



Dolan  
DNA Learning Center  
[www.dnalc.org](http://www.dnalc.org)

# Bubbling Liver



## Background

Most of the 100 trillion cells in the human body (except mature red blood cells and reproductive cells) contain the entire human genome--all the genetic information necessary to build a human being. This information is encoded in 3.2 billion base pairs, which are subunits of DNA. Inside the cell nucleus, 2 meters (about 6 feet) of DNA are packaged into 23 pairs of chromosomes (one chromosome in each pair is inherited from each parent). Human cells have 46 chromosomes that contain the DNA for approximately 30,000 individual genes (the units of heredity). Each gene is a segment of double-stranded DNA that holds the recipe (or code) for making a specific molecule, usually a protein.

In eukaryotes, DNA never leaves the nucleus. For the DNA instructions to reach the rest of the cell, an enzyme called RNA polymerase must make an RNA copy of a gene, called mRNA (messenger RNA). The mRNA copy leaves the nucleus and travels to a ribosome where the message is read. The ribosome then makes the protein coded for by that gene. To make a strand of RNA, the DNA first unzips, or pulls apart. RNA polymerase, an enzyme, builds an RNA strand complementary to one half of the unzipped DNA. For every C in the DNA strand the RNA polymerase inserts a G; for every G a C; for every T an A. For every A, RNA polymerase inserts a U, or Uracil. RNA does not contain Thymine. The ribosome “reads” the message 3 bases at a time. Each combination of bases letters is called a “codon.” For example, “UCG” is an mRNA codon. Codons code for amino acids, the building blocks of proteins. The UCG codon codes for the amino acid Serine (Ser). Transfer RNA (tRNA) molecules attach and deliver the proper amino acids to the ribosome to create a long chain. This chain of amino acids folds up to form a protein.

## Proteins

There are four main types of proteins: structural proteins, transport proteins, messenger proteins and enzymes. Structural proteins provide our bodies with support. Examples include actin and myosin, which make up much of our muscle tissue, and keratin, which is found in both hair and fingernails. Transport proteins carry molecules throughout our bodies. One example includes hemoglobin, the protein in red blood cells that carries oxygen. Messenger proteins allow cells in different parts of the body to communicate. Some hormones, such as insulin and human growth hormone (HGH) are messenger proteins.

Enzymes act as the construction workers of the protein world. They can build molecules as well as break them apart by catalyzing (speeding up) chemical reactions. The molecule that a given enzyme breaks down is called its substrate. Like a lock and key, enzymes will only react with substrates that fit their shape correctly. Once the substrate is broken apart or digested, the enzyme is free to catalyze further chemical reactions. Unless the shape of the enzyme is altered, it can catalyze reactions indefinitely. The process that changes the shape of an enzyme is called denaturing. When an enzyme is denatured, it usually cannot alter its shape to function again. Some factors that can cause denaturing include changes in acid levels or pH and temperature fluctuations.

## Human Digestive Enzymes

Enzymes are produced throughout the digestive system. Each digestive enzyme targets a specific substrate. For example, lipases target fats, amylases target starch, and proteases target proteins.

Most of the chemical digestion of food occurs in the small intestine by enzymes produced in the pancreas or in the intestine itself. **Lactase** enzyme is secreted in the lining of the small intestine. Individuals who are lactose intolerant have a lactase deficiency, and as a result, the dairy sugar lactose doesn't get digested. Normally, lactose is digested into its two constituent ingredients, glucose and galactose. When lactose isn't digested, it can cause abdominal discomfort and bloating, vomiting and or diarrhea. This enzyme deficiency can be treated through supplementation. Individuals who are lactose intolerant can take a pill containing the enzyme when they eat to aid in the digestion of the dairy sugar lactose. Individuals can also purchase lactose reduced dairy products, such as milk or ice cream. Harnessing the reusable nature of enzymes, with very small amounts of lactase large quantities of lactose free milk can be produced.

Surprisingly, it is quite normal for lactase levels to decrease with age. Lactose intolerance is actually an ancestral trait. As mammals, the natural production of this enzyme is only necessary in infancy when a baby nurses from its mother. After this stage, most mammals discontinue ingesting lactose, and the enzyme production drops and/or stops. Naturally, because humans are mammals, this happens to us too! In many of us though, lactase production continues beyond infancy thanks to our nomadic ancestors. When humans began to collect and drink milk from other animals, lactase production began to change.

