Students learn about enzyme function, enzyme specificity and the molecular basis of lactose intolerance through experiments with the enzyme lactase and analysis and discussion questions. Students engage in the scientific practices of designing and carrying out experiments and interpreting data. Students also analyze how lactase functions in the digestive system and how the digestive and circulatory systems cooperate to provide cells all over the body with molecules that provide the energy for cellular processes.

Before beginning this activity, students should have a basic understanding of atoms, molecules and chemical formulae. This activity can be used in an introductory unit on biological molecules and scientific method or later in the course during a discussion of enzyme function. This activity will probably require two 50-minute laboratory periods. Or your students may be able to complete the three experiments in one 50-minute laboratory period, if you have your students complete the "Introduction to Sugars and Enzymes" as a pre-lab and pages 6-7 of the Student Handout as a post-lab.

Learning Goals

Specific Learning Goals

- An enzyme is a molecule (usually a protein) that speeds up a specific chemical reaction. Without the enzyme, the reaction typically occurs extremely slowly or not at all.
- Digestive enzymes break down (digest) larger molecules in our food to smaller molecules that can be absorbed into our blood. For example, lactase breaks down the disaccharide lactose into the monosaccharides glucose and galactose.
- An enzyme acts on substrate(s) to produce product(s). The substrate binds to the active site of the enzyme.
- Each enzyme acts only on a specific substrate or several chemically similar substrates because only that specific substrate fits into the enzyme’s active site. For example, lactase digests lactose but not sucrose. Because of enzyme specificity, many different enzymes are needed to digest food (e.g. lactase and sucrase).
- An enzyme molecule returns to its original state after acting on the substrate, so each enzyme molecule can be reused over and over again. For example, a single molecule of lactase can break down many many molecules of lactose.
- A person who produces very little lactase can only digest very small amounts of lactose at a time. Consumption of larger amounts of dairy products in a short time period can result in the symptoms of lactose intolerance. This example illustrates that proteins are not just abstract concepts in biology textbooks, but real parts of our bodies that have observable effects on our characteristics and health.
- In the small intestine, lactase and other enzymes digest disaccharides to produce monosaccharides which are absorbed into the blood which carries the monosaccharides to cells all over the body. In the cells monosaccharides are used to provide the energy needed for cellular processes such as synthesizing molecules and muscle contraction. This illustrates how different body systems (e.g. the digestive and circulatory systems) cooperate to accomplish important body functions.

1 These Teacher Preparation Notes and the related Student Handouts are available at http://serendip.brynmawr.edu/sci_edu/waldron/#enzymes.
In accord with the Next Generation Science Standards:\(^2\):

- This activity helps students to prepare for the Performance Expectations:
  - HS-LS1-1. "Construct an explanation based on evidence for how... proteins... carry out the essential functions of life...."  
  - HS-LS1-2. "Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms."
- Students engage in recommended Scientific Practices, including "planning and carrying out investigations", "interpreting data", and "constructing explanations".
- Students learn the Disciplinary Core Idea (LS1.A), "... proteins... carry out most of the work of cells."
- This activity provides the opportunity to discuss the Crosscutting Concepts: "Structure and function" and "Cause and effect: Mechanism and explanation".

**Equipment and supplies:**

- Lactose solution: 5 g lactose per 200 mL water (20 mL for each group of 3-4 students); lactose is readily available from a variety of suppliers on the web*  
- Sucrose solution 5 g sucrose per 200 mL water (10 mL per group)*  
- Milk (20 mL per group)*  
- Lactase solution: 1 g lactase per 50 mL water (3 mL per group) you can order lactase from Fisher (https://www.fishersci.com/shop/products/mp-biomedicals-lactase-aspergillus-oryzae/p-4605366 ). Store the lactase in the refrigerator until you make the solution on the day of the activity. When you make the solution you will need to smoosh the lumps and stir a lot. A cheaper alternative is to purchase lactase pills from a web supplier or your pharmacy (1 g of lactase has roughly 12,000-18,000 lactose units, so if you have lactase pills with 9000 units, you can dissolve two pills in 50 mL of water).*  
- Beakers+  
- 25 mL graduated cylinders to measure lactose solution, sucrose solution, and milk +  
- 1 mL transfer pipet for lactase solution+  
- 15 milliliter test tubes* (2 per group if students will be able to rinse these between uses; otherwise 5 per group) and test tube rack or something else to keep the test tubes upright (1 per group)  
- Visually readable glucose test strips (5 per group; 2 for experiment 1; 1 for experiment 2; 2 for experiment 3); glucose test strips are available from http://www.amazon.com/Betachek-Visual-Blood-Glucose-Strips/dp/B00HTVECL6 or http://www.carolina.com/catalog/detail.jsp?prodId=695960&s_cid=ppc_gl_products&gclid=CIWU8MHxr8YCFY09gQodSjUITA or search on the web for urine glucose test strips; you will need to provide your students with instructions for using the particular type of glucose test strip you have ordered and you will want to have the color chart for reading the test strips available for your students  
- Gloves (3 per group)  
- Permanent markers and tape or labels for labeling test tubes (1 set per group)

