

# Looking Into Lactase

## A Medical Biotechnology Enzyme Lab

Maryland Loaner Lab Teacher Packet  
**Structured Inquiry Version**



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through a Dwight D. Eisenhower Professional  
Development Program.*

# Looking Into Lactase – Structured

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# Looking Into Lactase – Structured

## Equipment and Supplies

The Looking Into Lactase lab has two parts:

- A pre-laboratory activity that allows students to learn about the relationship between lactose intolerance and enzyme function.
- Three laboratory activities that allow students to first identify three different milk types and then determine how changes in pH and temperature alter enzyme activity. During these activities, students apply laboratory science to the field of medical biotechnology.

### Equipment and Supplies

Supplied By Maryland Loaner Lab Program:

Description	Quantity	Must Be Returned?
<b>Teacher Binder</b> with student worksheets	1	Return
<b>Step Strips for Structured Inquiry Version Envelope</b> (Parts 1-3)	1 envelope (10 sets each for Parts 1, 2 and 3)	Return used and unused sets
<b>Lactex Bag</b> <ul style="list-style-type: none"><li>- Lactex (Lactase Enzyme Powder), 1.44g/class set</li><li>- (10) Lactex Tubes</li><li>- (1) Plastic bottle (empty) labeled “Lactex Solution”</li></ul>	1 bag	<ul style="list-style-type: none"><li>- Return empty and unused enzyme powder tubes</li><li>- Clean, dry, and return (10) Lactex Tubes</li><li>- Clean, dry, and return plastic bottle</li></ul>
<b>Rice and Soy Milk Bag</b>	1 bag (1 box each)	Discard
<b>Part 1 Plastic Test Tubes Bag</b>	1 bag (30 tubes)	Discard
<b>Part 2 pH Plastic Test Tubes Bag</b>	1 bag (60 tubes)	Discard
<b>Part 3 Temperature Test Tubes Bag</b>	1 bag (60 tubes)	Discard
<b>pH Solutions Bag:</b> 6 pH solutions (2, 4, 7, 10, 12, dH <sub>2</sub> O) . 10ml each solution/class set requested.	1 bag (6 tubes)	Rinse with water only (don’t use soap or other chemicals), dry, and return
<b>Glucose Test Strip Bag.</b> An extra refill bottle will be included if multiple class sets are requested.	1 bag (10 containers)	Return all containers and unused test strips
<b>Cow Milk Tubes Bag</b>	1 bag (10 tubes)	Discard
<b>Extra Conical Tubes Bag</b> (for Guided Inquiry Version)	1 bag (10 tubes)	Return unused tubes

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## Equipment and Supplies

<b>Pipette Bag</b> - (10) Teacher Aliquoting Pipettes - (10) Student Lactex Pipettes - (10) Student Cow Milk Pipettes	1 bag (30 pipettes)	Discard pipettes, return labeled bags
<b>Pipette Pump Bag</b>	1 bag (10 pumps)	Return
<b>Thermometer, Plastic Tube Floater, Test Tube Brush Bag</b>	1 bag	Return all
<b>White Test Tube Racks</b>	10	Clean, dry, and return
<b>Container of Disinfecting Wipes</b>	1	Return

### Supplied by the Teacher:

Description	Quantity/Class	Comments
Cow milk	220 ml per class	
dH <sub>2</sub> O	175 ml	For making Lactex (Lactase Enzyme Solution)
Paper towels	10	One for each station
Goggles	1 per student	Must be used in Part 1, Part 2, and Part 3
Gloves	1 pair per student	
Ice in containers (or use of freezer in the lab)	Enough to hold tubes from 10 stations	Used in Part 3
Water bath or hot plate	1	Used in Part 3
1000 ml glass beaker	1	Used in Part 3

**SAFETY:** The classroom teacher must instruct students with basic laboratory safety rules and provide gloves and goggles for student use with the laboratory activity.

### *Return Kit Directions*

Before packing the kit ensure that all returnable containers and pipettes are clean and dry. Refer to return list on the equipment and supplies table if you are unsure about what needs to be returned. Re-pack all items in the same manner as received. Follow the FedEx procedure included in your paperwork to ship the kit.

A Microsoft Word version of this document is available upon request by emailing [mdll@towson.edu](mailto:mdll@towson.edu).

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## Next Generation Science Standards

<p><b>Performance Expectations:</b> Students’ ability to complete the following performance expectation(s) will be supported by participation in this activity.</p> <p><b>HS-LS1-3:</b> Plan and carry conduct an investigation to provide evidence that feedback mechanism maintain homeostasis.</p> <p><b>HS-LS3-2:</b> Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.</p> <p><b>HS-LS3-3:</b> Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.</p>		
Dimension	NGSS Code or citation	Corresponding student task in activity
<p><b>Disciplinary Core Idea</b></p>	<p>LS1.A Structure and Function</p> <ul style="list-style-type: none"> <li>Feedback mechanisms maintain a living system’s internal conditions within certain limits and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range. Feedback mechanisms can encourage (through positive feedback) or discourage (negative feedback) what is going on inside the living system. . (HS-LS1-3)</li> </ul>	<p>Students explore how enzyme function is affected by changes in pH and temperature.</p>
	<p>LS3.B Variation of Traits</p> <ul style="list-style-type: none"> <li>In sexual reproduction, chromosomes can sometimes swap sections during the process of meiosis (cell division), thereby creating new genetic combinations and thus more genetic variation. Although DNA replication is tightly regulated and remarkably accurate, errors do occur and result in mutations, which are also a source of genetic variation. Environmental factors can also cause mutations in genes, and viable mutations are inherited. (HS-LS3-2)</li> <li>Environmental factors also affect expression of traits, and hence affect the probability of occurrences of traits in a population. Thus the variation and distribution of traits observed depends on both</li> </ul>	<p>In the extension activities, students explore how different populations of people differ in their ability to break down the sugar lactose. These differences are caused by mutations in select populations that allow adults to continue to digest lactose into adulthood.</p> <p>Extension activities allow students to explore the ideas that mutations that allowed humans to retain the ability to digest lactose into adulthood were selected for in populations of humans that relied on dairy as a significant source of energy into adulthood.</p>

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## Next Generation Science Standards

	genetic and environmental factors. (HS-LS3-2), (HS-LS3-3)	
<b>Practice</b>	<p>Planning and Carrying out Investigations</p> <ul style="list-style-type: none"> <li>Plan an investigation individually and collaboratively and in the design identify independent and dependent variables and controls, what tools are need to do the gathering, how measurements will be recorded, and how many data are need to support a claim.</li> <li>Conduct an investigation and/or evaluate and/or revise the experimental design to produce data to serve as the basis for evidence that meet the goals of the investigation.</li> </ul>	<p>Students identify the independent and dependent variables to be tested.</p> <p>Students plan and conduct investigations to test how temperature and pH affect enzyme function. Students are supported in developing their protocols through the use of a ‘steps’ activity where all the steps of the investigation are written on separate pieces of paper, and students must decide what order to conduct the steps in.</p>
	<p>Constructing Explanations</p> <ul style="list-style-type: none"> <li>Make a quantitative and/or qualitative claim regarding the relationship between dependent and independent variables.</li> <li>Construct and revise an explanation based on valid and reliable evidence obtained from a variety of sources (including students’ own investigations, models, theories, simulations, peer review) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future</li> </ul>	<p>Students construct a scientific explanation about what temperature and pH enzymes work best. They are asked to provide evidence and reasoning to support their claims.</p>
<b>Crosscutting Concept</b>	<p>Cause and Effect</p> <ul style="list-style-type: none"> <li>Cause and effect relationships can be suggested and predicted for complex natural and human designed systems by examining what is known about smaller-scale mechanisms.</li> </ul>	<p>Students will explore how changes in temperature and pH can affect an enzyme’s ability to function.</p>