**Catalase** is another example of a human digestive enzyme. It is produced in the liver. As blood is filtered through the organ, catalase digests toxic hydrogen peroxide that is present in low levels as a byproduct of cellular respiration. The products of this chemical reaction are water and oxygen. This reaction can easily be reproduced in the classroom, but with calf's liver instead of human liver! There is a visible reaction when liver is placed in hydrogen peroxide. Violent bubbling is a sign that gas is being released, and an increase in temperature is a direct indicator of an exothermic reaction taking place.

## Enzymes in Industry

In cheese production, enzymes are used to separate the curds and whey in milk. Cheese is the solid portion of milk (curd) that has been separated from the liquid portion (whey). The cheese-making process was actually discovered by our nomadic ancestors. They would travel great distances, carrying liquids in the stomachs of dead animals. When milk is placed in a stomach the enzymes of the stomach cause the milk to curdle, thus producing clumps of curds or cheese. By combining the enzyme **emporase** with milk, this chemical reaction can easily be observed.

Enzymes are also used in the production of juice. In the flesh of an apple, apple juice is stored in tiny particles surrounded by walls of pectin. Pectin is a sugar molecule, and must be destroyed to release the juice trapped within. The enzyme **pectinase** is used to perform this chemical reaction in juice factories today. Years ago, apples would be pressed and crushed to extract their juices. Today, with the help of pectinase, juice factories are able to increase their yield.

## **Enzymes and Health**

The absence of a single enzyme can have a drastic effect on an individual's health. For example, a single enzyme deficiency in white blood cells can lead to immune disorders such as SCIDS (Severe Combined Immunodeficiency Syndrome) also known as the "boy in the bubble" disease. Sadly, David Vetter, the young boy for whom the disease was nicknamed passed away following a bone marrow transplant at the age of 11. Since his passing, 2 young girls suffering from the same disorder have been successfully treated with gene therapy. Their white blood cells were genetically engineered and transfused back into their bodies, bringing with them a trait they didn't have before: the ability to fight infection!



## Description of Activity

This one-hour laboratory provides children in grades 5-8 with the opportunity to observe an enzymatic reaction. By placing a small piece of calf's liver into a cup of hydrogen peroxide, students will see the enzyme catalase chemically change hydrogen peroxide into water and oxygen. Upon observation, several conclusions can be drawn about enzymes and the chemical reactions that they catalyze.

## Learning Outcomes

Students will:

- discuss the relationship between genes, proteins and enzymes.
- observe an enzymatically catalyzed chemical reaction.
- define the terms chemical reaction and catalyze.
- understand that various factors can affect the rate of an enzymatic reaction.

## Assumptions of Prior Knowledge

Students should be familiar with the structure and function of DNA and understand the relationship between genes and proteins.

## Misconceptions

Students often associate enzymes with the digestion of food, but fail to understand that they play a much larger role in the life of a cell. When first learning about chemical reactions, many students don't understand the difference between physical and chemical changes in matter.

## Lesson

### Materials and Equipment

1 large package of calf's liver  
1 scalpel or sharp cutting knife  
2 large bottles of 3% hydrogen peroxide  
1 package of clear plastic disposable cups  
1 package of small disposable plastic plates  
30 small thermometers  
1 cup of vinegar  
1 glass beaker  
1 hot plate, microwave or stovetop  
40 wooden tongue depressors  
1 book of matches  
15 molecular model kits

## Before Class

### The day before:

- Place one (1" x 1") piece of calf's liver in the freezer.
- Place one (1" x 1") piece of calf's liver in a cup of vinegar, and cover with plastic wrap.

### The day of the lab:

- Use a scalpel or knife to cut the calf's liver into 1" x 1" pieces. Place one piece of liver on a plastic plate for each student.
- Place one piece of liver into a glass beaker with water and boil.
- Fill one plastic cup half-full with hydrogen peroxide for each student.

