly, there are covalent bonds called disulfide bridges which form between those amino acids that have sulfydryl groups (--SH) on their sides. These disulfide bridges reinforce the shape of the protein (5).

Proteins are grouped according to their tertiary structure. One group is the fibrous proteins in which the amino acids are arranged in sheets and thus are very tough in nature. Tendons and bones are composed primarily of these types of proteins. The other group of proteins is the globular proteins. These are the proteins that are spherical in shape. Enzymes belong to this group of proteins. Because enzymes are globular proteins, each enzyme has a very specific shape.

In some cases several tertiary structures become associated together to function as a single protein or enzyme. It is then referred to as **quaternary structure.** Hemoglobin, the molecule that carries oxygen in our blood is this type of protein. Catalase, the enzyme that students will be working with, is also this type of protein. Catalase is composed of four polypeptides, each containing over 500 amino acids (4). In addition to the quaternary structure that these two proteins have in common, catalase and hemoglobin also share a Heme group. This Heme group contains an iron atom. It is the iron atom in this heme group that the catalase uses to help it break the bonds of the hydrogen peroxide, thus releasing water and oxygen gas (8).

Enzyme Function

Each enzyme has a pocket or cleft called the active site. This is the area of the molecule that will combine with the reactant molecules. The reactant molecules are referred to as substrates.

One theory that helps to explain exactly how an enzyme functions to reduce the activation energy of a chemical reaction is the lock- and – key theory (5). The catalyzed decomposition of hydrogen peroxide is our example of enzymatic action. The enzyme catalase has a specific active site that will only fit the substrate, hydrogen peroxide. The hydrogen peroxide will fit into the active site of the catalase and will be held there with various bonds that include both hydrogen and ionic bonds. When this occurs the structure is referred to as the enzyme-substrate complex. While the hydrogen

peroxide is combined with the catalase, changes occur in the molecule of hydrogen peroxide such as stretching of the bonds or causing other stresses on the molecule that would move it toward its transition state (moving toward reaching the activation energy required for the decomposition to occur). When the reaction is complete, water and oxygen are formed. The catalase releases these products and remains unchanged. The catalase can be recycled and can act on an average of 1000 molecules of hydrogen peroxide per second (4).

A modified version of the lock-and-key theory is the induced-fit theory. In this model the enzyme is able to change its shape to enfold the substrate model. The enzyme substrate complex in the induced-fit model also causes stress and straining on the bonds of the substrate and causes a reduction in the activation energy of the reaction. It is more likely that most enzymes act in this manner since it would increase the likelihood of a fit between the substrate and the enzyme (9).

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Factors That Affect Enzyme Function

There are several factors that affect enzyme function. Students must understand these factors since this is what their hypothesis will be based on. Substrate concentration affects enzymes such that the rate is directly proportional to the substrate concentration until it reaches a maximum rate. After the maximum rate is reached all of the active sites of the enzyme molecules are filled, thus adding more substrate will have no effect. Enzyme concentration also affects the rate. The higher the concentration of the enzyme, (keeping all other factors constant) the higher the reaction rate will be... This is because there are more enzymes with available active sites to combine with the reactant. Incubation time is also a factor. Reaction rates will decrease as incubation time increases even if more substrate is added. This is due to the fact that as time goes on the protein molecule loses its specific shape and the active site and substrate no longer fit together.

Temperature is a second factor that affects the rate of an enzyme-catalyzed reaction. As the temperature increases, up to the point of the optimum temperature for that enzyme, the rate will increase. If the temperature is raised above the optimum temperature the reaction rate falls quickly. This is because at high temperatures the bonds that hold the protein molecule in its 3-dimensional shape break and the protein shape becomes distorted. If the shape is changed the enzyme can no longer fit its substrate and the rate will decrease. This fact of high temperature causing denaturation (shape change) is the main reason why enzymes are necessary in living systems. Living systems maintain a stable body temperature, which is the optimum temperature for the enzymes. Organisms cannot raise their body temperatures to increase the rate of the reactions of metabolism. Therefore enzymes are needed for reactions to occur at an extremely high and efficient rate.