# Looking Into Lactase – Structured

## Next Generation Science Standards

	<p>Scale, Proportion and Quantity</p> <ul style="list-style-type: none"> <li>Some systems can only be studied indirectly as they are too small, too large, too fast, or too slow to observe directly.</li> </ul>	<p>Students use an indirect measure of enzyme function (glucose test strips) to explore how temperature and pH affect the enzyme lactase.</p>		
	<p>Structure and Function</p> <ul style="list-style-type: none"> <li>The functions and properties of natural and designed objects and systems can be inferred from their overall structure, the way their components are shaped and used, and the molecular substructures their various materials.</li> </ul>	<p>Students explore how the physical structures of enzymes allow them to function. They also explore how factors that affect enzyme protein shape can affect the ability of the enzyme to function.</p>		
	<p>Stability and Change</p> <ul style="list-style-type: none"> <li>Change and rates of change can be quantified and modeled over very short or very long periods of time. Some system changes are irreversible.</li> </ul>	<p>Students learn that some environmental conditions can alter the shape and functionality of an enzyme. Sometimes the changes are temporary, other times the changes are permanent (denaturation).</p>		
<p><b><u>Nature of Science</u></b></p> <p>Scientific investigations use a variety of methods</p> <ul style="list-style-type: none"> <li>Scientific investigations use diverse methods and do not always use the same set of procedures to obtain data.</li> <li>Scientific investigations use a variety of methods, tools, and techniques to revise and produce new knowledge.</li> </ul>				
<p><b><u>Connections to Common Core State Standards</u></b></p> <table border="0" style="width: 100%;"> <tr> <td style="vertical-align: top;"> <p><u>English Language Arts/Literacy</u></p> <p>RST.9-10.3</p> <p>RST.9-10.7</p> <p>RST.9-10.9</p> <p>RST.11-12.3</p> <p>RST.11-12.4</p> <p>RST.11-12.7</p> </td> <td style="vertical-align: top; border-left: 1px solid black;"> <p><u>Mathematics</u></p> <p>PRACTICE.MP1</p> <p>PRACTICE.MP3</p> <p>PRACTICE.MP4</p> </td> </tr> </table>			<p><u>English Language Arts/Literacy</u></p> <p>RST.9-10.3</p> <p>RST.9-10.7</p> <p>RST.9-10.9</p> <p>RST.11-12.3</p> <p>RST.11-12.4</p> <p>RST.11-12.7</p>	<p><u>Mathematics</u></p> <p>PRACTICE.MP1</p> <p>PRACTICE.MP3</p> <p>PRACTICE.MP4</p>
<p><u>English Language Arts/Literacy</u></p> <p>RST.9-10.3</p> <p>RST.9-10.7</p> <p>RST.9-10.9</p> <p>RST.11-12.3</p> <p>RST.11-12.4</p> <p>RST.11-12.7</p>	<p><u>Mathematics</u></p> <p>PRACTICE.MP1</p> <p>PRACTICE.MP3</p> <p>PRACTICE.MP4</p>			

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## Setting Up the Lab

### *Student Worksheets*

- Each student will require their own worksheet packet. For each student, make copies of pages S-1 – S-20.

### *Solution Preparation and Aliquoting*

#### **Part 1: Identifying the Milk Type**

Add (1) tube of lactase powder (labeled Lactase 1.44g) to bottle labeled “Lactex” (Lactase Enzyme Solution)

- Add 175 ml dH<sub>2</sub>O.
- Mix thoroughly - the powder will not completely dissolve.
- Aliquot 15 ml into the (10) conicals labeled “Lactex” (Lactase Enzyme Solution).
- Make <12 hours before use, refrigerate when not in use.

#### **Unknown Milk Samples**

(Tubes #1, #2, and #3)

- Aliquot 2 ml rice milk into each of 10 student test tubes labeled #1.
- Aliquot 2 ml cow milk into each of 10 student test tubes labeled #2.
- Aliquot 2 ml soy milk into each of 10 student test tubes labeled #3.

#### **Part 2: Determining the Effect of pH on Lactase Activity**

- Fill (10) empty cow milk conicals with 20 ml of cow milk for each work station.

#### **pH Solutions**

- Aliquot 1 ml of each of the appropriate pH solutions into the respective pre-labeled student test tubes.
- You will end up with a total of 60 filled student test tubes.
- There will be 1 test tube per student workstation for each pH (2, 4, 7, 10, 12, and dH<sub>2</sub>O).

#### **Part 3: Determining the Effect of Temperature on Lactase Activity**

- Fill (10) empty cow milk conicals with 20 ml of cow milk for each work station.

### *Prepare 10 Student Workstations*

You have been sent enough equipment to set up 10 student workstations, and the activities are designed to have students work in small groups (group size will depend on the total number of students in class). If you have requested materials for multiple classes, you will need to rinse tubes and refill reagents at student workstations between classes.

#### **Each student station should include:**

- Paper towel
- 1 Pipette pump

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## Setting Up the Lab

- 1 “Lactex” pipette
- 1 “Cow Milk” pipette
- 1 container glucose test strips
- 1 conical Lactex (Lactase Enzyme Solution) solution (filled by teacher)
- Safety goggles (1/student: to be worn at all times)
- 1 “Cow Milk” tube (filled by teacher)
- Student copies of the laboratory protocol (1/student)
- 1 Test tube rack
  - Front row (Part 1): Unknown milk samples #1, #2, #3 (filled by teacher)
  - Middle row (Part 2): pH solutions (2, 4, 7, 10, 12) and water control (filled by teacher)
  - Last row (Part 3): 6 test tube caps and 6 test tubes labeled HL (Hot Lactex), HM (Hot Milk), RTL (Room Temperature Lactex), RTM (Room Temperature Milk), CL (Cold Lactex), and CM (Cold Milk)

### Shared Equipment for Multiple Groups:

- Thermometer
- Ice in buckets/containers (or use of freezer)
- Water bath or hot plate with a 1000 ml glass beaker (white plastic tube holder used in either one)

### *Helpful Hints*

#### **Part 1: Identifying the Milk Type**

- This part uses reagents at room temperature. Allow milk samples and Lactex (Lactase Enzyme Solution) to warm to room temperature before starting the lab.
- Make sure to have students invert capped Lactex (Lactase Enzyme Solution) tubes to suspend powder before pipetting.
- When students are inverting tubes, remind them to hold the cap on to prevent leakage.

#### **Part 2: Determining the Effect of pH on Lactase Activity**

- Any experiment testing pH uses reagents at room temperature. Allow milk samples, Lactex (Lactase Enzyme Solution), and pH solutions to warm to room temperature before starting the lab.
- Instruct students to notify a teacher if the pH solutions have spilled and to wash hands immediately.

#### **Part 3: Determining the Effect of Temperature on Lactase Activity**

- Milk must be at desired temperature before adding the enzyme.
- It is possible to not use ice and place tubes in a rack that will be kept in a freezer, if nearby.
- A water bath with an adjustable heater or a hot plate with a 1000 ml glass beaker with hot water can be used for the hot temperature section.
- The teacher should monitor the hot water bath at all times, keeping student safety in mind.

# Looking Into Lactase – Structured

## Teacher Background

The Looking Into Lactase laboratory is an activity to facilitate learning about enzymes and activities and factors that affect them. It also connects laboratory science and the study of medical biotechnology.

This laboratory contains 3 parts:

### **Part 1: Identifying the Milk Type**

Students will confirm Lactex’s ability to break down the sugars found in cow milk.

### **Part 2: Determining the Effect of pH on Lactase Activity**

Students will investigate the effect of pH on enzyme activity.

### **Part 3: Determining the Effect of Temperature on Lactase Activity**

Students will investigate the effect of temperature on enzyme activity.

The pre-laboratory activity includes a storyline that involves Pharmex Pharmaceuticals, which is a fictitious company. As the teacher, you are to act as the Principal Investigator working in this company overseeing the various research teams (students). You are to tell the students that this company is currently working on the development of a medicine, Lactex (Lactase Enzyme Solution), and that it is to be used by people who suffer from symptoms of lactose intolerance.

Activity	Time needed
Preparing samples & student stations	60 minutes
Pre-Lab activity	30 - 45 minutes
Laboratory activity	60-90 minutes each activity
Extension Activities	Varies

### ***Teaching Scientific Inquiry: Explore Before Explain***

Teaching the process of scientific inquiry is as important as teaching content-related material. The process of scientific inquiry involves generating questions, designing investigations to answer questions, making predictions based on scientific concepts, gathering data, using evidence to propose explanations, and communicating scientific explanations.

Students are often given all the answers through background information or a lecture before they have a chance to explore a concept. When using an inquiry-based method for instruction, remember to keep inquiry in the foreground and scientific content in the background. Or EXPLORE before EXPLAIN! We want students to have a general understanding of enzymes before, during, and after their investigation. But we have withheld specific information about how enzymes function in different environments until after they have collected their data.

**The purpose for the EXPLORE stage is to get students involved in the topic and provide them with a chance to build their own understanding.** In the exploration stage the students have the opportunity to get directly involved with phenomena and materials. As they work together

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## Teacher Background

in teams, students build a set of common experiences, which prompts sharing and communicating. Instances where students are exploring in this lab are:

- Students are given general background knowledge about enzymes in Medical Fact Sheet #1 to help guide their investigation but not give them all the answers.
- Students explore how the lactase enzyme works by identifying the milk types in Part 1.

**The purpose for the EXPLAIN stage is to provide students with an opportunity to communicate what they have learned so far and figure out what it means.** Once students build their own understanding, they may use background information to help summarize or explain their own ideas. These segments introduce vocabulary in context and correct or redirect misconceptions. An instance where students are explaining in this lab after exploring is when they use Medical Fact Sheet #2. This fact sheet has answers for students regarding how enzyme effectiveness changes with different environmental factors like temperature and pH. Giving this fact sheet to students before they have had an opportunity to complete Part 2 and Part 3 provides the answers for them before they have had a chance to actively learn it themselves.

The following detailed background provided is for you as the teacher, but it is not meant for your students to see.

### *What are Enzymes?*

Enzymes are organic catalysts that control the rate of chemical reactions in cells while not being permanently altered themselves. In general, enzymes speed up the rate of reactions by lowering the activation energy required to start reactions.