* In order to conserve materials and thus reduce the cost of purchasing lactase, you can use smaller test tubes and correspondingly smaller amounts of each solution. If you do this, you will need to modify the instructions in the Student Handout.

+ If you keep the solutions at your desk, you will need four beakers (for each solution and the milk) and a minimum of three graduated cylinders and one transfer pipette for measuring these.

---

Instructional Suggestions and Background Information
A key is available upon request to Ingrid Waldron (iwaldron@sas.upenn.edu). The following pages provide instructional suggestions and additional background information – some for inclusion in your class discussions and some to provide you with relevant background that may be useful for your understanding and/or for responding to student questions.

Introduction to Sugars and Enzymes in the Student Handout
Sucrose is commonly called "sugar". Common sources of sucrose in our diet are sugar cane, sugar beets, and fruits. Fruits also contain the monosaccharides, glucose and fructose.

If your students are not entirely comfortable with molecular diagrams, you may want to insert the following question:

1b. Use the diagrams of the monosaccharides to add to the sucrose diagram all the carbon and hydrogen atoms that are not explicitly shown.

You may find the following figure useful if you want to explain to your students that enzymes speed up biological processes by reducing the activation energy required to get to the transition state in chemical reactions. An enzyme can lower the required activation energy by stressing particular chemical bonds of a substrate or bringing two substrates together in the correct orientation to react with each other. Without an enzyme, the activation energy typically is so high that very few molecules have sufficient thermal energy to undergo the reaction; in contrast, with an enzyme, many more molecules will have sufficient thermal energy to meet the lower activation energy requirement and the reaction will proceed at a biologically useful rate.

![Diagram of enzyme action on sucrose](image)


Experiment 1 – Can the sugar lactose be digested without any enzyme?
Question 5 in the Student Handout should help your students to understand why testing for glucose is a reasonable method for evaluating whether the enzyme lactase is needed to digest the sugar lactose.

3
Glucose test strips are used by people with diabetes to test for glucose in their urine or blood; when glucose is present in the urine this indicates that blood glucose levels are too high, which can be harmful to their health. Note that glucose test strips do not react with glucose when the glucose is part of the disaccharides lactose or sucrose. The glucose test strip only reacts with the monosaccharide glucose.

To calculate the number of lactose molecules per lactase molecule in question 10a of the Student Handout, we used the amount of lactose and lactase solutions added to the test tube, the concentrations of lactose and lactase in the solutions, and the molecular weight of lactose (342) and lactase (approximately 150,000-300,000).

You may want to ask your students to suggest improvements in the design of Experiment 1. For example, it might be useful to add 1 mL of water to Tube 1 for greater comparability to Tube 2.

Experiment 2 – Can the same enzyme digest lactose and sucrose?
Students can use the procedure provided for Experiment 1 to guide them in designing the procedure for Experiment 2. Some enzymes (e.g. lactase) act on only a single substrate. Other enzymes act on a specific type of chemical bond flanked by specific chemical structures (e.g. pepsin; http://osp.mans.edu.eg/medbiochem_mi/Cources/Biochemistry/1st_year_nevicine/Enzymes/files/Lecture_02.pdf).

Experiment 3 – Does the enzyme lactase digest the sugar in milk?
Answering question 17 should help students understand why they need to test for glucose both in 10 mL of milk without enzyme and in 10 mL of milk with 1 mL of lactase solution.

The Digestive System and Lactose Intolerance
The first paragraph on page 6 of the Student Handout mentions the extreme differences in size between molecules and a human body. You may want to link these extreme size differences to the:
- very large numbers of molecules in each cell (roughly 20 billion protein molecules per human cell and trillions of water molecules)
- many many cells in each human body (roughly 40 trillion cells).
If you want to reinforce student understanding of size differences and different levels of organization in biology, you may want to have your students do the Card Sort Activity – From Coffee to Carbon (available at http://teach.genetics.utah.edu/content/cells/CoffeeToCarbon.pdf). This activity has students sort the cards (each with a molecule, organelle or cell) according to size. To use this activity to reinforce student understanding of different levels of organization, I recommend that you begin by having your students sort the cards into four categories: molecules, organelles, cells, and other. After you have discussed this initial card sort, then have your students organize the cards from smallest to largest. (Depending on your students, you may want to omit some cards such as adenine, influenza virus, baker’s yeast.) After students have completed the card sort by size, discuss the results and show the animation which illustrates the relative sizes (available at http://learn.genetics.utah.edu/content/begin/cells/scale/).