## During Class

- Begin the lesson by reviewing the central dogma of molecular biology. DNA is transcribed into RNA and RNA is then translated to make proteins. Make the connection between proteins and traits. There are several classes of proteins, for example:  
Structural: make up physical structures (keratin in hair and nails)  
Hormones: Insulin (controls metabolism of glucose)  
Enzymes: Amylase (digests starch)
- Not all enzymes are proteins, but most are. They are the workhorses of the cell, and they catalyze chemical reactions.
- Discuss what a chemical reaction is. This should include a quick review of the periodic table of elements, and a brief discussion of chemical bonding between atoms to create molecules such as water (H<sub>2</sub>O), carbon dioxide (CO<sub>2</sub>) and table salt (NaCl). Use molecular models to demonstrate simple molecular structures. Give students the opportunity to build their own simple molecules, using the *Simple Molecules* worksheet. For a challenge, they can also attempt to build simple sugars, such as glucose, using the *Simple Sugars* worksheet.
- A chemical reaction is when molecules are changed (chemical bonds are broken or built). In a chemical reaction there are reactants and products. The products are chemically different than the reactants.
- Demonstrate this principle with a piece of paper. Ripping it or dissolving it in water can physically change the paper, but to change it chemically, the paper would need to be burned, soaked in acid or maybe even eaten!
- Define catalyze. Enzymes can catalyze chemical reactions. In layman's terms, this means the reaction



will proceed faster than without the enzyme. This happens because the amount of energy needed for the reaction (activation energy) to occur is lowered by the presence of the enzyme. A great example is digestion. For example, if a potato was left out on a table, it could take weeks or months for it to be completely decomposed. If that same potato was ingested and exposed to the enzymes of the digestive system, it would be completely digested in just hours.

- Point out that in this lab, students will observe a chemical reaction that occurs in the human digestive system all the time. The liver produces a digestive enzyme called catalase. Like all enzymes, catalase has a specific substrate, or substance, with which it will react. This is a great time to review some of the enzymes produced along the human digestive system and their substrates, such as salivary and pancreatic amylase (digest starch), pepsin (digests proteins), and lipase (digests fats). Mention that the names of most enzymes end in “ase”.
- Hydrogen peroxide is a naturally produced by-product of digestion. It also happens to be toxic! To remove this toxin from the body, catalase (which is produced in the liver) digests it. The end products of this chemical reaction are water and oxygen. Ask students to build a model of hydrogen peroxide before beginning the lab.
- Pass out the *Bubbling Liver* protocol, and review the chemical reaction. Catalase is the enzyme and hydrogen peroxide is the substrate. At the end of the reaction, hydrogen peroxide has been chemically changed, but the enzyme remains unchanged. This means that it can be re-used, which will be demonstrated in the lab.
- Ask a student to read steps 1-3 out loud, and begin the lab. Explain that recording the temperature will help to demonstrate whether a chemical reaction has occurred. Usually during digestion, there is a release of heat energy when chemical bonds are broken, so the temperature should increase. This is called an exothermic reaction.
- Once the initial temperature readings have been recorded, use a popsicle stick to slide a piece of liver from a plate into the cup of hydrogen peroxide. Observe and record observations. The temperature should rise about 10 degrees, and there should be a lot of bubbling. Discuss what gas should be in the bubbles (oxygen), and what liquid should be left in the cup (water). How can one demonstrate that it really is oxygen in the bubbles? The flame test is useful. Using a match, light the end of a broken popsicle stick. Gently blow out the flame until it is just a glowing splint. Hold the splint in the bubbles rising out of the cup. If there is indeed oxygen in the bubbles, the splint will re-light.

- To demonstrate some of the other properties of enzymes students can transfer a piece of used liver into a clean cup of hydrogen peroxide (step 4). The used liver should begin to bubble again. This is proof that enzymes remain unchanged after chemical reactions, and that they can be re-used.
- Students can transfer liver into a different substrate, such as water or juice. There should be no reaction, because enzymes only react with specific substrates. This specificity is determined by the three dimensional shapes of enzymes and their substrates. They fit together like a “lock and key”.
- Test the affect of temperature and pH on enzymatic activity by placing boiled, frozen or acid (vinegar) soaked liver into fresh cups of peroxide (step 5). There should be decreased or no activity in these cups. Extreme changes in temperature and pH will denature enzymes. When an enzyme is completely denatured, it will no longer fit with its substrate, and is rendered inactive. Every enzyme requires an optimal temperature and pH to function efficiently.