Enzymes are extremely efficient. They can catalyze reactions at rates up to 10 billion times higher than comparable non-catalyzed reactions. Due to their specific configuration, they hold the reactant molecules in close proximity and in the correct orientation for the reaction to occur. Each enzyme has a specific site (the active site) where the substrate and enzyme combine. Enzymes also show specificity in that each specific type of enzyme acts on a particular substrate or on a certain kind of chemical bond. The specificity of enzymes is due to their structures. Each enzyme has a characteristic three-dimensional (3-D) shape. The analogy of a “lock and key” is often used to illustrate the temporary joining of a specific enzyme to a specific substrate in an enzyme-substrate complex.

When an enzyme is subjected to high temperatures, the enzyme ceases to function. (There are exceptions, such as the enzymes in thermophilic bacteria). This is due to the denaturing of the enzyme (unfolding of the 3-D structure). At lower temperatures, the 3-D structure is intact, but there may not be enough energy for a reaction to occur. In this case the enzyme is inactive, but not permanently denatured.

### *What is Lactase?*

Lactase is an enzyme used by the body to hydrolyze lactose, a disaccharide unique to mammalian milk, into the monosaccharides, glucose and galactose. Lactose has been shown to aid in the absorption of several minerals, including calcium, magnesium, and zinc.

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## Pre-Laboratory Activity: Teacher Guide

*Time Requirement: 30-45 minutes*

### ***Pre-Laboratory Activity***

Pass out a student worksheet packet (pages S-1-S-20) to each student.

1. Read the **Medical Case Study** to familiarize students with the type of patient who would benefit from the use of this drug. This is found on pages S-1 and S-2.
2. Ask students for suggestions about helping the patient manage his lactose intolerance. This could include avoiding foods that contain the sugar lactose. Ask students to think about how difficult it can be to avoid certain foods. Guide students to think about what breaks down lactose (lactase). Ask students if they think there is some way the lactase enzyme could be ingested by the patient and have them brainstorm their ideas.
3. Introduce the scenario to students that they are working for a pharmaceutical company and read out loud **Email #1** from the Principal Investigator (S-3). Tell students that if effective, this drug, Lactex, can be used by people who experience the symptoms of lactose intolerance.
4. Direct the students to the **Medical Fact Sheet #1** in their worksheet packet (S-4). Have the students read this fact sheet and answer the following questions: What are enzymes? (*Enzymes are organic catalysts, speeding up chemical reactions while not being permanently altered themselves.*) Why are they important in the human body? (*They help catalyze reactions.*) What does the lactase enzyme have to do with lactose intolerance? (*The lactase enzyme is needed to break down the sugar lactose. People with lactose intolerance do not produce enough/ any lactase to catalyze this reaction.*)
5. Have students read **Email #2** from the Principal Investigator (S-5). Have students discuss how the sugars lactose, sucrose, and glucose differ. Ask students to point out which sugar would need the addition of an enzyme to break down and which would not and why.

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## Laboratory Activity Part 1: Teacher Guide

*Time Requirement: 60-90 minutes (can be broken into 2 - 45 minute sessions)*

### ***Part 1: Identifying the Milk Type***

The purpose of the laboratory activity is for students to determine the effect of the lactase enzyme on three types of milk by conducting an investigation.

Students are testing unknown milk samples (rice, cow, and soy milk) before and after the addition of lactase to determine the identity of each.

Begin by reviewing the types of sugar present in different types of milk (see S-5 of student handout). Break the students up into pairs or small groups and challenge each group to identify the milk type in three test tubes by putting the steps in order. Have each group work on the steps activity, and then have one or two groups share their order with the rest of the class. Allow students to discuss which order they think is correct if there is disagreement. Let the students know that scientists don't always know the proper procedure before they begin an investigation. Trial and error is a part of the scientific process, and it is okay if groups do things differently.

Once students have put the steps in order to help them identify the different milk types, have them carry out their investigation for Part 1. This activity allows students to become familiar with the equipment and reagents that will be used in the following two activities.

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## Laboratory Activity Part 1: Teacher Guide

The order of the steps for Part 1 is listed below:

1. Label three glucose test strips individually as #1, #2, and #3.  
**Note: Test strips are sensitive to light. Do not take them out of the brown container until ready to use. Avoid touching the blue pad on the end of the strip.**
2. Dip glucose test strip #1 into test tube #1 and lay it down on the paper towel. Dip glucose test strip #2 into test tube #2 and lay it down on the paper towel. Dip glucose test strip #3 into test tube #3 and lay it down on the paper towel.
3. Wait 30 seconds for each test strip, and then record the color of the test strips in Table 1.
4. Record the concentration of glucose in Table 1. To do this, compare the color that develops on the test strip to the color chart on the test strip container.
5. Gently mix the Lactex solution. Then using the pipette labeled “Lactex”, add 1 ml of the solution to tubes #1, #2, and #3.
6. Cap tubes #1, #2, #3 and turn upside down to mix a few times, but do not shake vigorously. **Hold cap on tube to avoid leakage.** Wait 3 minutes.
7. Remove 3 new glucose test strips. Label as #1, #2, and #3.
8. Dip new glucose test strip #1 into test tube # 1 and place on the paper towel. Dip new glucose test strip #2 into test tube #2 and place on the paper towel. Dip new glucose test strip #3 into test tube #3 and place on the paper towel.
9. Wait 30 seconds for each test strip, and then record the color of the test strips in Table 1.
10. Record the concentration of glucose in Table 1. To do this, compare the color that develops on the test strip to the color chart on the test strip container.
11. Identify the milk type based on your results in Table 1.

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## Laboratory Activity Part 2: Teacher Guide

*Time Requirement: 60-90 minutes (can be broken into 2 - 45 minute sessions)*

### ***Part 2: Determining the Effect of pH on Lactase Activity***

The purpose of this laboratory activity is for students to test a range of pH solutions mixed with cow milk to determine if there is a change in lactase activity.

First, assess student prior knowledge on what conditions most affect enzyme activity (pH, temperature, amount of enzyme are possible ideas). Remember to try and have students explore before explain. If possible, wait to give them Medical Fact Sheet #2 until after their investigation.

Begin by discussing what neutral, acidic, and basic (or alkaline) pH values are and where they are found in the pH range (0-14). Challenge students to come up with reasons why a drug company would be interested in finding out the optimum pH level for their newly developed drug. Examples include influencing interactions with food (i.e., highly acidic food, such as orange juice) or ensuring the enzyme functions within the pH range of the human stomach (acidic) and intestines (neutral - basic).

Break students up into pairs or small groups and challenge each group to put the steps in order for Part 2. Have each group work on the steps activity, and then have one or two groups share their order with the rest of the class. Allow students to discuss which order they think is correct if there is disagreement. Let students know that scientists don't always know the proper procedure before they begin an investigation. Trial and error is a part of the scientific process, and it is okay if groups do things differently.

Once students have put the steps in order, have them carry out their investigation for Part 2.

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## Laboratory Activity Part 2: Teacher Guide

The order of the steps for Part 2 is listed below:

1. Gently mix the tube labeled “Lactex”.
2. Using the pipette labeled “Lactex”, add 1 ml of the Lactex solution to the 6 test tubes labeled: pH 2, 4, 7, 10, 12, and dH<sub>2</sub>O.
3. Cap all 6 test tubes and turn upside down to mix a few times, but do not shake vigorously. **Hold cap on tube to avoid leakage.** Wait 3 minutes.
4. Using the pipette labeled “Cow Milk”, add 2 ml of cow milk to all 6 test tubes.
5. Cap all 6 test tubes again and turn upside down to mix a few times, but do not shake vigorously. **Hold cap on tube to avoid leakage.** Wait 3 minutes.
6. Label 6 glucose test strips with the labels found on the test tubes: 2, 4, 7, 10, 12, and dH<sub>2</sub>O.
7. Take glucose test strip labeled “2” and dip it into the tube with pH solution “2” and lay it down on the paper towel.
8. Repeat the above step for the other samples (pH 4, pH 7, pH 10, pH 12, and dH<sub>2</sub>O). Keep the strips separated on the paper towels.
9. Wait 30 seconds for each test strip.
10. Record the color of the test strips in Table 2. Also, record the concentration of glucose in Table 2. To do this, compare the color that developed on the test strips to the color chart on the test strip container.

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## Laboratory Activity Part 3: Teacher Guide

*Time Requirement: 60-90 minutes (can be broken into 2 - 45 minute sessions)*

### ***Part 3: Determining the Effect of Temperature on Lactase Activity***

The purpose of this laboratory activity is for students to test the efficacy of the lactase enzyme at varying temperatures: room temperature, boiling, and freezing.

First, assess student prior knowledge on what conditions most affect enzyme activity (pH, temperature, amount of enzyme are possible ideas). Remember to try and have students explore before explain. If possible, wait to give them Medical Fact Sheet #2 until after their investigation.

Begin by challenging students to come up with reasons why a drug company would be interested in finding out how the drug works at different temperatures, such as determining proper storage conditions for a solution, ensuring the solution works on temperatures of the human body, and ensuring the enzyme would work when taken with food such as coffee or ice cream.

