

Looking Into Lactase

A Medical Biotechnology Enzyme Lab

Maryland Loaner Lab Teacher Packet



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Development Program.*

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Loaner Lab Overview

The Looking Into Lactase lab has two parts:

- A Pre-Laboratory activity that allows students to explore the condition of lactose intolerance and becoming familiar with the symptoms associated with it.
- A laboratory activity that allows students to work with an enzyme and to investigate the changes in physical conditions that may alter its activity. During this activity, students make a connection between laboratory science and an application for medical biotechnology.

Equipment and Supplies

List of equipment and reagents supplied by MDLL included in clear plastic sheet protector in binder.

Supplied By Maryland Loaner Lab Program:

Description	Quantity	Must Be Returned?
Teacher packet with student worksheets	1	Return
Career DVD	1	Return
Lactex Powder	1	Return empty and unused bags
Plastic bottle (empty) labeled "Use to mix 1 bag Lactex Powder with 175 ml dH ₂ O"	1	Clean, dry, and return
Conical Tubes Labeled "Lactex"	10	Clean, dry, and return
Rice and Soy milk	1 box each	Discard
Part 1 Test Tubes (#1, #2, #3) with caps	30	Discard
Part 2 Test Tubes (pH tubes) with caps	60	Discard
Part 3 Test Tubes (temperature) with caps	60	Discard
(6) pH solutions (2, 4, 7, 10, 12, dH ₂ O)	10mL; each in marked 15 ml conical tube	Clean, dry, and return
Glucose Test Strips (10 brown containers)*	~150	Return all containers and unused test strips
Cow Milk Tubes	10	Clean, dry, and return
Teacher Aliquoting Pipettes (Labeled "Cow milk", "Rice milk", "Soy milk", pH 2, 4, 7, 10, 12, dH ₂ O, and "Lactex")	10	Clean, dry, and return

Loaner Lab Overview

Student Plastic Pipettes (Labeled “Cow milk” & “Lactex”)	20	Clean, dry, and return
Pipette Pumps	10	Return
Plastic Tube Floater	1	Return
Thermometer	1	Return
Test Tube Brush (for cleaning)	1	Return
White Test Tube Racks	10	Clean and return
Refill Glucose Test Strips (~500 test strips per bag)	1	Return bag and unused test strips

***Note: The glucose test strips are sensitive to light and touch. In order to insure best results, avoid exposing strips to light until ready to use, and avoid touching the testing end (blue end) of the test strip.**

IMPORTANT: The teacher must provide enough cow milk for the entire lab (Approximately 220 ml per class).

Supplied by the Teacher:

Description	Quantity/Class	Comments
Cow milk	220 ml. per class	
dH ₂ O	175mL	For making Lactex Solution
Paper towels	10	One for each station
Goggles	1 per student	Must be used in Part II and Part III
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 III
Water bath or hot plate	1	Used in Part III
1000 ml glass beaker	1	Used in Part III

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. Remember to return all original packaging, including labeled zip-lock bags. Re-pack all items in the same manner as received. Follow the FedEx procedure included in your paperwork to ship the kit.

Maryland Science Core Learning Goals

Goal 1.0 Skills and Processes

1.4.1 *The student will organize data appropriately using techniques such as tables, graphs, and webs. (for graphs: axes labeled with appropriate quantities, appropriate units on axes, axes labeled with appropriate intervals, independent and dependent variables on correct axes, appropriate title.)**

1.1.5 The student will explain factors that produced biased data (incomplete data, using data inappropriately, conflicts of interest, etc.)

1.2.1 The student will identify meaningful, answerable scientific questions.

1.2.6 The student will identify appropriate methods for conducting an investigation (independent and dependant variables, proper controls, repeat trials, appropriate sample size, etc.).

1.2.7 The student will use relationships discovered in the lab to explain phenomena observed outside the laboratory.

1.3.1^{NTB} The student will develop and demonstrate skills in using lab and field equipment to perform investigative techniques.

1.3.2 The student will recognize safe laboratory procedures.

1.3.3^{NTB} The student will demonstrate safe handling of the chemicals and materials of science.