### Analysis and Discussion

Upon completion of the lab, make a chart of enzyme facts learned during the lesson. The list should include the following:

- Enzymes catalyze chemical reactions.
- Enzymes have specific substrates.
- Enzymes and their substrates fit together like a “lock and key”.
- The names of most enzymes end in “ase”.
- Most enzymes are proteins.
- Extreme changes in temperature and pH can denature enzymes and affect the rate of an enzymatic reaction.
- Enzymes remain unchanged upon completion of a chemical reaction.
- Enzymes can be re-used.

As a group, the class can refer to the specificity of the enzymes in the human body. At which temperature do our enzymes function most efficiently? Why is it not healthy to have a high fever for an extended time? At what pH do our enzymes function most efficiently? This varies, depending on what part of the body they function. For example, the enzymes in the stomach function very well in a very acidic environment, but in the small intestine the pH borders on slightly basic. Are digestive enzymes the only enzymes produced in the human body? Can students think of any enzymes that actually build chemical bonds? DNA polymerase and RNA polymerase (found in the cells of all organisms) do just that!



## Further Explorations

### Group Presentations

Can the absence or deficiency of a single enzyme affect the health or well being of an individual? There are many genetic disorders associated with mutations of various enzyme genes. These disorders range from lactose intolerance to Severe Combined Immunodeficiency Syndrome. Have your students research some of these disorders and give presentations to the rest of the class.

### Discussion

There are several enzymes found in all living things. What does this mean about the relationships among various organisms. What enzymes do all organisms share, and what do they do? Are these enzymes necessary for life?

### Enzymes in Molecular Biology

Restriction enzymes are used to cut the DNA molecule, and ligase enzymes are used to glue DNA fragments together. Using the *DNA Interactive* website, investigate the use of enzymes in recombinant DNA technology, genetic engineering and DNA fingerprinting.

Go to:

[www.dnai.org](http://www.dnai.org) < Manipulation < Techniques < Cutting and Pasting to learn more about restriction enzymes.

Go to:

[www.dnai.org](http://www.dnai.org) < Manipulation < Techniques < Amplifying to learn about amplifying DNA for techniques such as DNA fingerprinting.

## Resources

### Books:

Discovering Enzymes. Dressler, David, and Potter, Huntington. Scientific American Library, NY. 1991. A great resource on the discovery, anatomy, physiology and evolution of enzymes.

DNA Science. Micklos, David A., Freyer, Greg A., and Crotty, David A. Cold Spring Harbor Press, NY. 2003. A laboratory manual on the history of recombinant DNA technology.

Have a Nice DNA. Balkwill, Fran and Rolph, Mic. Cold Spring Harbor Laboratory Press, NY. 2002. An elementary level introduction to basic cell processes such as DNA replication and protein production.

### Internet Sites:

<http://www.dnai.org/index.htm>

*DNA Interactive*: a commemorative Internet site devoted to the discovery of the double helix.

A Dolan DNA Learning Center Internet site

<http://www.ygyh.org/>

*Your Genes, Your Health*: A multimedia guide to genetic disorders.

A Dolan DNA Learning Center Internet site

<http://www.rcsb.org/pdb/>

The Protein Data Bank, by the Research Collaboratory for Structural Bioinformatics.

This site contains a database of the molecular structures of proteins, including enzymes.



## Correlations

### New York State

NYS Standard 4: Science

The Living Environment

- Organisms inherit genetic information in a variety of ways that result in continuity of structure and function between parents and offspring.
- Organisms maintain a dynamic equilibrium that sustains life.

### National

Content Standard C: Life Sciences

Structure and Function in Living Systems

- Living systems at all levels of organization demonstrate the complementary nature of structure and function. Important levels of organization for structure and functions include cells, organs, tissues, organ systems, whole organisms and ecosystems.
- All organisms are composed of cells—the fundamental unity of life. Most organisms are single cells; other organisms, including humans, are multi-cellular.
- Cells carry on the many functions needed to sustain life. They grow and divide, thereby producing more cells. This requires that they take in nutrients, which they use to provide energy for work that cells do and to make the materials that a cell or an organism needs.
- Specialized cells perform specialized functions in a multicellular organism. Groups of specialized cells cooperate to form a tissue, such as a muscle. Different tissues are in turn grouped together to form larger functional units, called organs. Each type of cell, tissue, and organ has a distinct structure and set of functions that serve the organism as whole.
- The human organism has systems for digestion, respiration, reproduction, circulation, excretion, movement, control and coordination and for protection from disease. These systems interact with each other.