Break students up into pairs or small groups and challenge each group to put the steps in order for Part 3. Have each group work on the steps activity, and then have one or two groups share their order with the rest of the class. Allow students to discuss which order they think is correct if there is disagreement. Let the students know that scientists don't always know the proper procedure before they begin an investigation. Trial and error is a part of the scientific process, and it is okay if groups do things differently.

Once students have put the steps in order, have them carry out their investigation for Part 3.

# Looking Into Lactase – Structured

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## Laboratory Activity Part 3: Teacher Guide

The order of the steps for Part 3 is listed below:

1. Gently mix the tube labeled “Lactex”. Using the pipette labeled “Lactex”, add 1 ml of Lactex solution to the three test tubes labeled: HL, RTL, and CL.
2. Using the pipette labeled “Cow Milk”, add 2 ml of cow milk to the test tubes labeled: HM, RTM, CM. Cap all test tubes EXCEPT for the hot test tubes. Leave those uncapped.
3. Place the test tubes in their respective temperatures for 10 minutes:
  - hot tubes, HL and HM, into the hot water (remember, no caps)
  - cold tubes, CL and CM, into the ice
  - room temperature tubes, RTL and RTM, leave in the rack at your station

Record the hot water temperature, the ice temperature, and room temperature in degrees Celsius in Table 3.

4. Carefully pour the contents of the HM tube into the HL tube. Tubes and contents will be hot, so use appropriate laboratory safety techniques and hold tubes at the top. Carefully pour the contents of the CM tube into the CL tube. Carefully pour the contents of the RTM tube into the RTL tube.
5. Cap all the tubes and gently mix a few times. **Hold cap on tube while mixing to avoid leakage.**
6. Place each of the three full tubes back into its appropriate temperature for 5 minutes (No caps for hot tubes).
7. Label 3 glucose strips “H” for Hot, “C” for Cold, and “RT” for Room Temperature.
8. Dip glucose test strip “H” into the hot test tube and lay it down on the paper towel. Dip glucose test strip “C” into the cold test tube and lay it down on the paper towel. Dip glucose test strip “RT” into the room temperature test tube and lay it down on the paper towel.
9. Wait 30 seconds for each test strip.
10. Record the color of the test strips and the concentration of glucose in Table 3. To do this, compare the color that developed on the test strips to the color chart on the test strip container.

# Looking Into Lactase – Structured

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## Laboratory Activity Results: Teacher Guide

### *Interpretation of Results*

#### *Part 1: Identifying the Milk Type*

Sample #1 is **Rice Milk**, which contains Glucose.

- Turns the test strip a shade of brown BEFORE the addition of lactase due to the presence of glucose.
- Turns the test strip a shade of brown AFTER the addition of lactase because it still contains glucose; although, it will probably be a lighter shade of brown because students added lactase solution to the test tube, effectively diluting the contents of the test tube and thus decreasing the concentration of glucose.

Sample #2 is **Cow Milk**, which contains lactose, a disaccharide that is broken down by lactase into glucose and galactose.

- No effect on the test strip BEFORE the addition of lactase (contains only lactose, no glucose).
- Turns the test strip a shade of brown AFTER the addition of lactase because lactose is broken down into glucose and galactose.

Sample #3 is **Soy Milk**, which contains the sugar sucrose.

- No effect on the test strip BEFORE the addition of lactase (contains only sucrose, no glucose).
- No effect on the test strip AFTER the addition of lactase (contains only sucrose, no glucose).

#### *Part 2: Determining the Effect of pH on Lactase Activity*

- Lactase is effective at pH 2 – 7 (including dH<sub>2</sub>O) and therefore breaks down the lactose sugar in milk into glucose and galactose. As a result, the test strips will turn a shade of brown because glucose is present.
- Lactase is not effective at pH 10 – 12. At the high pHs the enzyme lactase is denatured and is no longer able to break down the sugar lactose in milk. Therefore, no glucose is present and the test strips remain blue.
- The optimum range for the activity of lactase would be considered pH 2-7.

#### *Part 3: Determining the Effect of Temperature on Lactase Activity*

- The lactase enzyme works best at room temperature, resulting in the glucose test strip turning a shade of brown.
- The test strip at cold temperatures may detect some glucose, but at a lesser amount than at room temperature. This happens because at low temperatures, enzymes have very little energy and the rate of the reaction slows down but does not stop entirely.
- At very hot temperatures, the lactase enzyme will not work at all. The enzyme is denatured at very hot temperatures and is inactivated. The test strip at hot temperatures will not detect glucose and so the test strip will remain blue.

# Looking Into Lactase – Structured

## Equipment Directions and Reagent Notes

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**Teachers, please read the following information. As the facilitator, it is your responsibility to demonstrate to the students how to properly use the equipment and reagents in this lab. Proper use of the equipment and reagents is necessary to obtain accurate results and to ensure student safety.**

### How to Use a Pipette Pump

Pipette pumps will be used with plastic 10 ml pipettes to measure liquids. Secure the plastic pipette into the pump by using a pushing and twisting motion. Use the wheel to draw liquid into the pipette by rolling it forward, reverse the wheel's direction to let the liquid out. Always hold the pipettes upright when they are attached to the pump or the liquid will go inside the pump and become contaminated and volume will be lost.



The 10 ml plastic pipettes have two scales on them that run in opposite directions. When measuring liquid, use the scale that has the “1 ml” at the bottom tip and “10 ml” at the top. Also, make sure to use the bottom of the meniscus (the curved part of the liquid in the pipette) to determine the volume level. When transferring liquid, make sure the container you are transferring the liquid into is nearby or liquid may start to drip out of the pipette tip. The transfer must take place quickly.

The labeled pipettes should be used only with the corresponding liquids (they can be reused with the same liquid only), otherwise the pipettes and samples risk contamination. When using the pipettes, the tip shouldn't touch the insides of tubes you are dispensing the liquid into to also prevent contamination.

### Glucose Test Strips

Glucose test strips are used to detect the presence of glucose. The pad will turn various shades of brown in the presence of glucose – the greater the concentration of glucose, the darker the shade of brown. There is a scale on the outside of the containers that indicates concentration. Test strips are sensitive to light, which is why they must be stored in the containers, and not removed from the containers until ready to use. Test strips are also sensitive to touch, so students should avoid touching the pad on the end of the strip. Test strips need only be submerged briefly to moisten the pad, they do not need to soak.

### Lactase Powder (Lactex)

The lactase powder is made up of commercially available name brand lactase enzyme pills. The solution will not completely dissolve. When aliquoting the solution, make sure to gently mix the solution occasionally to ensure an even distribution of the lactase.

# Looking Into Lactase – Structured

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## Answers to Part 1

### Part 1: Identifying the Milk Type

4. Identify the independent and dependent variables in this activity.

Independent Variable: **Unknown Milk Samples**

Dependent Variable: **Lactase Activity (measured by production of glucose)**

7. Create a data table in the space provided below, so you can record the data you collect.

**Title of Table:** **Sample of table shown below**

**Table 1: Glucose Results Before and After Adding Lactase Enzyme**

Sample Number	Color of test strip BEFORE adding Lactex	Concentration of glucose (mg/dl) BEFORE adding Lactex	Color of test strip AFTER adding Lactex	Concentration of glucose (mg/dl) AFTER adding Lactex	Milk Type
1					
2					
3					

# Looking Into Lactase – Structured

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## Answers to Part 1

### *Answers to Analysis Questions – Part 1*

9. What is the purpose of the glucose test strips? What does the glucose concentration tell you?

*The strips serve as an indicator for the presence of glucose, which is one of the products of lactase activity. The glucose concentration tells you how much glucose was produced when the Lactex (Lactase Enzyme Solution) was used.*

10. What effect does Lactex (Lactase Enzyme Solution) have on the cow milk?

*The Lactex contains the lactase enzyme, which catalyzed the hydrolysis of the disaccharide, lactose, producing the two monosaccharides, glucose and galactose.*

11. Which sample is the cow milk? Provide your evidence and reasoning.

*Claim – Sample # 2 contains cow milk.*

*Evidence – Before adding Lactex, the glucose test strip stayed blue. After adding Lactex, the glucose test strip turned brown.*

*Reasoning – The glucose test strip only detected the presence of glucose after adding Lactex to the milk sample because cow milk contains the disaccharide lactose, which was broken down into the monosaccharides glucose and galactose by the enzyme lactase.*

12. Which sample is the rice milk? Provide your evidence and reasoning.

*Claim – Sample #1 contains rice milk.*

*Evidence – Before and after adding Lactex, the glucose test strip turned brown.*

*Reasoning – The glucose test strip detected the presence of glucose in the original milk sample and in the milk sample after adding Lactex. Glucose was still detected in the milk sample after adding the lactase enzyme because rice milk only contains the monosaccharide glucose with or without the presence of the lactase enzyme.*

13. Which sample is the soy milk? Provide your evidence and reasoning.

*Claim – Sample # 3 contains soy milk.*

*Evidence – Before and after adding Lactex, the glucose test strip stayed blue.*

*Reasoning – The glucose test strip did not detect the presence of glucose before or after adding Lactex to the milk sample because soy milk contains the disaccharide sucrose, which can only be broken down into the monosaccharides glucose and fructose by the enzyme sucrase. Sucrase was not added to the milk samples, so the sugar in the soy milk could not be broken down.*

# Looking Into Lactase – Structured

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## Answers to Part 2

### *Answers to Questions - Part 2*

1. Where does the lactase enzyme need to work in the human body? (Hint: review Medical Fact Sheet #1)

*The lactase enzyme works in the small intestine.*

2. How might the conditions at the above location differ or be the same from where you tested the lactase enzyme?

*The small intestine is likely to have a different temperature and pH from the lab where this enzyme was tested. (Answers may vary.)*

3. What factor(s) would a pharmaceutical company likely test to simulate the environment where the lactase enzyme will be used?