1.3.4^{NTB} The student will learn the use of new instruments and equipment by following instructions in a manual or from oral direction.

1.4.2 The student will analyze data to make predictions, decisions, or draw conclusions.

1.4.6 The student will describe trends revealed by data.

1.5.1 The student will demonstrate the ability to summarize data (measurements/observations).

1.7.5 Students will investigate career possibilities in the various areas of science.

Goal 3.0 Concepts of Biology

3.1.2 *Discuss factors involved in the regulation of chemical activity as part of a homeostatic mechanism. (enzyme regulation) **

3.1.1 The students will be able to describe the unique characteristics of chemical substances and macromolecules utilized by living systems. (specifically enzymes)

* Italicized CLG's are the primary focus of the laboratory activity. The other indicators are addressed, but can also be used as a guide to determine the focus of the lesson plans for the pre-lab and post-lab extension activities.

Middle School Voluntary State Curriculum Indicators

1.A.1 Design, analyze, or carry out simple investigations and formulate appropriate conclusion based on data obtained or provided.
1.A.1.h Use mathematics to interpret and communicate data.
1.B.1 Review data from a simple experiment, summarize the data, and construct a logical argument about the cause and effect relationship in the experiment.
1.B.1.d Describe the reasoning that lead to the interpretation of data and conclusions drawn.
3.B.2 Recognize and provide examples that human beings, like other organisms have complex body systems of cells, tissues and organs that interact to support an organism's growth and survival.
3.C.1.b Interpret tables and graph produced by others and describe in words the relationships they show.
3.D.1 Recognize and describe that evolutionary change in species over time occurs as a result of natural variations in organisms and environmental changes.
3.B.1.b Identify and describe that different individual people or groups of people are affected by an issue in different ways.
4.D.2.a Based on data from investigations and research, identify and describe chemical properties of common substances. (reacts with acids, reacts with bases)

Setting Up the Lab: Before the Students Come

Student Worksheets

- Each will require their own worksheet packet. For each student, make copies of pages S-1 – S-16. (Laminated copies of these pages have been included to facilitate the copying process.)

Solution Preparation and Aliquoting

Cow Milk

- Fill (10) empty cow milk conicals with 20 ml of cow milk

Lactase Enzyme (Lactex Solution)

- Add (1) bag lactase powder to bottle labeled “Lactex Solution”
- Add 175 ml dH₂O
- Mix thoroughly - the powder will not completely dissolve
- Aliquot 15 ml into the (10) conicals labeled “Lactex”

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 10 student tubes for each pH (2, 4, 7, 10, 12, and dH₂O)

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”

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 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
- 1 “Lactex” pipette
- 1 “Cow Milk” pipette
- 1 container glucose test strips

Setting Up the Lab: Before the Students Come

- 1 conical “Lactex” solution (filled by teacher)
- Safety goggles (1/student; Parts II and III can pose risk)
- 1 “Cow Milk” tube (filled by teacher)
- Student copies of the laboratory protocol (1/student)
- 1 Test tube rack
 - Front row (Experiment #1): Unknown milk samples #1, #2, #3 (filled by teacher, see below)
 - Middle row (Experiment #2): pH solutions and water control “2, 4, 7, 10, 12, dH₂O” (filled by teacher)
 - Last row (Experiment #3): Tubes labeled “RTM, RTL, CM, CL, HM, HL” (should all have same group number)

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:

- Experiments #1 and #2 need to have reagents at room temperature. Allow milk samples, Lactex solution, and pH solutions to warm to room temperature before starting the lab.
- Make sure to have students invert capped Lactex tubes to suspend powder before pipetting.
- When students are inverting tubes, remind them to hold the cap on to prevent leakage.
- 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
- Instruct students to notify teacher if the pH solutions are spilled and to wash hands immediately.
- The teacher should monitor the hot water bath at all times, keeping student safety in mind.
- Each part of this experiment can be performed as a stand-alone activity or delivered as multiple lessons, if desired.