Reproduction and Heredity

- Every organism requires a set of instructions for specifying traits. Heredity is the passage of these instructions from one generation to another.

### AAAS Benchmarks

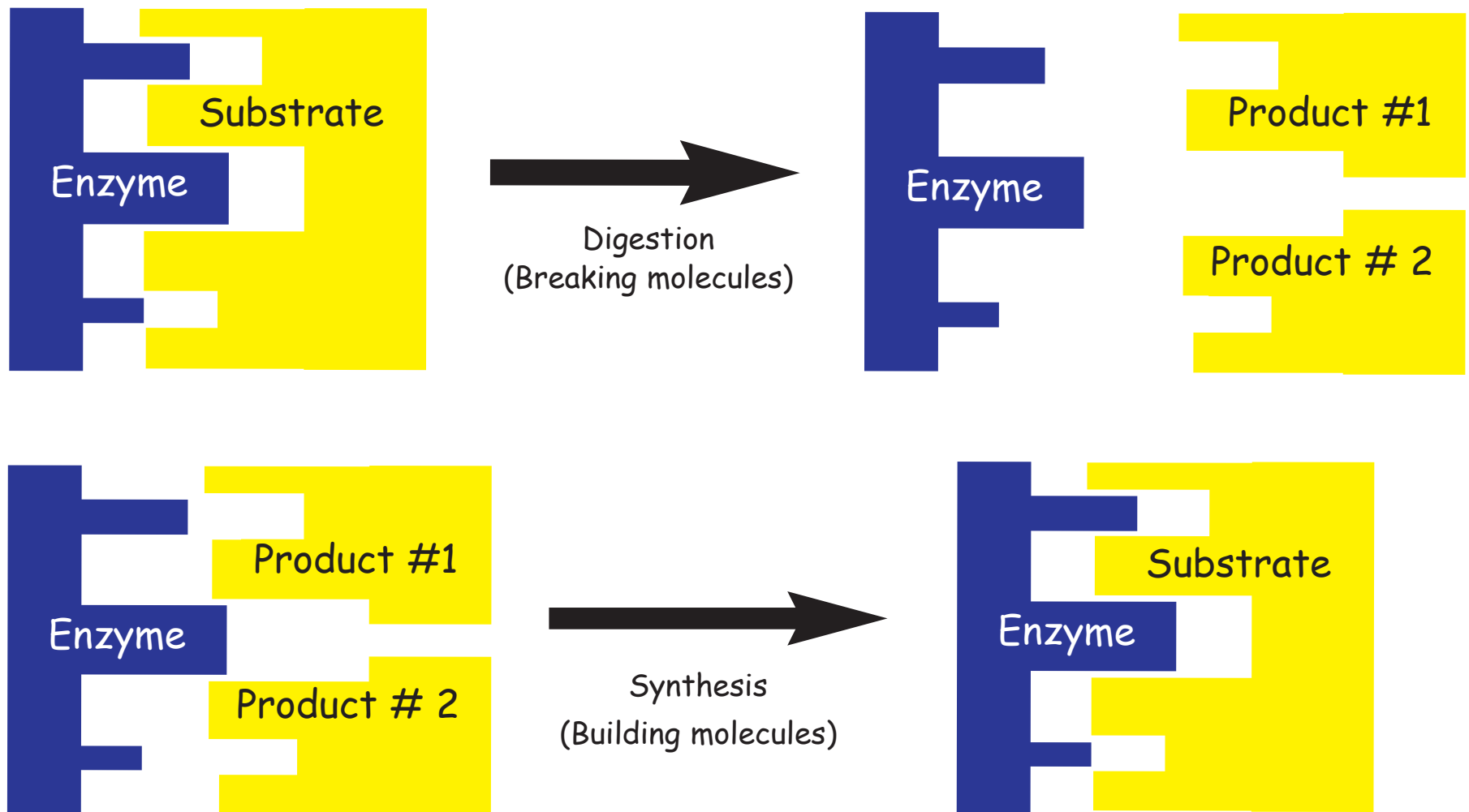
Standard C: Cells

- Some living things consist of a single cell. Like familiar organisms, they need food, water and air; a way to dispose of waste; and an environment they can live in.
- Microscopes make it possible to see that living things are made mostly of cells.
- Some organisms are made of a collection of similar cells that benefit from cooperating. Some organisms' cells vary greatly in appearance and perform very different roles in the organism.