The pH of the environment also is a factor in enzyme function. pH is a number used to quantify an acid's strength. An acid's strength is determined by measuring the concentration of hydronium ions (H_3O^+) in the solution. Students may be familiar with the pH scale (5). The scale lists common substances and their pH value . Water is neutral in pH and is assigned a pH value of 7. Other substances fall along the scale as the concentration of hydronium ions is measured. Substances that have a high concentration of hydronium ions are organized, according to strength, below 7 on the

scale, while substances that have a low concentration of hydronium ions are organized, according to strength, above 7 on the scale. Substances that are below 7 on the pH scale are known as acids while those above 7 are known as bases. Common acids include vinegar and citrus juices. Common bases include soaps and cleaning products (5). Most enzymes function best at a very narrow range of the pH scale. For example, gastric acids in the stomach create a very acidic environment, an ideal environment for the work of digestive enzymes which digest our food. If these enzymes were placed in an environment that was more acidic or basic than the stomach, they would not function. When an enzyme is placed in an environment that is either above or below its optimum pH value, the enzyme will denature. Denaturation will disrupt the active site and the enzyme can no longer catalyze the reaction that it is intended for.

Leading students toward experimental design and experimentation

Activities that will be included in this unit are: Day 1, student created models to foster understanding of protein structure and enzyme lock and key. Day 2, a laboratory activity that introduces the students to the enzymatic action of catalase on the decomposition of hydrogen peroxide. Day 3, a student centered day of learning to manipulate the materials and equipment that will lead them to a hypothesis. They will also use what they have learned in the development of a procedure to test that hypothesis. (It should be noted that this part of the unit may require extra days. It is a crucial part of the unit since it allows the student to examine different aspects of the reaction and to practice using the materials.) Day 4, discussion on the development of a hypothesis and the design of a laboratory procedure. I have added a list of ideas to help them with this task. After a class discussion, they take the list home and develop their plan. Day 5, discussion and feedback centered on student generated hypothesis and experimental design. Day 6, supervision and encouragement as students set out to gather data that will either support or refute their hypothesis. Day 7, discussion of expectations for the formal lab report and distribution of the laboratory report rubric (3).

Activity 1. Information on the production of hydrogen peroxide in cells can be covered after the unit of cellular respiration. Teachers can present the above background in lecture format, or they can create a Power Point presentation. An effective method of checking for understanding of this information is for students to complete a poster of a labeled diagram of a cell with the mitochondria. Enlarged mitochondria can then be drawn and the overall steps of aerobic cellular respiration can be written inside of this organelle. Special emphasis should be placed at the site of the production of hydrogen peroxide. Students could then present their posters to the class. They should also be prepared to answer questions about their work. Posters can be done in small groups, or students can make them individually. A study that was recently published describes the importance of students sketching diagrams. This approach can be used in many instances throughout this unit, especially in the first 2 activities of the unit (10).

Activity 2. Students will understand the structure of proteins and the lock and key model of enzymatic action. In this lesson students will be making models. There are two parts to this model making activity. For the first part, the materials that are needed are several packages of small or large pipe cleaners in various colors and a container or plastic bag to store them. The teacher then

cuts the pipe cleaners into assorted sizes. After mixing the pieces of pipe cleaner, the teacher can then randomly place them in the containers. After preparation, none of the containers will have the same color, size or amount of pipe cleaners. These pieces of pipe cleaner will represent the amino acids. The first thing that the students will do is to make the primary structure of the amino acids by twisting some or all of the pipe cleaners into a linear chain. This is a model of a polypeptide chain. After the students make their chains, it should be pointed out that none of the chains are exactly alike. This illustrates the great variety of proteins even though there are only 20 amino acids that exist in organisms. The next step would be for the students to form a cork-screw shape or to fold their chain into a fan. Both secondary structures can be achieved on the same chain as some students will discover. They can be reminded that hydrogen bonding between parts of their chain is responsible for these shapes. It should also be noted that the secondary structures vary between students, again indicating the variety of proteins. The tertiary structure is accomplished by bending and folding the model. Hydrogen bonding and di-sulfide covalent bonds are responsible for this step. After each student has a model of the tertiary form of a protein, they need to form groups of 4 and combine their models to make the quaternary structure. It can be noted that catalase is a protein composed of 4 polypeptide chains. Although students will not make an actual model of catalase, a general model of how enzymes work in a chemical reaction will help them understand what is happening in their reaction vessels. After the students make their models, the teacher can refer to the following website to show them the actual model of the catalase molecule: http://www.pdb.org/pdb/101/motm.do?momID=57 (8).