Introduction

The Looking Into Lactase laboratory is an activity to facilitate learning about substances called enzymes (their activities and factors that affect them) and the connection of laboratory science and the study of medical biotechnology. This laboratory contains an activity that teaches students how to use the lactase enzyme to break down lactose in milk samples. It also investigates the effects of pH and temperature on enzyme activity. Finally, there is a connection throughout the laboratory to the medical condition of lactose intolerance with an explanation and understanding of its symptoms.

The pre-laboratory activities include 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 (brand name “Lactex”) that contains the enzyme lactase to be used by people who suffer symptoms of lactose intolerance.

Activity	Time needed
Preparing samples & student stations	60 minutes
Pre-Lab activity	30 - 45 minutes
Laboratory activity	90 - 120 minutes (can be split into 3 lessons)
Extension Activities	Variable

Background

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 reaction 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.

Pre-Laboratory Activity: Teacher Guide

Time Requirement: 30-60 minutes

Pass out student worksheet packet to each student (directions for copying student worksheet packets in “Setting up Lab: Before Students Come” section on page 6).

Introduce the scenario to the students that they are working for a pharmaceutical company and read out loud the Memo from the Principal Investigator. Develop further the scenario of a pharmaceutical company involved in the development of a new product (a solution containing the lactase enzyme) that can be used by people who experience the symptoms of lactose intolerance. This lab is an example of Medical Biotechnology.

1. Direct the students to the Medical Fact Sheet in their worksheet packet. Have the students read the sheet and engage them in a short discussion (personal experiences, people they know, what they might have already learned about the topic, etc.) about lactose intolerance.
2. Discuss and/or review information about enzymes including their structure, function, and how they are affected by various conditions. Have the students gain a deeper understanding of enzyme structure and function which would include the following information about enzymes:
 - Enzymes are organic catalysts, speeding up chemical reactions while not being permanently altered themselves.
 - 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” can be used to illustrate the temporary joining of an enzyme to a specific substrate.
 - When an enzyme is subjected to high temperatures, the enzyme ceases to function. This is due to the unfolding of the 3-D structure and the enzyme is said to be denatured. 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.
3. Have students read the first paragraph from each of the three experiments (in their worksheet packet) to introduce the scenarios. Ask the students to identify the goals of the three experiments, and have the students make predictions (hypotheses) for each.

Laboratory Activity: Teacher Guide

Time Requirement 90 – 120 minutes (can be broken into 2-3 45 minute sessions)

The purpose of the laboratory activity is to apply the concepts developed in the Pre-Laboratory to an investigation about the function of enzymes and the connection of laboratory science (research and development of products) to medical biotechnology. The objectives of the laboratory activity are as follows:

- Students will determine the effect of the lactase enzyme on 3 types of milk by conducting an experiment
- Students will determine the effect of pH on enzyme function
- Students will explore the effect of temperature on the activity of the lactase enzyme

Experiment #1: Identifying the Milk Type has students testing unknown milk samples (rice, cow, and soy milk) before and after the addition of lactase, to determine the identity of each. They will be provided with a chart that lists the type of sugar contained in each type of milk sample. After completing experiment #1, the students should be able to identify the three unknown milk samples.

Begin by reviewing the types of sugar present in different types of milk (see chart on student worksheet). Break the students up into pairs or small groups and challenge each group to come up with a way to determine milk type with the materials provided: glucose test strips and lactase enzyme. Have each group report out their ideas and have the class evaluate their logic. Students should come up with the ideas behind the protocol in Part I. That is, they dip the glucose test strips into each unknown milk sample BEFORE adding any lactase enzyme. This will allow them to identify the rice milk, which naturally contains glucose. Next, they will add lactase to all three samples and dip new test strips. This will reveal which one is sucrose (no glucose present still) and which is cow milk (the lactose sugar having been broken down into glucose and galactose). Note that, in this experiment, the students are asked to add lactase enzyme to the rice milk even though they have already identified it. That is so they can check for any ‘side effects’ that might be caused by the newly developed Lactex solution.

Once students have identified a procedure to identify the different milk types, have them complete the protocol listed in Part 1. This activity allows students to become familiar with the equipment and reagents that will be used in the following activities.

Experiment #2: Determining Effect of pH on Lactase Activity will involve students testing a range of different pH solutions mixed with cow milk to see if there is a change in the lactase activity.

