Dolan
DNA Learning Center
www.dnalc.org

Better Milk for Cats

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

In this one-hour activity, students in grade 5-8 will simulate an enzymatic reaction that normally occurs in the human digestive system. Using lactase enzyme, the dairy sugar lactose will be split into the two simple sugars glucose and galactose.

Learning Outcomes

Students will:

- discuss the connection between genes, proteins and enzymes.
- explore the function of enzymes and substrates.
- understand lactose intolerance and how it relates to genetics
- observe a chemical reaction that occurs daily within the human digestive tract.

Assumptions of Prior Knowledge

Students should be familiar with the structure and function of DNA and the relationship between genes and proteins. A basic understanding of the concept of digestion is also helpful.

Misconceptions

Although most students have heard of lactose intolerance, some aren't familiar with the symptoms or cause of the disorder. Many individuals that happen to be lactose intolerant are embarrassed, and think that there is something wrong with them, when in actuality it is quite normal for a mammal to be lactose intolerant beyond infancy.

Lesson

Materials and Equipment

45 clear plastic cups
15 large oral syringes
15 plastic droppers
15 coffee filters
15 glucose test strips
1 quart of whole milk

Reagents

15 tubes of 4ml of 1% sodium alginate solution
15 tubes of 2ml of lactase enzyme
1 liter of 1.5% Calcium Chloride ($Cacl_2$) solution

Recipes

1% Sodium Alginate solution (makes 100ml)

- In a large flask, slowly add 1g sodium alginate powder to 100mL dH_20 using a stir bar and lowest possible heat setting.
- Using a magnetic stir bar, et solution stir for approximately 1-1.5 hours, until the sodium alginate is completely dissolved.
- Store covered in the refrigerator.

1.5% Calcium Chloride ($CaCl_2$) solution

- Dissolve 15g of $CaCl_2$ salt in 1000ml (1 liter) of dH_2O.
- Store covered in refrigerator.

Purchasing

- Glucose test strips – Fisher Scientific
- Sodium Alginate powder – Health food store
- Lactase Enzyme (Beta Galactosidase) – Sigma
- Calcium Chloride - Fisher Scientific
- Plastic Droppers – Fisher Scientific
- Oral Syringes – Fisher Scientific
- 15ml Corning tubes – Fisher Scientific

Before Class

The day before the lesson:

- Prepare the sodium alginate solution and aliquot 4ml into 15ml Corning tubes (15 total).
- Aliquot 2ml of lactase enzyme into 15m Corning tubes (15 total).
- Prepare Calcium Chloride solution.

The day of the lesson:

- Prepare lab set-ups for each pair of students which include: 2 plastic cups, one dropper, one syringe, one coffee filter, one tube of sodium alginate, one tube of lactase and a half cup of calcium chloride solution.

During Class

- Begin with a discussion of lactose intolerance. What is lactose intolerance? What are the symptoms? If someone is lactose intolerant, does this mean he/she can never enjoy dairy products if they so choose? Make sure to point out that this is NOT the same as a milk allergy, which is caused by an immune response to milk, but is actually a digestive disorder.
- Continue with a discussion of digestion. When food is digested, what is happening to it? Why does food need to be digested? What role do enzymes play in digestion?
- Enzymes catalyze, or speed up, the chemical digestion process (food can also be partially digested mechanically by chewing and the churning of the stomach). They are produced throughout the digestive tract. Diagram the entire digestive tract and point out that various enzymes are produced in different parts of the digestive tract, from the mouth to the intestines. For example, salivary amylase digests starch in food that is being chewed, pepsin digests proteins in the stomach and lipases digest fat in the small intestine.
- All dairy products contain lactose, a compound sugar comprised of one unit of glucose bound to one unit of galactose. When lactose reaches the small intestine, pancreatic lactase enzyme breaks the bond between glucose and galactose, and individually they can separately be absorbed through the lining of the intestine.
- If an individual is lactose intolerant, the lactose isn't digested, which leads to symptoms such as vomiting, bloating, cramping, diarrhea and/or hives. Why would someone's body not properly digest lactose? The enzyme isn't being properly produced. Why?
- Review the central dogma of molecular biology. Genes carry "recipes" to make proteins, such as enzymes. If there is a mutation in a gene, it can affect the production of a protein. Interestingly enough, thousands of years ago humans were genetically pre-programmed to stop producing the lactase enzyme after infancy. Once our nomadic ancestors began to collect and drink milk from other animals, humans slowly became lactose tolerant. Through evolution, the genetic programming of lactase production continues beyond infancy in most individuals. It is not uncommon though, for humans to become lactose intolerant later in life. It's an ancestral trait!
- If someone does happen to be lactose intolerant, they can still enjoy dairy products. There are digestive supplements that can be taken before eating dairy products that contain the lactase enzyme. There are lactose free and soy based products that can be substituted.
- In this lab, students "manufacture" milk that is lactose-reduced using a technique similar to that used by the *Lactaid*® company; through exposure to the lactase enzyme.
- Pass out the *Better Milk for Cats* protocols, and ask a student to read steps 1, 2 and 3 out loud. Demonstrate each step. Students will be mixing lactase enzyme with sodium alginate solution. When the sodium alginate/enzyme solution is placed in a salt solution (calcium chloride) one drop at a time, it solidifies; thus creating small beads containing enzyme.
- After completing steps 1- 3, read step 4 out loud. To determine if the lactose was digested, the milk can be tested for the presence of a product of the chemical reaction (glucose). Demonstrate how to test the results of the experiment with a glucose test strip. Make sure to have a control! Prove that prior to exposure to the enzyme, the milk doesn't contain glucose using s clean test strip.