*The pharmaceutical company might want to test conditions that are similar to the human body, like pH and temperature, since Lactex will be consumed by humans.*

### **Part 2: Determining the Effect of pH on Lactase Activity**

4. Identify the independent and dependent variables in this activity.

Independent Variable: **pH**

Dependent Variable: **Lactase Activity (measured by production of glucose)**

7. Create a data table in the space provided below, so you can record the data you collect.

**Title of Table:** *Sample of table shown below*

**Table 2: pH and Glucose Results**

pH	Color of strip after adding Lactex	Concentration of glucose (mg/dl) after adding Lactex
2		
4		
7		
10		
12		
dH <sub>2</sub> O (control)		

# Looking Into Lactase – Structured

## Answers to Part 2

### 12. Claim, Evidence, Reasoning

#### What is the optimum pH range for Lactex?

<b>Claim</b> (a statement that answers the question)  Lactex is effective at pH 2-7.  The optimum pH range for Lactex is pH 2-7.  (Answers may vary depending on the data from each investigation.)	
<b>Evidence</b> (the scientific data you collected that supports your claim)  Evidence should be in the form of a graph or data can be listed in the form of a table.	<b>Reasoning</b> (your explanation for how the evidence supports your claim)  Examples of appropriate reasoning include: <ul style="list-style-type: none"><li>• The lactase enzyme in the Lactex breaks down the lactose sugar in cow milk into glucose and galactose when the pH ranges from 2-7. This is shown when the glucose test strips turned brown indicating the presence of glucose at these pH levels.</li><li>• The lactase enzyme in Lactex is not effective at pH 10-12 because at high pHs the lactase enzyme is denatured and cannot break down the sugar lactose in the cow milk. This is shown by the absence of a brown color on the glucose test strip at pHs 10 and 12 indicating no glucose was present.</li></ul> Examples of inappropriate reasoning include: <ul style="list-style-type: none"><li>• No reasoning given.</li><li>• Students just repeat the results from their investigation.</li></ul>

# Looking Into Lactase – Structured

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## Answers to Part 3

### Part 3: Determining the Effect of Temperature on Lactase Activity

1. Identify the independent and dependent variables in this activity.

Independent Variable: **Temperature**

Dependent Variable: **Lactase Activity (measured by production of glucose)**

4. Create a data table in the space provided below, so you can record the data you collect.

**Title of Table:** **Sample of table shown below**

**Table 3: Temperature and Glucose Results**

Temperature Condition	Actual Temperature (°C)	Color of strip	Concentration of glucose (mg/dl) after adding Lactex
Ice			
Room temperature			
Hot water			

# Looking Into Lactase – Structured

## Answers to Part 3

### 6. Claim, Evidence, Reasoning

#### At what temperature does Lactex work best?

<b>Claim</b> (a statement that answers the question)  Lactex works best at room temperature.	
<b>Evidence</b> (the scientific data you collected that supports your claim)  Evidence should be in the form of a graph or data can be listed in the form of a table.	<b>Reasoning</b> (your explanation for how the evidence supports your claim)  Examples of appropriate reasoning include: <ul style="list-style-type: none"><li>• The lactase enzyme in the Lactex breaks down the lactose sugar in cow milk into glucose and galactose when the enzyme is at room temperature. This is shown when the glucose test strip turned brown indicating the presence of glucose.</li><li>• The lactase enzyme is not effective at very hot temperatures because the lactase enzyme is denatured and becomes inactivated, which is shown when the glucose test strip remained blue indicating no glucose was present.</li></ul> Examples of inappropriate reasoning include: <ul style="list-style-type: none"><li>• No reasoning given.</li><li>• Students just repeat the results from their investigation.</li></ul> Common misconceptions: <ul style="list-style-type: none"><li>• Heating kills enzymes. <i>No, heating denatures enzymes. Enzymes are not living organisms, so they cannot be killed.</i></li><li>• Enzymes are denatured at lower temperatures. <i>At low temperatures, enzymes have very little energy so the rate of the reaction slows down, but does not stop entirely. Enzymes are not denatured (unraveled) at cold temperatures.</i></li><li>• Enzymes are depleted in the course of a reaction. <i>Enzyme molecules will not be used up in a reaction. The decrease in enzymatic rate is due to the gradual decrease in the substrate concentration or denaturation of the enzyme at high temperatures.</i></li></ul>

# Looking Into Lactase – Structured

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## Answers to Brief Constructed Response Questions

### *Answers to Assessment Questions*

1. b
2. c
3. d
4. a
5. d

### *Answers to Brief Constructed Response Questions*

6. How does your data compare to that of others in your class? Explain.

*Answers will vary but should discuss why experimental data may vary when similar questions were asked.*

7.

- A. Which enzyme works better in an alkaline environment? Provide your evidence and reasoning?

*Enzyme B works better in an alkaline environment because its peak reaction rate was at pH 8, which is alkaline (basic). Enzyme A did not work at all in an alkaline environment (it stopped working at pH 6, which is considered an acidic environment).*

- B. At which pH do the two enzymes work equally well? Provide your evidence and reasoning?

*The two enzymes worked equally well at pH 5. At pH 5, both enzymes have the same reaction rate of 1 mg/sec.*

- C. What is the optimum pH for Enzyme A? Provide your evidence and reasoning?

*The optimum pH for Enzyme A is pH 3 because that is where the reaction rate is the greatest (3 mg/sec).*

- D. Describe the overall reaction rate pattern shown by both enzymes.

*Both enzymes show a bell-shaped curve with a low reaction rate at the lower and upper levels of their ranges and an optimum rate approximately midway between each of their two extremes.*

# Looking Into Lactase – Structured

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## Answers to Extension Activities

1. Similarities:

- All three groups show relatively high lactose tolerance at the earliest ages for which data is available.
- All three groups show a decrease in tolerance with age.  
(Note: Mestizo refers to offspring of European and S. American Indian parents.)

Differences:

- Peruvian mestizo children show the most rapid decrease in tolerance, being less than 20% tolerant by age 6.
  - U.S. Caucasian children maintain an 80+% tolerance to age 18 while African Americans decrease steadily to 30% at age 18.
  - Although U.S. Caucasian children show a decrease after the age of 4, they actually show a slight increase after the age of 16.
2. The student graphs should reflect the trends described in #1 above and the paragraph should include the information given for #1 above.
3. The letter should be written in standard business letter format. The letter should address the fact that the majority of students in the Baltimore City Public School system are African Americans and thus a high percentage (maybe up to 70%) is likely to be lactose intolerant. The letter should propose a solution such as offering calcium fortified orange juice as an alternative to the milk or providing lactase supplemented milk.
4. A. Since humans cannot digest cellulose, this carbohydrate passes into the large intestine where resident bacteria break down the cellulose and release gas.
- B. The bacteria and protozoa that live in the stomachs of herbivorous mammals, such as cows and sheep, are able to digest the cellulose of the grass. They can then absorb the glucose end product to use for energy. The bacteria and protozoa, in turn, get an appropriate supply of food and a place to live safe from predators. If the mammals did

# Looking Into Lactase – Structured

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## Answers to Extension Activities

not have these symbiotic organisms living in their stomachs, they would be more similar to humans and not be able to utilize the cellulose in the plant cell walls.

5. Students should discuss the usefulness of the lactase enzyme by breast-fed infants and how this would contribute to the survival rate of these children. As infants are weaned, the enzyme's activity seems to decrease universally. The exceptions are those areas of the world where mammals were domesticated for providing milk as a source of nourishment for all segments of the population. The discussion should include the evolutionary advantage of maintaining functioning lactase enzymes in humans in these regions and the possibility that having this enzyme was a selective advantage that would be passed on genetically from one generation to the next.