The students will also make models to represent the lock and key mechanism of enzyme action. Provide each student or small group of students with several colors of clay. The clay should be divided into one or two inch blocks. Students are given 4 different colors of clay. The general instructions for this activity are as follows: select one color of clay and use that material to create a model of an enzyme. They should create a model that has an obvious active site which would be similar to a three dimensional puzzle piece. Next, select a different color of clay that will represent the substrate. The shape of this should be such that it fits into the active site of the enzyme model, similar to the way two puzzle pieces fit together. The third model includes a reproduction of the enzyme and the substrate but the enzyme and substrate are linked together to represent the enzyme substrate complex. The final model is a replica of the original enzyme model and a third color of clay that represents the product of the reaction. It is essential that the enzyme model at the end of the "reaction" appears the same as the enzyme model at the beginning of the reaction. This represents the fact that enzymes are unchanged during the course of the reaction. Students can then arrange their models from left to right to indicate an enzyme catalyzed reaction. Several points should be addressed during this activity. One is that the enzyme is specific for its substrate. This point can be shown by choosing one student's enzyme model and another student's substrate model and showing how the two do not fit together. Another point is that the enzyme does not change during the course of the reaction. An additional point would be to show how the substrate has changed and turned into product. The reaction involves changing one substance (clay of one color) to another substance (clay of a different color). Once the students make a general model, ask them to make the model of the reaction that they will be working with in the laboratory, $H_2O_2 \rightarrow H_2O + O_2$. Their enzyme can remain the same, representing catalase. Their substrate can represent the hydrogen peroxide. The

enzyme and substrate will come together to make the enzyme substrate complex. The final result will be the unchanged enzyme and two models of product, one being the water and the other being the oxygen.

Activity 3. This procedure can be done as a demonstration or by the students. The students will be observing the reaction as being evidenced by the formation of bubbles. These bubbles are oxygen gas which is being produced by the reaction and escaping from the liquid in the test tube.
Materials needed: 8 small test tubes, 3% hydrogen peroxide (this is the hydrogen peroxide that is commonly sold in pharmacies and supermarkets), container of chicken livers or potatoes, 20 mL of skim milk, 1% solution of hydrochloric acid, 2% solution of sodium hydroxide.

In order to show enzyme specificity carry out the following procedure:

Place 3 clean test tubes in a test tube rack.
Label them as test tubes 1-3.
To the first test tube add 4 mL of water, to the second add 4 mL of milk and to the third add 4 mL of hydrogen peroxide.
Drop a small piece of liver (approximately 1 cm^2) into each of the test tubes.
Record the results.

The results are that the only test tube that shows a reaction is the one that contains the substrate for catalase, which is the one with the hydrogen peroxide. The students should relate these results to their model of their enzyme and substrate.

In order to show that denaturation is caused by extreme heat, the following procedure can be carried out:

In the control test tube add 4 mL of water.
In the experimental test tube add 4 mL of hydrogen peroxide.
Obtain 2 small pieces of liver that have been boiled for five minutes and that have been allowed to cool.
Add one piece to each test tube.
Record the results.

The results should show that there is no reaction because the catalase was denatured in the boiling process.

In order to show the effects of pH on enzyme action carry out the following reaction:

Label 4 test tubes as follows: water, acidic, basic, and hydrogen peroxide.
In the first test tube add 4 mL of water.
In the next 3 test tubes add 4 mL of hydrogen peroxide.
In the test tube marked acidic, add 4mL of the hydrochloric acid. In the test tube marked basic, add 4 mL of the sodium hydroxide.
Using pH paper, test the pH of each test tube and record. If the pH of the acidic tube is not

below 3, add more acid. If the pH in the basic tube is not above 9, add more sodium hydroxide.

Add a small piece of liver to each test tube and record the results.

The results will show that there will be little if any reaction in the acidic or basic tubes thus indicating catalase has been denatured. Since the action of catalase occurs inside of the cell, its optimum pH is close to 7.