Access student prior knowledge on what conditions most affect enzyme activity (pH and temperature). Discuss what neutral, acidic, and basic (or alkaline) pH values are and where they are found in the pH range (0-14). Review the effect of pH on enzyme function. Students should understand that enzymes function best at specific pH levels (which vary depending on the enzyme). Non-optimal pH levels can affect the shape of the enzyme, thereby decreasing its effectiveness as a catalyst. Extreme pH levels can permanently denature the enzyme protein, whereas less extreme pH conditions may only temporarily alter

Laboratory Activity: Teacher Guide

the enzyme shape. 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. These reasons may include:

- Identifying potential interactions with food (e.g., highly acidic food, such as orange juice)
- Ensuring enzyme functions within pH range of human stomach (acidic) and intestines (neutral - basic)

Once students have identified the need for checking enzyme function at different pH ranges, have students complete the protocol listed for Part 2. This activity will allow students to directly observe the effect pH has on enzyme activity.

Experiment #3: Determining Effect of Temperature on Enzyme Activity has students testing the efficacy of the enzyme, lactase, at varying temperatures: room temperature, boiling, and near freezing.

Begin by accessing student prior knowledge on what conditions most affect enzyme activity (pH and temperature). Review with students how extreme high temperatures can permanently denature enzymes. Remind students that low temperatures temporarily reduce enzyme function due to lack of energy and not because they denature or change the shape of the protein. Challenge students to come up with reasons why a drug company would be interested in finding out how the drug works at different temperatures. These reasons may include:

- Determining proper storage conditions for solution
- Ensuring solution works in temperature range found in human body since it is meant to be taken orally
- Ensuring the enzyme would work when taken with hot or cold foods, such as coffee or ice cream

Once students have identified the need for checking enzyme function at different temperatures, have students complete the protocol listed for Part 3. This activity will allow them to directly observe the effect of temperature on enzyme activity.

Interpretation of Results

Experiment #1: Identifying the milk types

Sample #1 is Rice Milk which contains Glucose

- Turns test strip a shade of brown BEFORE addition of lactase due to presence of glucose
- Turns test strip a shade of brown AFTER addition of lactase because 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.

Laboratory Activity: Teacher Guide

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

- No effect on test strip BEFORE addition of lactase (contains only lactose, no glucose)
- Turns test strip a shade of brown AFTER 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 test strip BEFORE addition of lactase (contains only sucrose, no glucose)
- No effect on test strip AFTER addition of lactase (contains only sucrose, no glucose)

Experiment #2: Determining the effect of pH on enzyme activity

- Lactase is effective at pH 2 – 7 (including dH₂O) 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.
- 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. The water control tube should match the tube with a pH solution of 7, which should be a shade of brown on the test strip.

Experiment #3: Determining the effect of temperature on enzyme activity

Results for this experiment may vary if proper temperatures are not obtained.

- The lactase enzyme works best at room temperature, resulting in the glucose test strip turning a shade of brown.
- The test strip at cold temps should detect glucose, but at a lesser amount than at room temperature. As the test strip warms up, the enzyme will begin working again, so please read the results promptly.
- 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 will remain blue.

Equipment Directions and Reagent Notes:

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 from the experiments.

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 blue 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 brown containers that indicates concentration. Test strips are sensitive to light, which is why they must be stored in the brown containers, and not removed from the containers until ready to use. Test strips are also sensitive to touch, so students should avoid touching the blue pad on the end of the strip. Test strips need only be submerged briefly to moisten pad, they do not need to soak.

Lactase Powder

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

Answers

Answers to Analysis Questions from Student Worksheets

Part 1: Identify the Cow Milk

Identify the independent and dependent variable:

The independent variable is the set of conditions that is manipulated, in this case the unknown milk samples. The dependent variable is the set of conditions that is measured, in this case the lactase activity (as shown by the presence of glucose).

Analysis Questions

1. What is the purpose of the glucose test strips?

The strips serve as an indicator for the presence of glucose, which is one of the products of lactase activity.

2. What effect does the lactase have on the cow milk?

The lactase catalyzed the hydrolysis of the disaccharide, lactose, producing the two monosaccharides, glucose and galactose.