Analysis and Discussion

Upon completion of the lab, discuss why placing the lactase enzyme into beads would be beneficial if a large volume of this milk was being produced. This is a great demonstration of the fact that enzymes can be reused.

Ask students to propose a possible "cure" for lactose intolerance. Discuss the use of gene therapy to treat or cure genetic disorders.

Further Explorations

Research the enzymes of digestion. Find information on digestive disorders caused by the misproduction or absence of an enzyme.

Resources

Books:

DNA Science . Micklos, David A., Freyer, Greg A., and Crotty, David A. , Cold Spring Harbor Press, New York.

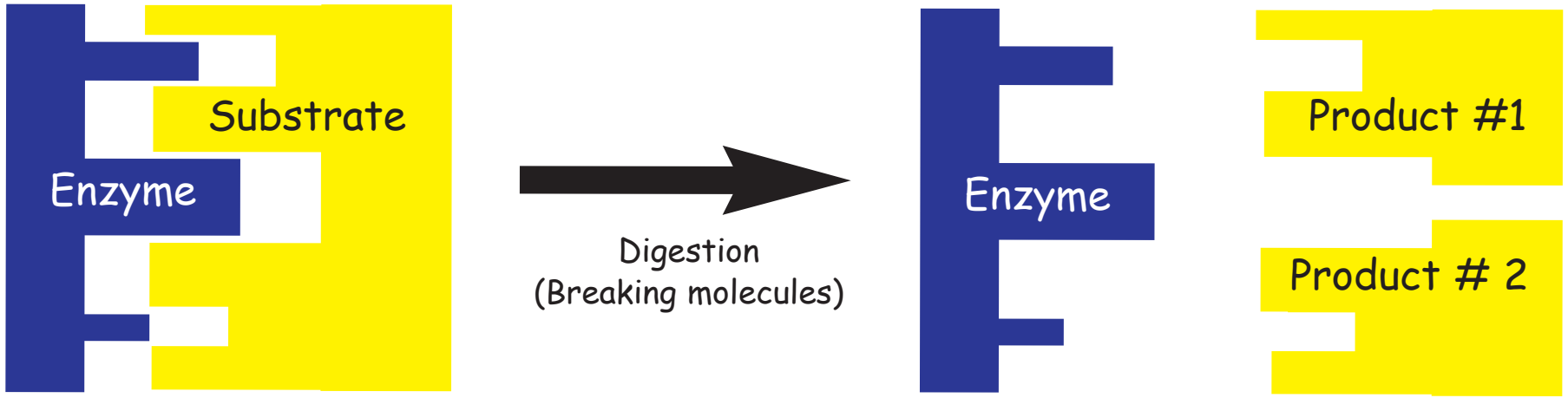
Internet Sites:

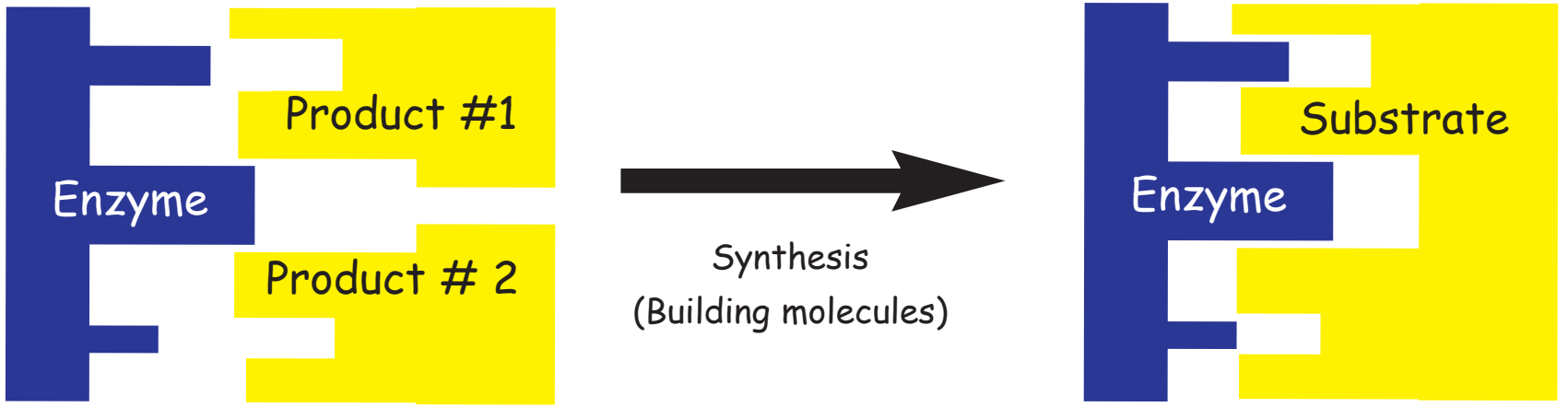
http://www.dnai.org/index.htm
A DNA Learning Center website

http://www.ygyh.org/
A DNA Learning Center website

Enzymes and Substrates

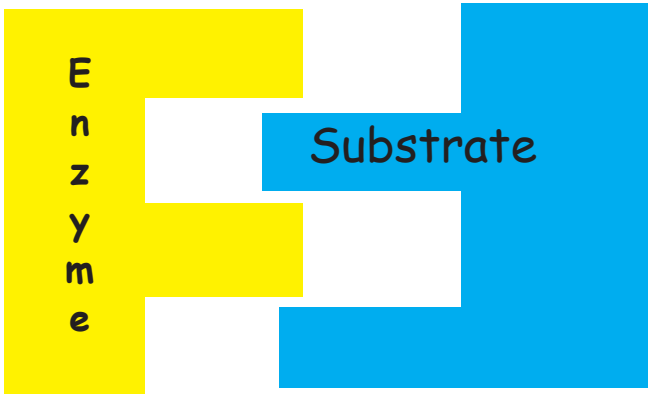
Enzymes, whether they break or build molecules, have specific substrates with which they can react. An enzyme and its substrate fit together like a lock and a key.



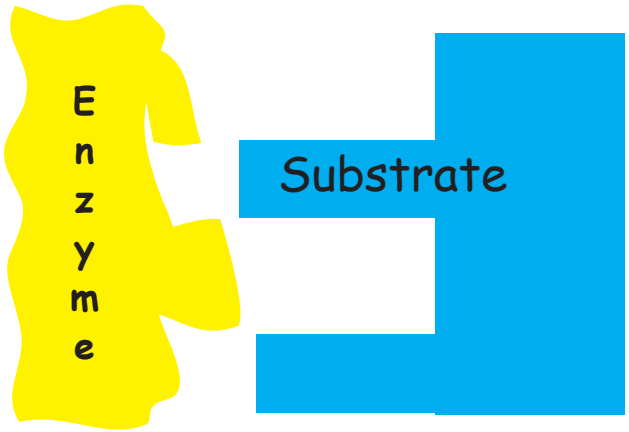


Enzymes and Substrates

If the shape of an enzyme is altered by a change in heat or pH, it can no longer bind with it's 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.



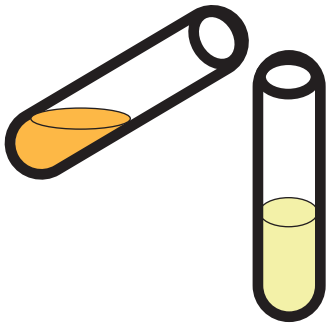
The fit of an enzyme and its specific substrate.