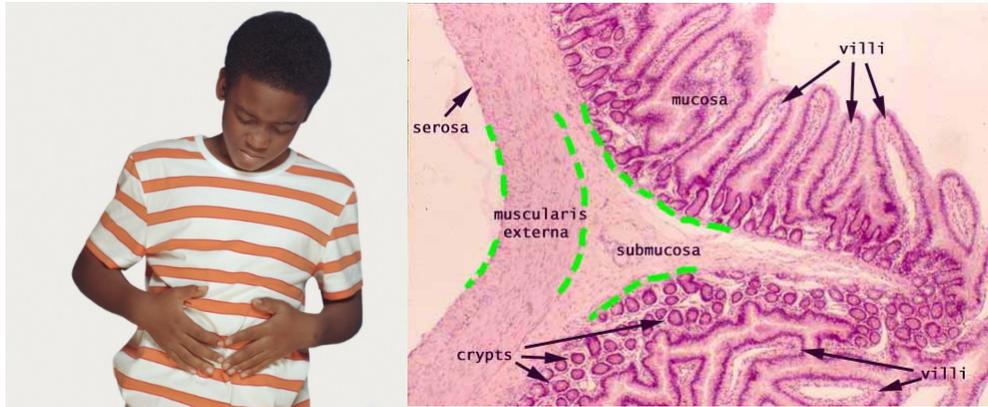
### Video Links:

1. Howard Hughes Medical Institute BioInteractive Short Film  
The Making of the Fittest: Got Lactase? The Co-evolution of Genes and Culture (15 minutes)  
<http://www.hhmi.org/biointeractive/making-fittest-got-lactase-co-evolution-genes-and-culture>
2. Evolution of life  
Good Milk/Bad Milk (7 minutes)  
<http://www.evolution-of-life.com/en/observe/video/fiche/good-milk-bad-milk.html>

# Looking Into Lactase – Structured

## Student Worksheets

### Medical Case Study



Duodenum (the first section of the small intestine) contains finger-like projections called villi that aid in nutrient absorption.

### Case Report

A 16-year-old student presented to his doctor with a 3-day history of vomiting and severe diarrhea. Based on a stool culture, a bacterial infection was diagnosed and the student was treated with an antibiotic. Eating yogurt or drinking milk seemed to worsen his diarrhea and the doctor told him to avoid milk until he felt better. The diarrhea started improving 3-4 days later, and once he finished taking the medication, his stools had normalized and his appetite returned.

On his return to school, he had lunch (tuna mayonnaise sandwich, yogurt, and a cup of coffee with milk) at the cafeteria. That afternoon, he experienced abdominal cramping but thought it was because he was hungry. To help this, he ate some chocolate and drank some flavored milk. Two hours later, the cramps worsened and he developed diarrhea. He went back to the doctor, concerned that he had another ‘stomach bug’.

What do you think is happening?

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# Looking Into Lactase – Structured

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## Student Worksheets

The doctor came up with the following options:

- Another bacterial infection
- Food poisoning
- An allergic reaction to something he ate
- An intolerance of some kind.

Stool cultures were negative. He had no history of previous food or environmental allergies and after discussing his intake that day, the doctor thought it could be possible lactose intolerance. He was told to eliminate all milk and dairy products for 7 days.

After severe diarrhea, the intestinal mucosa and the villi are damaged. Lactase is released by the villi. The damaged villi and mucosa need to be repaired before lactase will return to the small intestine. The doctor thinks the student's villi had not had enough time to repair and not enough lactase had been produced since his infection for him to tolerate the milk related food.

The student followed instructions, and the diarrhea and cramps resolved. He managed to reintroduce dairy foods and was able to follow his usual routine again.

Two weeks later he revisited the doctor, again with diarrhea, severe cramping, and bloating. The student's diet and lifestyle were evaluated. He had started drinking a flavored milk drink almost daily. A primary lactase deficiency was suspected. However, the student asked why he had previously been able to tolerate milk in his coffee, as well as chocolate and yogurt on various occasions.

Being of African descent, he has a high likelihood of having underlying primary lactose intolerance and could handle some dairy products. The diarrhea had damaged the villi and therefore affected lactase production, causing his tolerance to lower. Once the lactase had been replenished, he could once again tolerate small amounts of lactose, but when he increased the intake of lactose by drinking milk frequently, symptoms developed again.

What suggestions do you have to help the patient manage his lactose intolerance?

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### Email #1 to Students

## *Pharmex Pharmaceuticals*

**To:** All Research Team Members  
**From:** Principal Investigator  
**Re:** About Enzymes

Research Team Members,

I commend all research team members for your hard work to date. I thought it might help to remind you why it is so important for us to work diligently for the successful development of our product, Lactex (Lactase Enzyme). I have enclosed a handout that I picked up at a doctor's office, which includes **Medical Fact Sheet #1**. Please read and discuss this sheet with your team members.

*After reading the handout...*

1. What are enzymes?
2. Why are they important in the human body?
3. What does the lactase enzyme have to do with lactose intolerance?

# Looking Into Lactase – Structured

## Student Worksheets

### Medical Fact Sheet #1

**What are Enzymes?** Enzymes are proteins that act as organic catalysts (compounds capable of initiating or speeding up a chemical reaction). They control the rate of chemical reactions in cells without being permanently altered themselves.

In general, enzymes speed up the rate of reactions by lowering the activation energy required to start reactions (see Figure 1).

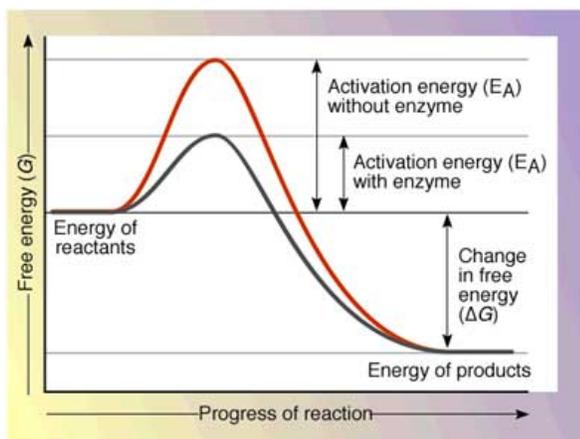


Figure 1: Graph depicting the activation energy required to start a reaction with an enzyme versus without one. (From: <http://163.16.28.248/bio/activelearner/06/images/ch06c1.jpg>)

Enzymes are extremely efficient. They can catalyze reactions so that their rates are up to 10 billion times faster than comparable non-catalyzed reactions. Because of their specific structure, enzymes hold the reactant molecules close to one another and in the correct position for the reaction to occur. Each enzyme has a specific site (the active site) where the substrate and enzyme combine. The analogy of a “lock and key” is often used to illustrate the joining of an enzyme to a substrate (see Figure 2).

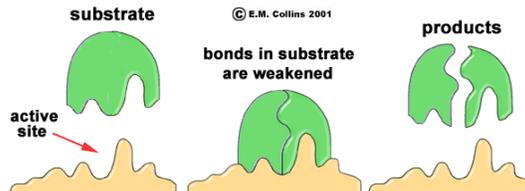


Figure 2: Diagram depicting how an enzyme combines with a substrate to form a product or products. (From: [waynesword.palomar.edu](http://waynesword.palomar.edu))

Each specific enzyme acts on a particular substrate or on a certain kind of chemical bond. The specificity of enzymes is due to their structure. Each enzyme has a characteristic three-dimensional (3-D) shape.

**What affects enzyme function?** Many environmental conditions can influence how an enzyme functions, including temperature and pH of the enzyme. In addition, the concentration of enzyme present can alter the rate of the reaction.



### s Lactose Intolerance?

Lactose intolerance is a medical condition caused by little to no activity of the enzyme, lactase. This prevents the sugar, lactose, from being broken down into glucose and galactose in the small intestine, resulting in undigested lactose being passed into the colon where bacteria ferment it into hydrogen gas and organic acids.

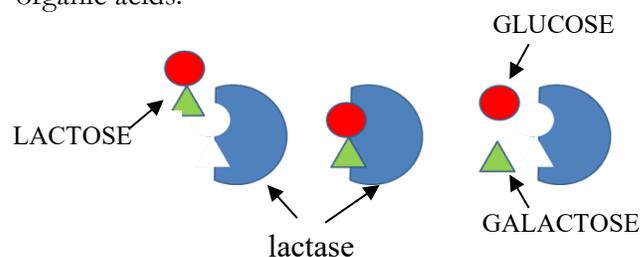


Figure 3: Without the lactase enzyme, the human body cannot break down lactose into glucose and galactose.

### Email #2 to Students

#### *Pharmex Pharmaceuticals*

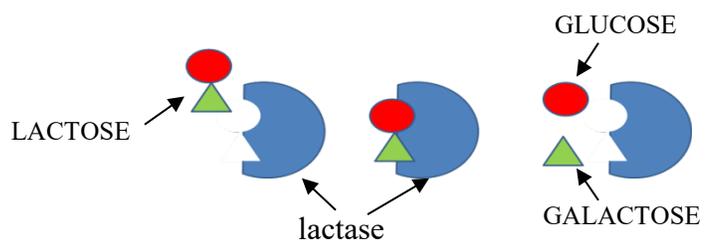
**To:** All Research Team Members  
**From:** Principal Investigator  
**Re:** Testing the Lactase Enzyme

Research Team Members:

Now that you have an understanding of enzymes, I need you to verify the activity of lactase found in the newly developed drug called Lactex. The drug must be tested on different types of milk that contain different sugars to verify that it works on lactose and only lactose. If the enzyme has additional activities that affect other sugars, it may not be approved for use by the FDA. Three milk samples will be tested: cow, soy, and rice. This is a blind test to avoid bias, so the samples are labeled #1, #2, and #3. It is your job to identify which milk sample is which and to determine that Lactex breaks down only lactose.