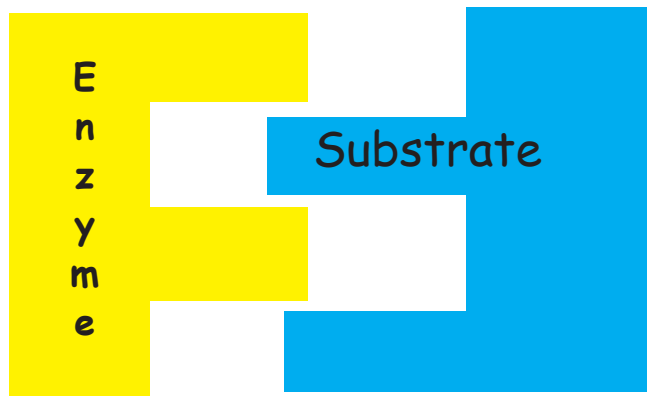
# Enzymes and Substrates

Enzymes, whether they break or build molecules, have specific substrates with which they can react. An enzyme and its substrate fit together like a lock and a key.

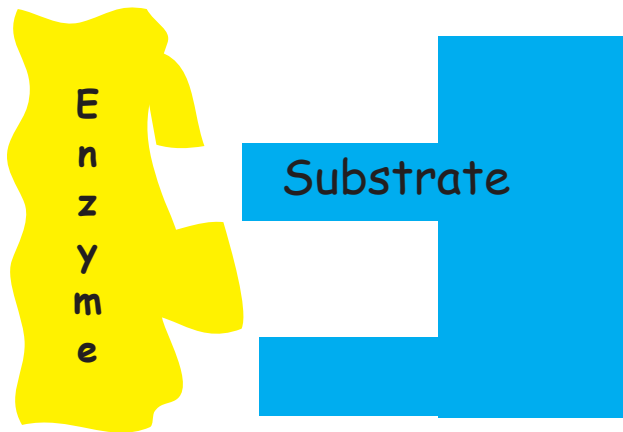


# Enzymes and Substrates

If the shape of an enzyme is altered by a change in heat or pH, it can no longer bind with its substrate. This change in shape is called denaturation. A denatured enzyme is like a melted key, it cannot fit with its substrate, and is non-functional.



The fit of an enzyme and its specific substrate.

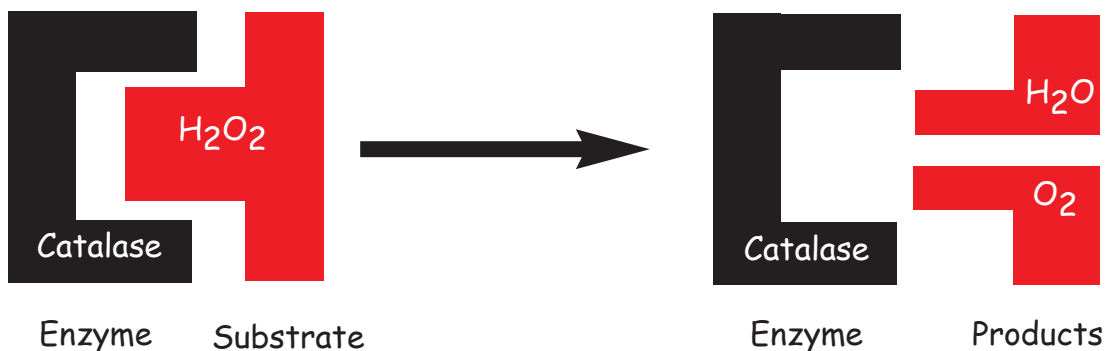


A denatured enzyme does not fit with its substrate.