If you would like to expand the discussion of levels of organization in biological organisms and relative sizes at these different levels of organization, the following resources and figure may be helpful. Brief introductions to levels of organization are available at:

- http://wps.pearsoncustom.com/wps/media/objects/3014/3087289/Web_Tutorials/01_A02.swf

http://www.protein-structure.net/images/Body-Systems.jpg
Lactose Intolerance

Lactose intolerance is a result of decreased production of lactase as a child grows toward adulthood. (Lactose intolerance in infancy is very rare—less than 1 in 60,000 newborns.) The decrease in production of lactase as a person gets older is called lactase nonpersistence.

The alleles for the gene for lactase differ in the nucleotide sequence in the regulatory DNA; this difference influences the age trend in the rate of transcription of the coding DNA for the protein, lactase, and thus influences the rate of production of lactase.

- **Lactase persistence alleles** result in substantial production of lactase throughout life.
- **The lactase nonpersistence allele** results in substantial production of lactase by infants, but very low levels of lactase in adults, resulting in lactose intolerance.

For virtually all infants and for adults with lactase persistence:
- in the small intestine:
  
  \[
  \text{lactase} \\
  \text{lactose} \rightarrow \text{glucose + galactose}
  \]

For the roughly two-thirds of adults worldwide who have lactase nonpersistence:
- in the small intestine, most lactose is not digested due to low levels of lactase
- so, in the colon of the large intestine, lactose is fermented by anaerobic bacteria:
  
  fermentation
  
  \[
  \text{lactose} \rightarrow \text{short-chain fatty acids + gases (e.g. CO}_2\text{)}
  \]

  the mixture of water, partially digested food, etc. in the colon is hypertonic

  \[
  \text{osmotic influx of water} \rightarrow \text{diarrhea} \rightarrow \text{discomfort}
  \]

  flatulence and lactose intolerance

Dairy products are an important source of calcium, as well as protein and some vitamins. People with lactose intolerance can continue to consume dairy products but **minimize symptoms** by:

- using lactase supplements
- consuming dairy products with reduced lactose due to treatment with lactase (e.g. lactose-free milk) or fermentation by bacteria (e.g. traditionally made cheese or yogurt)
- consuming small amounts of dairy products at multiple times during the day
- adaptation of bacteria in the colon by gradually increasing regular lactose consumption of modest amounts of dairy products

The lactase persistence allele provides an example of natural selection in humans:

- Lactase nonpersistence alleles are nearly universal in mammals and were nearly universal in early humans.
- When some groups of humans began raising dairy animals, natural selection favored lactase persistence alleles which became more common in these groups.
- Different lactase persistence alleles are observed in European and African herding groups. This illustrates how similar characteristics can involve independently in different populations (convergent evolution).

Lactose intolerance is different from a milk allergy which happens when the body’s immune system reacts to proteins in milk. (A good summary of milk allergy is available at
What happens to the digested food molecules?

You may want to use one or both of these figures and explanations to help your students understand how the digestive and circulatory systems cooperate to provide cells all over the body with monosaccharide molecules that can be used to provide the energy for cellular processes.

This schematic diagram illustrates how the circulatory system is crucial for allowing the specialized digestive, respiratory and excretory systems to serve needed functions for all the cells in the body.

The left diagram shows the structure of the wall of the small intestine with many villi on the inner surface. The right diagram shows a much magnified view of one of the villi; each of the villi is only about 1 mm long.

The enzymes lactase and sucrase are located on the surface of the cells that line each of the villi (enzymes indicated by e). This is useful since the monosaccharides are produced right near the surface of the villi which facilitates transport of the monosaccharides to the blood in the numerous capillaries inside the villi.

Additional resources for introducing your students to body systems and digestion include:
http://www.ivyroses.com/Revise/AnatomyPhysiology/index.php and
Related Activities

"Enzyme Investigation" is presented on the last two pages of these Teacher Preparation Notes. This inquiry activity can be used as an extension activity.