The last part of the experiment shows the recyclable nature of an enzyme.

Place 4 mL of hydrogen peroxide into each of two test tubes.

Add a small piece of liver to the first test tube and observe until most of the bubbling has stopped. This could take several minutes.

Ask the students what the liquid in the test tube is after the bubbling has ceased. They should reply that it is water.

Pour the water out of the test tube and retain the same piece of liver.

Place the "used" piece of liver in the second test tube of hydrogen peroxide.

Record the results.

The reaction in the second test tube will be the same as the reaction in the first because enzymes are unaltered during a chemical reaction. The students should relate these results to their model of the lock and key process.

Activity 4.

Materials list:
A pressure probe and calculator;
Vernier or Nova equipment with the necessary software for data collection;
25 or 50 mL glass burette or 5mL volumetric syringe (without the needle);
Blender;
cheese cloth;
thermometers;
burettes;
250 and 50 mL beakers;
small medicine cups as an alternative to beakers;
distilled water;
1.5% hydrogen peroxide;
ice bath;
2% $KMnO_4$;
1M H_2SO_4;
1.5% hydrogen peroxide (3% hydrogen peroxide is found in most pharmacies, add equal amounts of 3% hydrogen peroxide and distilled water to a container to obtain a 1.5% solution).

A source for the enzyme may be several grams of liver or potato or a packet of yeast. Volumes of hydrogen peroxide need to be limited small amounts such as 2mL -5mL per test.

Students are provided with these materials. It is during this time that they create their catalase solution and experiment by varying the amounts of catalase and hydrogen peroxide. They will be recording qualitative data on the degree of bubbling as well as quantitative data on amounts of catalase and hydrogen peroxide used. If they are limited to 5mL of peroxide per test, they will find that they can use a dilute catalase solution. A very small amount of catalase is required to get a reaction that is measurable. It is required that the students measure the exact amount of liver, potato or yeast that they are using to make their catalase solution. They must also record the amount of water. This must be done since fresh catalase is needed each day. They must also record the volumes of the hydrogen peroxide and catalase that they decide to use. These are controlled during the experiment and must be constant for each trial. Students should be reminded that a control group is necessary. This would be a sample of H_2O_2 without catalase. At the end of the experiment the students can measure the amount of H_2O_2 in the control to compare with their experimental group.

Helping students learn a common technique does not interfere with inquiry. It simply gives them the skills necessary to accomplish their goal. Students will develop a ratio of enzyme to substrate mix that they can manipulate as they measure rate. Once the students discover the basics of manipulation of the equipment and materials that they need, they then can develop their hypothesis and design an experiment to test it. A sample of testable questions may be: What is the optimum temperature or pH for the action of catalase? How does enzyme concentration affect reaction rate? How does substrate concentration affect reaction rate. How does surface area affect reaction rate? Is there a difference between the actions of catalase that comes from different sources? These possiblities should not be given to the students; they should be allowed to think of their own. These are provided to the teacher so that if students are unable to formulate a research question, they can be given some direction.

As students become confident with the background information they should also become familiar with the materials that will be available for them to work with. There are several ways that students can measure reaction rate. Both of these techniques involve the course of the reaction over a specified time period. They can measure the amount of hydrogen peroxide that remains as a function of time (that is after several time intervals) or they can measure the amount of oxygen produced using a pressure probe and the appropriate equipment that may available such as those available from Vernier, Nova or other device. The first experience that students have with design should be that of getting familiar with the reaction itself and deciding how they will measure rate. This involves working with differing volumes of 1.5% hydrogen peroxide and a catalase extract. Students should make their own catalase extract from either cow liver, potatoes or yeast. A small quantity of liver or potato with 50 to 100 mL of distilled water placed in a blender, pureed, then strained will yield a catalase solution. Mixing a packet of yeast, a pinch of sugar and 200-250 mL of warm water will also produce a catalase solution. Since standardizing the enzyme solution is challenging, it is important that the student work this out with minimal assistance from the teacher. There are some instances where they can be guided. They may have to be reminded of the effect of temperature on reaction rate. Enzyme solution should be kept in an ice bath, the temperature of which must be noted and recorded as a control. Since they will have been instructed to use small

volumes, between 2mL and 5mL of hydrogen peroxide, they will have to add their enzyme to the hydrogen peroxide very slowly, perhaps drop by drop and study how fast the reaction occurs. They may then have to dilute the enzyme accordingly.