# Bubbling Liver

Catalase is an enzyme found in many plants and animals. It has the important job of breaking poisonous hydrogen peroxide molecules into simple, harmless molecules. We can observe catalase at work by placing a piece of calfs liver which contains the enzyme, into a cup of hydrogen peroxide. When these two ingredients are combined, there will be several signs that the chemical reaction is taking place....keep your eyes open!

**Catalase performs the following chemical reaction:**



## Procedure:

1. Fill half of a plastic cup with 3% hydrogen peroxide.
2. Place a thermometer in the cup and record the temperature.

**Temperature before chemical reaction:** \_\_\_\_\_

3. Place a small (1" x 1") piece of liver into the cup of hydrogen peroxide, and **observe the reaction**. Record the temperature after one minute.

**Temperature during chemical reaction:** \_\_\_\_\_

In addition to a change in temperature, what other signs are there that a chemical reaction has occurred?

4. Pour a fresh cup of hydrogen peroxide, and place the used piece of liver from your first experiment into the cup. Do you think the enzyme still works?
5. NOW, place a piece of boiled, frozen or acid treated liver into a fresh cup of hydrogen peroxide. Is there a reaction?

# Building Molecular Models

Black

Carbon (C)

Yellow

Hydrogen (H)

Red

Oxygen (O)

Blue

Nitrogen (N)

Green

Chlorine (Cl)

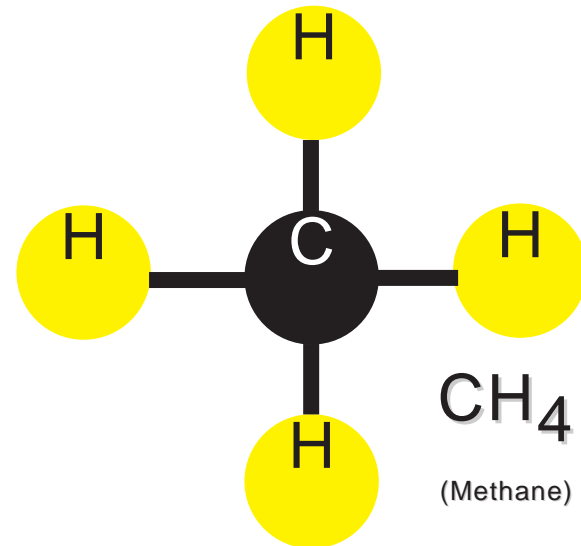
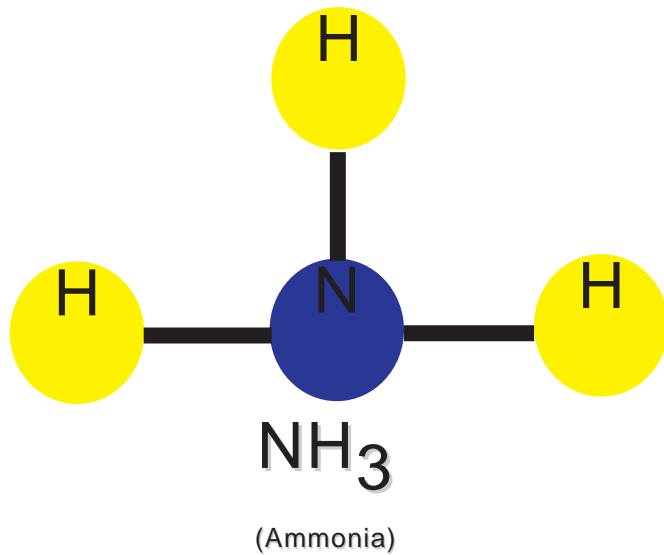
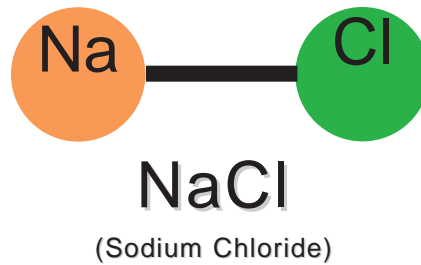
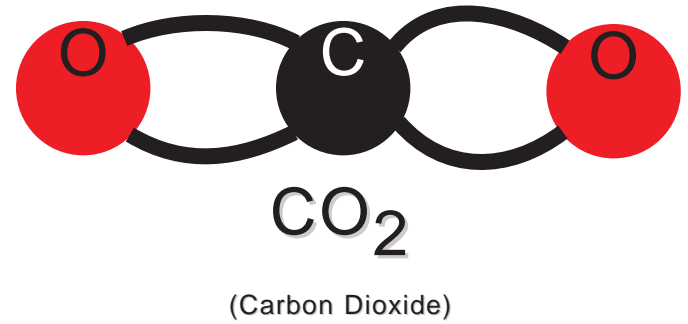
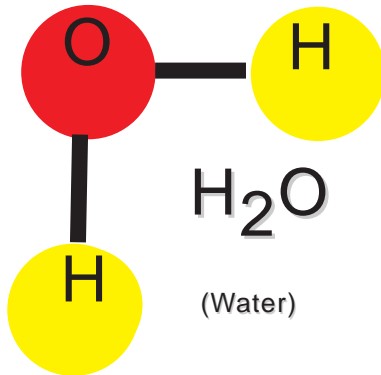
Purple