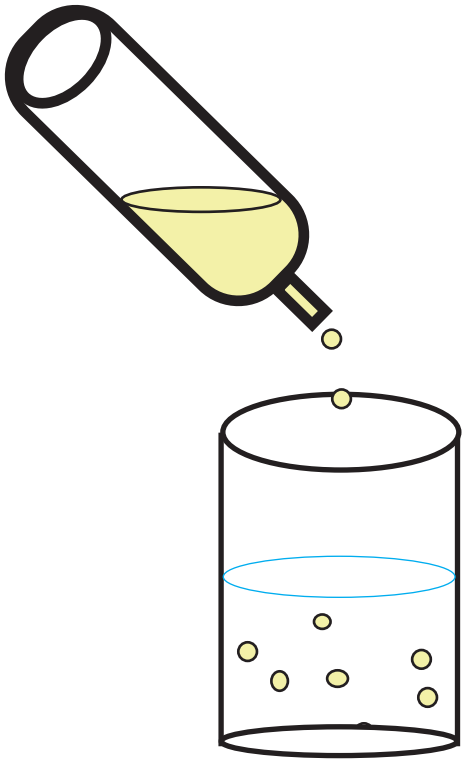
A denatured enzyme does not fit with it's substrate.

Better Milk for Cats

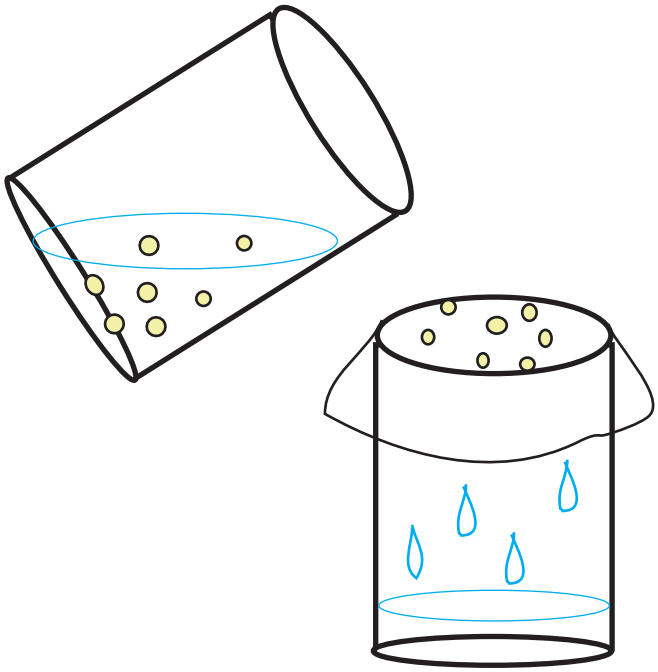
1. Combine 2 ml lactase enzyme with 6 ml sodium alginate solution.



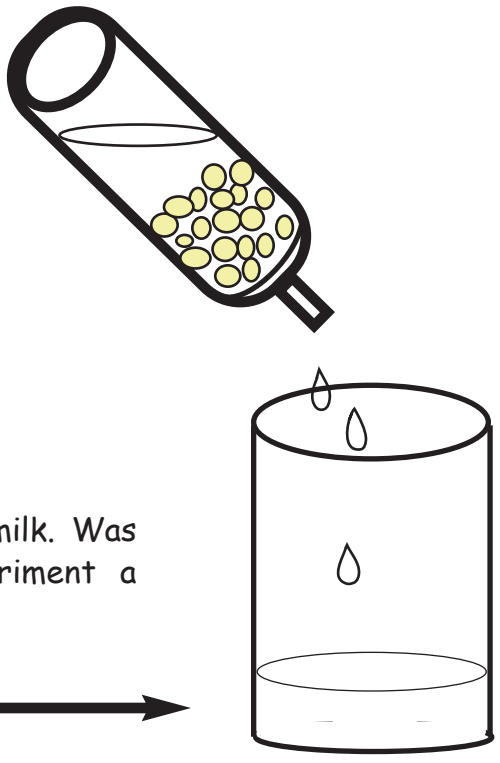
2. Pour the lactase/alginate mixture into a clean syringe, and add it one drop at a time to a calcium chloride solution.



3. Separate the beads from the calcium chloride with a coffee filter.



4. Pack the beads into the syringe barrel and filter milk through the beads into a clean cup.



5. Test the milk. Was the experiment a success?