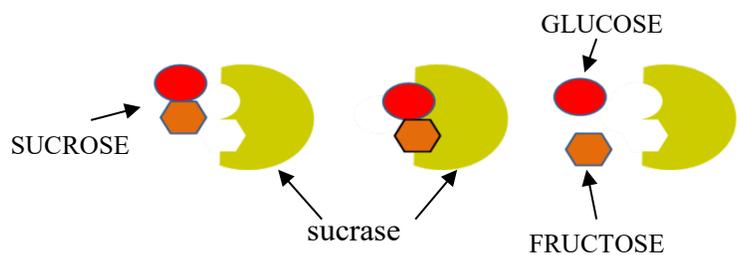
Cow milk contains the sugar LACTOSE.

- The disaccharide LACTOSE is broken down into the monosaccharides glucose and galactose by the enzyme LACTASE.



Soy milk contains the sugar SUCROSE.

- The disaccharide SUCROSE is broken down into the monosaccharides glucose and fructose by the enzyme SUCRASE.



Rice milk contains the sugar GLUCOSE.

- GLUCOSE is a monosaccharide.



# Looking Into Lactase – Structured

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## Student Worksheets

### Part 1: Identifying the Milk Type

**You have been given the following materials with which to determine the identity of each milk sample:**

- 3 unknown 2 ml milk samples in test tubes labeled #1, #2, and #3
- Glucose test strips (in container – see side for usage instructions)
- Plastic pipette – Lactex
- Pipette pump
- Blue-capped tube filled with Lactex (Lactase Enzyme Solution)

(**Note:** If you plan on adding any Lactex to your milk samples, please use 1 ml of Lactex.)

#### **Procedure:**

4. Identify the independent and dependent variables.

Independent Variable: \_\_\_\_\_

Dependent Variable: \_\_\_\_\_

5. Locate the set of strips of paper in an envelope labeled Part 1. Each strip has a step of the procedure written on it. Read through each strip of paper and work with your lab partner(s) to determine the order of the steps.
6. Make a prediction for the outcome of your investigation.

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# Looking Into Lactase – Structured

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## Student Worksheets

7. Create a data table in the space provided below, so you can record the data you collect.

**Title of Table:** \_\_\_\_\_

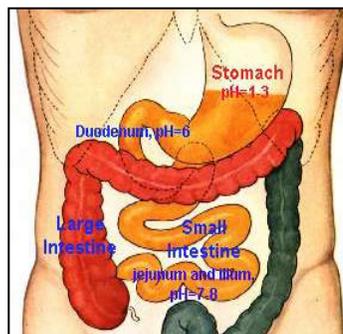
8. Once you have created your data table, proceed with the investigation!



# Looking Into Lactase – Structured

## Student Worksheets

### Part 2: Determining the Effect of pH on Lactase Activity



### *Pharmex Pharmaceuticals*

**To:** All Research Team Members

**From:** Principal Investigator

**Re:** Determine the Effect of pH on Lactase Activity

Research Team Members,

Once a drug has been determined effective at laboratory conditions, drug companies think about the conditions “in vivo”, or within the living organism. We want to make sure our drug works in the conditions it will experience in the human body. Consider the questions below and then determine the effect of pH on Lactex (Lactase Enzyme Solution).

1. Where does the lactase enzyme need to work in the human body? (Hint: review Medical Fact Sheet #1)

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2. How might the conditions at the above location differ or be the same from where you tested the lactase enzyme?

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3. What factor(s) would a pharmaceutical company test to simulate the environment where the lactase enzyme will be used?

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# Looking Into Lactase – Structured

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## Student Worksheets

### Part 2: Determining the Effect of pH on Lactase Activity

You have been given the following materials with which to determine the effect of pH on Lactex:

- Test tubes with solutions of pH 2, 4, 7, 10, 12, and dH<sub>2</sub>O (distilled water)
- Glucose test strips (in container – see side for usage instructions)
- Plastic pipette – Lactex
- Plastic pipette – Cow milk
- Pipette pump
- Blue-capped tube filled with Cow Milk
- Blue-capped tube filled with Lactex (Lactase Enzyme Solution)

#### Procedure:

4. Identify the independent and dependent variables.

Independent Variable: \_\_\_\_\_

Dependent Variable: \_\_\_\_\_

5. Locate the set of strips of paper in an envelope labeled Part 2. Each strip has a step of the procedure written on it. Read through each strip of paper and work with your lab partner(s) to determine the order of the steps.
6. Make a prediction for the outcome of your investigation.

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# Looking Into Lactase – Structured

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## Student Worksheets

7. Create your own data table in the space provided below so you can record the data you collect.

**Title of Table:** \_\_\_\_\_

# Looking Into Lactase – Structured

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## Student Worksheets

8. Make a graph to help represent your data.



# Looking Into Lactase – Structured

## Student Worksheets

### 9. Claim, Evidence, Reasoning

**What is the optimum pH range for Lactex?**

**Claim** (a statement that answers the question)

**Evidence** (the scientific data you collected that supports your claim)

**Reasoning** (your explanation for how the evidence supports your claim)

# Looking Into Lactase – Structured

## Student Worksheets

### Part 3: Determining the Effect of Temperature on Lactase Activity

#### *Pharmex Pharmaceuticals*

**To:** All Research Team Members

**From:** Principal Investigator

**Re:** Determine the Effect of pH on Lactase Activity

Research Team Members,

As research scientists at Pharmex, we are certain the Lactex solution breaks down lactose and have determined at which pH range the solution works best. You have been asked to determine under what temperature conditions the Lactex solution will work. The solution may sometimes be ingested with cold foods (such as ice cream) and then go into the stomach (a generally warm place). It must be determined whether or not the Lactex solution can remain active in a variety of temperatures. Using cow milk, your research team will test the activity of the Lactex solution at different temperatures. The temperatures being tested in the lab are: freezing cold (ice), very hot (boiling water), and room temperature.

**You have been given the following materials with which to determine the effect of temperature on Lactex:**

- Glucose test strips (in container – see side for usage instructions)
- Plastic pipette – Lactex
- Plastic pipette – Cow milk
- Pipette pump
- Blue-capped tube filled with Cow Milk
- Blue-capped tube filled with Lactex (Lactase Enzyme Solution)

#### **Procedure:**

1. Identify the independent and dependent variables.

Independent Variable: \_\_\_\_\_

Dependent Variable: \_\_\_\_\_

# Looking Into Lactase – Structured

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## Student Worksheets

2. Locate the set of strips of paper in an envelope labeled Part 3. Each strip has a step of the procedure written on it. Read through each strip of paper and work with your lab partner(s) to determine the order of the steps.
3. Make a prediction for the outcome of your investigation.

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4. Create your own data table in the space provided below so you can record the data you collect.

**Title of Table:** \_\_\_\_\_

# Looking Into Lactase – Structured

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## Student Worksheets

5. Make a graph to help represent your data.



# Looking Into Lactase – Structured

## Student Worksheets

### 6. Claim, Evidence, Reasoning

**At what temperature does Lactex work best?**

**Claim** (a statement that answers the question)

**Evidence** (the scientific data you collected that supports your claim)

**Reasoning** (your explanation for how the evidence supports your claim)

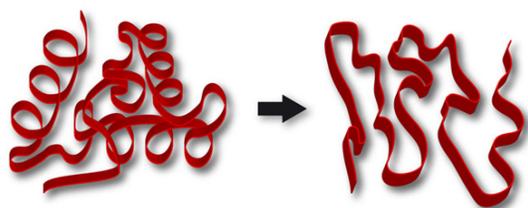
# Looking Into Lactase – Structured

## Student Worksheets

### Medical Fact Sheet #2

#### What effect does temperature change have on enzymes?

An enzyme is a protein, and when an enzyme is subjected to high temperatures, the enzyme will cease to function. This is due to the breaking of weak bonds in the enzyme's structure, especially in the active site, causing the protein to unravel so it can no longer function. This is called denaturation.



Active (functional) protein

Denatured protein

Figure 4: An enzyme can be denatured (unraveled) by exposure to high temperatures or extreme pH change. (<http://www.rsc.org/learn-chemistry/resources/chemistry-in-your-cupboard/vanish/8>)

At lower temperatures, the enzyme structure stays intact, but there may not be enough energy for a reaction to occur. In this case, the enzyme is inactivated not denatured.

#### What effect does change in pH have on enzymes?

Extremely high or low pH values will cause most enzymes to no longer function. Because enzymes normally work within a very narrow pH range, a sudden drop or increase in pH will cause enzyme denaturation. A minor change in the pH will not cause an enzyme to denature; the bonds will be disrupted but they can still reform. Extreme pH changes may lead to denaturation of the enzyme.

#### What if the concentration of the enzyme increases?

As the enzyme concentration increases, more active sites become available to form enzyme-substrate complexes with substrates. As more and more of them form, the reaction rate increases. By adding more and more enzymes, the reaction will reach a point where all the substrate molecules are occupying enzymes' active sites. This will result in excess enzymes.

This is graphically represented in Figure 5.