3. Which sample is the cow milk? How do you know?

Sample #2. There was no measurable glucose in the original sample, but after adding the lactase there was measurable glucose. This was the only solution that had these results. (Sample #1 was rice milk and #3 was soy milk).

4. Can Lactex be used specifically on lactose? Are there any effects on the other sugars?

Yes, because there was no change in the amount of glucose measured on the rice and soy milk samples. The lactase did not have any effects on the sugars in rice or soy milk.

Answers

Part 2: Determine Effect of pH on Lactase Activity

Identify the independent and dependent variable:

The independent variable is the pH and the dependent variable is the lactase activity (as shown by the presence of glucose). When graphing the results, the independent variable is on the X axis and the dependent variable is on the Y axis.

Analysis Questions

1. Why do we use the water sample?

The water sample represents the control (the pH is 7) against which the other samples can be compared.

2. What is the optimum pH range for Lactex? How do you know?

The optimum pH for the enzyme appears to be pH 2 through pH 7 because there were glucose readings in that range and above pH 8 there was no glucose.

3. What effect does changing the environment from an acidic environment to an alkaline (basic) environment have on the activity of the lactase enzyme?

The enzyme ceases to function as the environment becomes increasingly alkaline.

4. Why would pH affect the enzyme's ability to function?

pH levels can affect the noncovalent forces (ionic interactions and hydrogen bonds) that hold the enzyme in a very specific shape. Changes to the shape of an enzyme therefore affect its ability to bind to specific substrates, in this case sugars.

Part 3: Determine Effect of Temperature on Lactase Activity

Identify the independent and dependent variable:

The independent variable is the temperature and the dependent variable is lactase activity (as shown by the presence of glucose). When graphing the results, the independent variable is on the X axis and the dependent variable is on the Y axis.

Answers

Analysis Questions

1. Which temperature represents the control?

Room temperature represents the control. Experiments #1 and 2 were performed at room temperature.

2. What is the optimum temperature for Lactex? How do you know?

The enzyme appears to work best at room temperature. The highest glucose reading occurred at room temperature.

3. Explain the difference in enzyme activity at varying temperatures. What happened to the enzyme?

At room temperature, the enzyme worked very well. At a colder temperature, the enzyme worked, but not as well as it did at room temperature because there was not enough energy for the enzyme to work. At the highest temperature, the enzyme did not work at all. At the higher temperature, the enzyme denatured which destroyed the 3-D structure making it incapable of interacting with lactose.

4. Do you think it is possible to recover the enzyme activity from the ones treated to extreme temperature conditions? Why or why not?

It is possible to recover the enzyme activity from the cold temperature since the structure didn't change. By adding energy (heat), the enzyme should work well again. The enzyme exposed to extreme heat is not recoverable since it was denatured. Cooling it down does not allow the enzyme to reform the proper 3-D structure to make it functional again.

Answers to Assessment Questions

1. b

2. c

3. d

4. a

5. d

Answers

Brief Constructed Response Questions

6. At which temperature did the Lactex solutions work best to break down the lactose in the milk—freezing, room temperature, or hot? What does this suggest about how effective the Lactex solutions would be for someone who likes ice cream compared with someone who drinks coffee?

The enzyme worked best at room temperature, which is closest (of the temperatures used) to that of body temperature. It may not work as well with the cold ice cream in which the enzyme is inactive, but not denatured. However, by the time the solution and the ice cream reach the stomach, the temperature should be warm enough for the enzyme to function. Hot coffee (depending on how hot is it) may denature the enzyme making it ineffective.

7.

- A. Which enzyme works better in an alkaline environment? How do you know?

Enzyme B 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 pH6 which is considered an acidic environment).

- B. At which pH do the two enzymes work equally well? How do you know?

pH 5. At pH5, both enzymes have the same reaction rate of 1 mg/sec.

- C. What is the optimum pH for Enzyme A? How do you know?

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 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.

Answers

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 4, while the two other groups still show nearly 100% tolerance.
- U.S. Caucasian children maintain an 80+ % tolerance to age 18 while African Americans decrease steadily to less than 40%.
- 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.