Potassium (K)

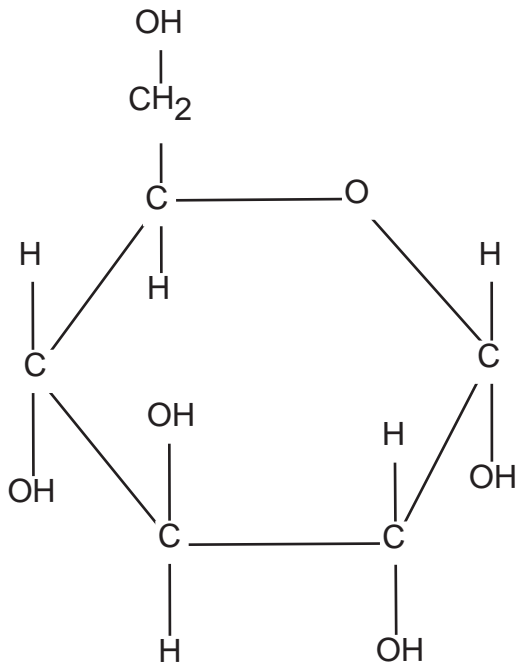
Orange

Sodium (Na)

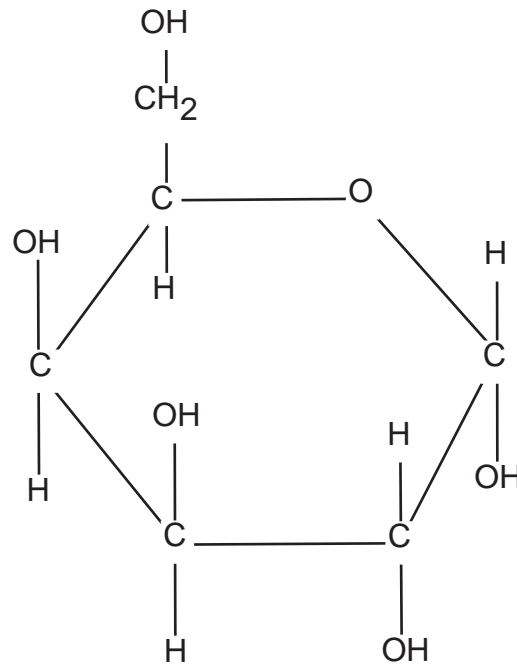
# Simple Molecules



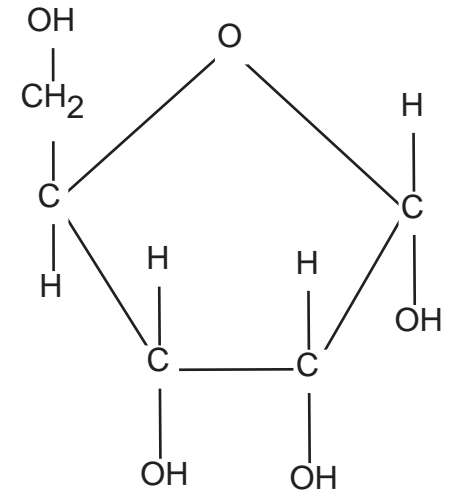
# Simple Sugar Molecules



Galactose  
C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>



Glucose  
C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>



Ribose  
C<sub>5</sub>H<sub>10</sub>O<sub>5</sub>