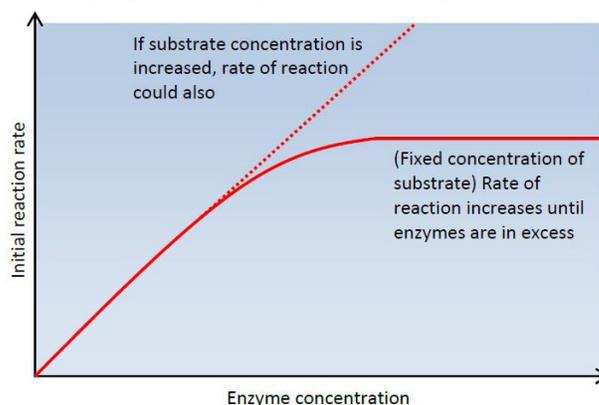


Figure 5: Increasing enzyme concentration (solid line) will increase the rate of the reaction as more enzymes are colliding with substrate molecules. The reaction rate will level off when there are free enzymes around with no substrate to bind to. (<http://asbiology101.files.wordpress.com/2009/09/3-9-factors-affecting-enzymes1.pdf>)

#### What is the lifespan of an enzyme?

Each enzyme has a finite lifespan. Some work for twenty minutes or less, while others can live for many weeks.

# Looking Into Lactase – Structured

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## Student Worksheets

### Assessment Questions

1. The enzyme \_\_\_\_\_ can break down \_\_\_\_\_ into \_\_\_\_\_ and galactose.
  - a. lactose, glucose, lactase
  - b. lactase, lactose, glucose
  - c. glucase, lactase, lactose
  - d. lactase, glucose, lactose
2. Enzymes are \_\_\_\_\_ molecules that \_\_\_\_\_ specific chemical reactions.
  - a. carbohydrate, inhibit
  - b. lipid, speed-up
  - c. protein, speed-up
  - d. nucleic acid, inhibit
3. The ability of an enzyme to function is influenced by the
  - a. indicator
  - b. temperature
  - c. enzyme shape
  - d. both b and c
4. The presence of glucose can be shown by
  - a. a glucose test strip turning from blue to brown
  - b. a lactose test strip turning from brown to blue
  - c. a sample of milk turning from blue to yellow
  - d. a sample of milk turning from blue to red
5. Which of the following groups of people retain into adulthood, with the largest percent, the natural ability to digest lactose?
  - a. Asians
  - b. Africans
  - c. Latinos
  - d. Northern Europeans

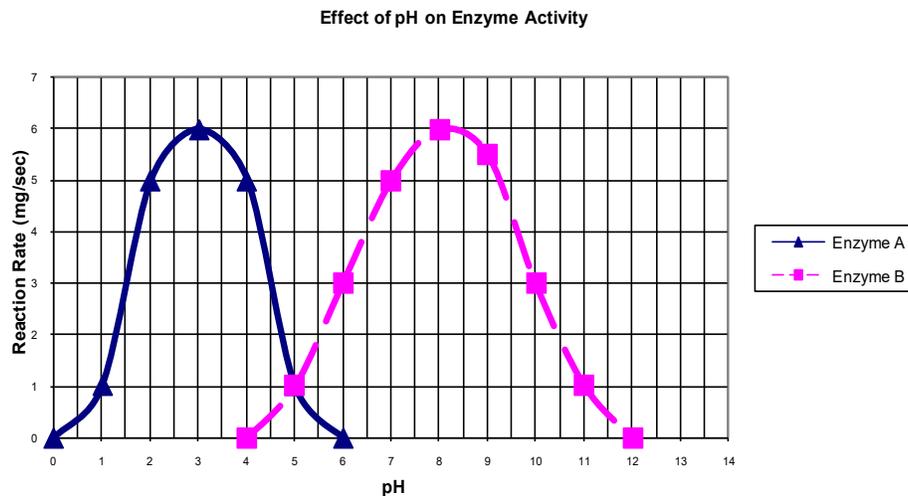
### Brief Constructed Response Questions

6. How does your data compare to that of others in your class? Explain.

# Looking Into Lactase – Structured

## Student Worksheets

7. Look at the following graph showing the reaction rates of two enzymes.



A. Which enzyme works better in an alkaline environment? Provide your evidence and reasoning?

B. At which pH do the two enzymes work equally well? Provide your evidence and reasoning?

C. What is the optimum pH for Enzyme A? Provide your evidence and reasoning?

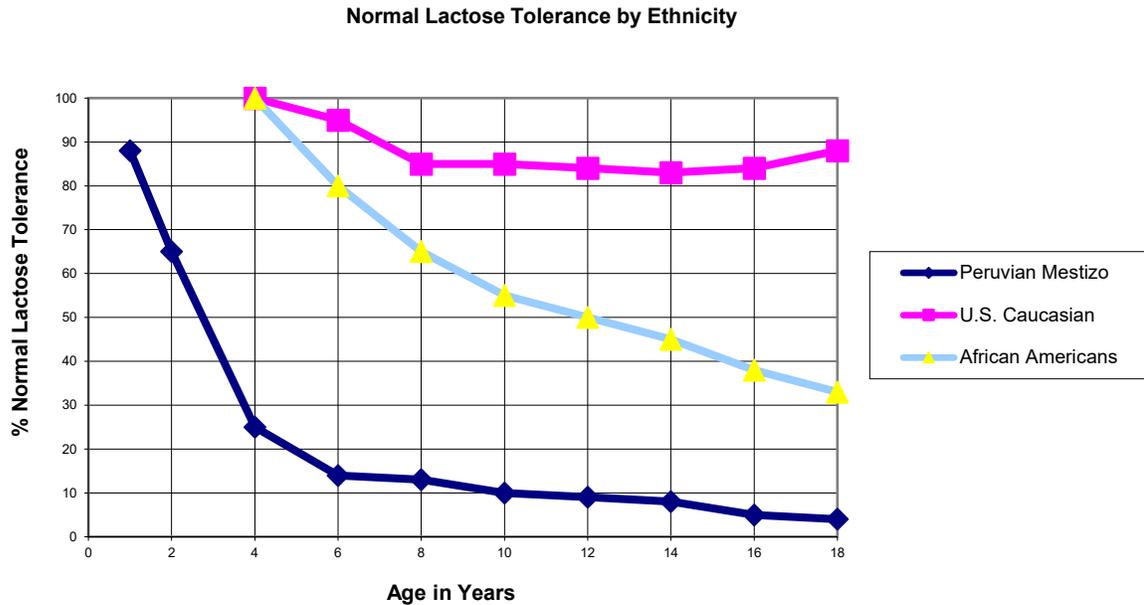
D. Describe the overall reaction rate pattern shown by both enzymes.

# Looking Into Lactase – Structured

## Student Worksheets

### Suggested Extension Activities

1. Using the graph, write a brief paragraph comparing the similarities and differences in the three populations in terms of normal lactose tolerance.



2. Using the data in the chart, construct a graph. Be sure to follow customary rules for graphing, including an appropriate title and labeled axes. Put the independent variable on the x axis and the dependent variable on the y axis. Then, write a paragraph comparing the similarities and differences in the three populations in terms of normal lactose tolerance.

Normal Lactose Tolerance in U.S. Caucasian and African American Children and Peruvian Mestizo Children (in %)

Age in Years	Caucasian	African American	Peruvian
2	No data	No data	65
4	100	100	25
6	95	80	14
8	85	65	13
10	85	55	10
12	84	50	9
14	83	45	8
16	84	38	5
18	88	33	4

# Looking Into Lactase – Structured

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## Student Worksheets

3. Propose a solution to the problem of school-age children in Baltimore City needing calcium and not making use of the milk provided with school lunches (due to their symptoms of lactose intolerance). Write this up in the form of a letter to the principal.
  
4. While on a field trip with your biology class to visit a local farm, you accidentally become separated from your classmates and miss lunch. After a while, you get so hungry that you pull up some grass and eat it. Shortly afterward, you were reunited with your classmates. On the bus ride home a few hours later, you developed terrible cramps and gas and nobody wanted to sit near you. You don't understand what happened. You see the cows, sheep, and goats eating grass, and they are mammals just like you. You asked your teacher about it, and she said that many herbivorous animals have compartmentalized stomachs that contain certain bacteria and protozoa. She also mentioned that humans don't have the ability to digest cellulose, a carbohydrate found in the cell walls of plants.
  - A. In terms of enzymes, explain why eating the grass caused you to have digestive problems.
  - B. Explain the role of bacteria and the protozoa in the stomachs of herbivorous mammals such as cows, sheep, and goats. What do you think would happen to these animals if they did not have this symbiotic relationship with the bacteria and the protozoa?
  
5. Discuss in terms of evolution why the ability to produce lactase seems to be universal among humans for the first two years of life and diminishes for many groups of people after that.
  
6. Have students write up a package insert for their new Lactex (Lactase Enzyme) product that would give the directions on how to take the solutions. This would include information regarding the types of foods you can ingest with the solutions (have the students consider acidic types of foods, basic or alkaline things such as antacid medicines, and also consider the temperatures of the food products to be taken with the solutions).