Students can expand their understanding of enzymes in the bioengineering design challenge included in "Alcoholic Fermentation in Yeast", available at http://serendip.brynmawr.edu/sci_edu/waldron/#fermentation. In the first part of this activity, students learn about the fundamentals of alcoholic fermentation and test for alcoholic fermentation by assessing CO₂ production by live yeast cells in sugar water vs. two controls. In the bioengineering design challenge, students work to find the optimum sucrose concentration and temperature to maximize rapid CO₂ production, using no more sucrose than needed for maximum CO₂ production. Structured questions guide the students through the basic engineering steps of applying the relevant scientific background, developing and systematically testing proposed design solutions, and then using initial results to develop and test improved design solutions.

Additional activities to help students understand the functions of proteins are presented in "Understanding the Functions of Proteins and DNA", available at http://serendip.brynmawr.edu/sci_edu/exchange/bioactivities. This overview provides a sequence of learning activities to help students understand that proteins and DNA are not just abstract concepts in biology textbooks, but rather crucial components of our bodies that affect functions and characteristics that students are familiar with. Students learn about how proteins contribute to the digestion of food and to characteristics such as albinism, sickle cell anemia and hemophilia. Then, students learn about the relationship between the genetic information in DNA and the different versions of these proteins. The discussion, web-based, and hands-on learning activities presented are appropriate for an introductory unit on biological molecules or as an introduction to a unit on molecular biology.

A hands-on activity for teaching about macromolecules is "Who took Jerell’s iPod? -- An Organic Compound Mystery", available at http://serendip.brynmawr.edu/sci_edu/waldron/#organic. In this activity, students learn how to test for triglycerides, glucose, starch, and protein and then use these tests to solve a mystery. The activity reinforces students understanding of the biological functions and food sources of these different types of organic compounds.

Another hands-on activity, "A Scientific Investigation – What types of food contains starch and protein?" is available at http://serendip.brynmawr.edu/sci_edu/waldron/#starch. In this activity, students learn about scientific investigation by carrying out key components of the scientific method, including developing experimental methods, generating hypotheses, designing and carrying out experiments to test these hypotheses and, if appropriate, using experimental results to revise the hypotheses. Students design and carry out two experiments which test whether starch and protein are found in some or all foods derived from animals or plants or both.

An analysis and discussion activity that will help students to understand levels of organization in biology and the function of the digestive system is "Structure and Function of Cells, Organs and Organ Systems", available at http://serendip.brynmawr.edu/exchange/bioactivities/SFCellOrgan.
In this activity, students learn how the structure of cells, organs and organ systems is related to their functions. Students analyze multiple examples of the relationships between structure and function in diverse eukaryotic cells and in the digestive system. In addition, students learn that cells are dynamic structures with constant activity and they learn how body systems interact to accomplish important functions.
Enzyme Investigation
by Deane Gordon, retired from Philadelphia Military Academy @ Leeds

Your task is to identify and name the unknown enzyme that is in the numbered bottle. Use your previous knowledge to plan and carry out an investigation to identify your enzyme. Then, complete a standard lab report with your findings.

You have previously observed that glucose test strips change color when glucose (as a monosaccharide) is present. Any color change indicates at least trace amounts of glucose and bigger color changes indicate more glucose.

Here are the possible enzymes.

Enzyme Sucrase, which catalyzes the reaction:
Sucrose → Glucose+ Fructose

Enzyme Lactase, which catalyzes the reaction:
Lactose → Glucose+ Galactose

There are also bottles with no enzymes, so you have three possibilities.

Here is a partial list of materials to help you get started:

One Numbered Bottle containing sucrase or lactase or no enzyme.
Test Tubes
Test Tube Rack
Glucose test strips
YOU COMPLETE THE LIST

Your lab report will be in the standard format. Please make sure to identify the unknown and explain why you came to that conclusion. Also, include possible sources of error.

Have fun.
This lab is intended as an extension activity for the Enzymes Help Us Digest Food activity. It may be used independently, depending on student readiness and their understanding of enzyme specificity.

Refer to the Teacher Preparation Notes for Enzymes Help Us Digest Food for solution concentrations and amounts.

Prepare one bottle for each student group:
- one third of the bottles with lactase enzyme (labeled Bottle #1)
- one third of the bottles with sucrase enzyme (available as invertase from Carolina Biological Supply) (labeled Bottle #2)
- one third of the bottles with water (Add some baking soda or other solute to make it look like Bottles #1 and #2.) (labeled Bottle #3)

The students should select the sucrose solution and either the lactose solution or milk to test their enzyme. They may also use a glucose solution and/or water as controls.