As was mentioned earlier, students may choose to measure the amount of oxygen gas produced by the reaction. This is done with equipment that includes a pressure probe. As the reaction proceeds, the pressure of the oxygen gas increases and is recorded by the probe. If they use a pressure probe and the reaction happens too quickly, they may not get a good reading from the instrument. The pressure probe apparatus includes a reaction vessel, with a 2 hole stopper. The hydrogen peroxide is placed in the vessel and the stopper is attached. One side of the stopper is attached to the probe; the other side provides a place for the introduction of the enzyme. The probe is attached to the device and when the machine is set; it will automatically record the changes in the pressure of the oxygen gas as the reaction proceeds over time. Students will need to experiment with volumes of hydrogen peroxide and catalase to determine a constant reading for rate. Once this is determined, they can then test the variable that they have chosen in their experimental design.

If students decide to measure the amount of the hydrogen peroxide that is remaining after the reaction has stopped, it is a more detailed procedure. After the students determine their catalase enzyme ratio they will be using, sulfuric acid is added to the mixture in order to stop the reaction. The volume of sulfuric acid must be recorded since it is a controlled variable and will be constant throughout the experiment. It is also important to remind the students that the reaction must be completely stopped. They will know when the reaction has stopped since there will no longer be a release of oxygen gas indicated by an absence of bubbles. After their specified time period they will be titrating with potassium permanganate. Suggested intervals may be 10,30,60,90,120,180 and 360 seconds. Of course since this is a student designed lab, their time intervals may vary. They should be reminded to take at least five consecutive readings. The first step of this procedure is to determine a base line. This is done so that the students will know how much permanganate is needed to reach the endpoint which signals the end of the reaction. Or, in other words, how much permanganate is needed to react with all of the available hydrogen peroxide. This is done as follows:

Pour 5 mL of 1.5% H_2O_2 into a small beaker or plastic medicine cup.
Add 1mL of H_2O.
Add 10 mL of H_2SO_4 (1M).
Mix well.
Remove a sample of this solution and place it in a small beaker or plastic medicine cup.

The volume of this sample must be equal to the volume of the peroxide catalase and acid mixture determined in part 1 of the lab. It should be between 2mL and 5mL.

The students will add potassium permanganate which reacts with sulfuric acid and hydrogen peroxide according to the following equation:

$5\ H_2O_2 + 2\ KMnO_4 + 3\ H_2SO_4 \rightarrow K_2SO_4 + 2\ MnSO_4 + 8\ H_2O + 5O_2$ (11)

Potassium permanganate is a dark purple color. When students add this to the colorless peroxide/acid/enzyme mix, the color will disappear as long as there is peroxide available to react. When all of the peroxide is gone, the mixture will stay a faint pink or light brown color. At this time students are to stop adding the permanganate and record the amount used. Since the amount of permanganate used is proportional to the amount of peroxide in the sample, according to the above equation, students can determine the amount of peroxide that remains after each trial probe. The procedure for students to follow for the permanganate titration is provided as follows: (note that the volumes of each substance are not given since these were predetermined by the student.)

Place hydrogen peroxide in a small beaker or plastic medicine cup.
Add the catalase.
Mix well.
After the specified time interval, add the sulfuric acid to the mixture.
Obtain the burette or syringe and add 10 mL of $KMnO_4$.
Record the initial volume of the burette or syringe.
Slowly, drop by drop, add the $KMnO_4$ until a pink or light brown color remains.
Record the final volume of $KMnO_4$ in the burette or syringe(11).

The amount of hydrogen peroxide consumed after each time period can be calculated by subtracting the amount of $KMnO_4$ used from the base line. All of these data is recorded by the student. (11).

At the conclusion of the experiment students must clean all materials. The burettes and syringes can be rinsed with H_2O_2 and water. All materials should be left in the manner that they were when the students entered the laboratory.

The following pages have information for development of a hypothesis and the experimental design. Guidelines for writing the formal laboratory report are also included. The following web site is an excellent resource to help students write a quality laboratory report: http://www.ncsu.edu/labwrite/pl/pl-homepage.htm.

Generating a hypothesis

1. Make an observation. In biology class, the observation is tied in to the current topic that is covered.
2. Learn more about the topic of the observation by doing research. This research should go beyond what is covered in class and should include previously published research that is related to the topic.
3. Make a list of factors that you think may have a relationship and choose a relationship that is interesting to you and one that you would like to explore in the laboratory.
4. Describe the type of relationship.
5. Make a prediction. If I change **A** in the relationship then **B** should happen.
6. Determine how you will measure the effects of **A** on **B**.
7. Restate as a hypothesis: include the specific information that you gathered in your research as well as the testable prediction and how it will be measured.

Experimental Design

1. List all materials that are needed.
2. State your dependent variable (this is what is being measured in the experiment).
3. State the independent variable (this is what is being manipulated in the experiment).
4. List constants (these are variables that are held constant in the experiment so that only the effect of the independent variable is measured).
5. Describe the experimental group for the experiment.
6. Describe the control group (in this lab it would be the H_2O_2 without catalase).
7. Describe the experimental groups for your experiment. Include details about the tests, such as basic procedure and sample size.
8. Describe a result that would cause you to reject your hypothesis.

SPECIFICS FOR LAB REPORTS:

The report should be neat and easy to read. All lines drawn must be done so using a ruler. The elements of the laboratory report are in order listed here:

Title

The title should be brief and appropriate.

Introduction

This is the hypothesis. It includes all relevant background information that is well researched as well as a succinct prediction of the outcome of the experiment based on the research.

Experimental design

State briefly how the experiment will achieve its purpose. Details of the procedure are not needed here.

Materials and Methods

Variables properly identified (independent, dependent, constants, and control).

May include figure to illustrate lab set-up

Procedure describes experimental design in enough detail that another scientist can reproduce the same experiment. It must include how to control variables and allow for the collection of both qualitative and quantitative data.

Data Collection

Collected data should be organized and easy to read. The first type of data is the raw data. This is the data that is recorded during the experiment. It includes both qualitative and quantitative data. The quantitative data can be arranged in a neat table. The data table must have a descriptive title so that the table can "stand alone". That being so the reader will not have to refer to the procedure to understand what is being recorded and why it is being recorded.

All variables must be labeled

All units of measure must be included.

Data Analysis

This includes any mathematical manipulation of the raw data.

These data may be organized in a neat table that is clearly titled. If calculations are done, one sample calculation should be included above the table. It may also include any graphical representation of the data. If graphs are included they must have a descriptive title, the x and y axis must be labeled with appropriate units. A brief interpretation of the graph must be written below the graph.

Conclusion and Evaluation

Students should write if there was support or non-support for their hypothesis. They should explain what their results mean using specific examples from their data and relate it back to their hypothesis.

Students should include a list of problems with experimental procedure. They should include possible solutions or changes that need to be made.

Students should expand on their knowledge gained on this topic and in the laboratory. They should also provide a list questions that have been raised or state ideas for future experiments.

Works Cited

Include all published work, including internet sources that were consulted in the research.

Implementing District Standards

The following list includes those sections of the North Carolina Standard Course of Study for Science and how they apply accordingly to the objectives of this curriculum unit.

The tenets of scientific literacy include:
*** Describe and explain, and predict natural phenomena.
In this unit students will study a reaction, make predictions based on their knowledge and explain the results of their experiment.
***Pose explanations based on evidence derived from one's own work.
In this unit students will design their own experiment, collect data and explain their results.

The standard course of study for science is designed to merge unifying concepts of science such as
***Evidence, Models and Explanation
This unit has students gathering scientific evidence in the laboratory. They are also using models to explain scientific concepts..
***Form and Function
This unit has students make the connection between the structure of the mitochondria and its function. It also allows students to understand the connection between the structure of an enzyme and its functions.

The standard course of study also lists strands. The strand that is evident in this unit is "Science as Inquiry". This is an inquiry based unit since students are designing an experiment to test a concept of their own choosing.

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" Science ." North Carolina Public Schools. http://www.ncpublicschools.org/curriculum/science/

This is the website that lists the North Carolina Standard course of study for science