Answers

- 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. The mammals 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 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.

MEMO to students

Pharmex Pharmaceuticals

To: All Research Team Members
From: Principal Investigator
Date: 11/22/11
Re: Testing the efficacy of our new enzyme product

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, which we are going to name Lactex. I have enclosed a flier that I picked up at a doctor's office, which includes a **Medical Fact Sheet**. Please share this flier with your team members.

Our newest product, Lactex, is nearly ready for the last phase of testing before submission to the U.S. Food and Drug Administration for approval. Once we get the FDA's approval, we can begin large scale manufacturing and marketing of our product. Before this, however, we need to verify Lactex's ability to break down the disaccharide lactose into glucose and galactose. We will also need to check Lactex's ability to function at different temperatures and different pH values.

You are to work in your teams to conduct three experiments. Please submit your final laboratory protocols, which include your results, after completion. Also remember to follow established safety procedures, as we do not want any fines assessed us by OSHA (Occupational Safety and Health Administration).

Medical Fact Sheet (continued)



What is Lactose Intolerance? Lactose intolerance is a medical condition caused by little to no activity of the enzyme, lactase, preventing 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.

Symptoms can include bloating, abdominal pain, cramps, gas, flatulence, and sometimes diarrhea. Symptoms usually appear anywhere from half an hour to 2 hours after the ingestion of dairy products. The severity of symptoms depends upon how much lactose is consumed, whether it's consumed alone or with other foods and the type of dairy products consumed. Some dairy products contain more or less lactose. For example, certain hard or aged cheeses, like cheddar and Swiss, contain less lactose than whole milk and, therefore, are easier to tolerate. Yogurt contains active enzymes that digest lactose and is better tolerated as well.

What are the Health Consequences?

Avoiding dairy products may result in inadequate intake of calcium, which can lead to various diseases, including rickets, osteoporosis, and hypertension. Dairy foods are an excellent source of natural calcium, protein, vitamins A and

D, and other nutrients. Calcium is especially important for healthy bones and teeth. Milk is the primary source of calcium for children. Children who are lactose-intolerant need to obtain the necessary calcium from supplements.

Who Is Lactose Intolerant? Both childhood- and adult-onset lactose intolerance are very common and are inherited as autosomal recessive conditions. Individuals who can digest lactose into adulthood have inherited at least one dominant gene that allows them to do so. Northern Europeans have the lowest prevalence of adult-onset lactose intolerance, at approximately 5%. However, about 30% of Central Europeans, and 70% of Southern Europeans, as well as Hispanic and Jewish populations, are lactose intolerant. Both Asian and African adults are nearly all (90%) lactose intolerant (see Figure 1).

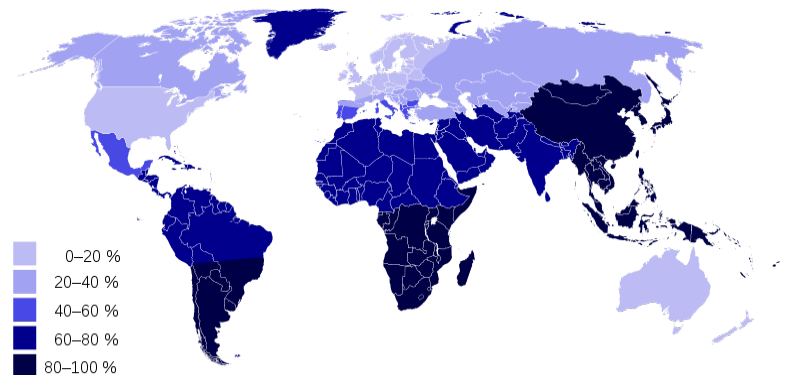


Figure 1: Map showing prevalence of lactose intolerance by region.

What Medical Treatments are Available? Treatment includes changing the diet by carefully eliminating certain milk-containing foods. Another form of medical care is the use of supplemental

Medical Fact Sheet (continued)

lactase; this can be in the form of liquid drops or chewable pills, which can be taken prior to the ingestion of lactose-containing foods. Lactase-supplemented milk and dairy products are now also available.

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 2).

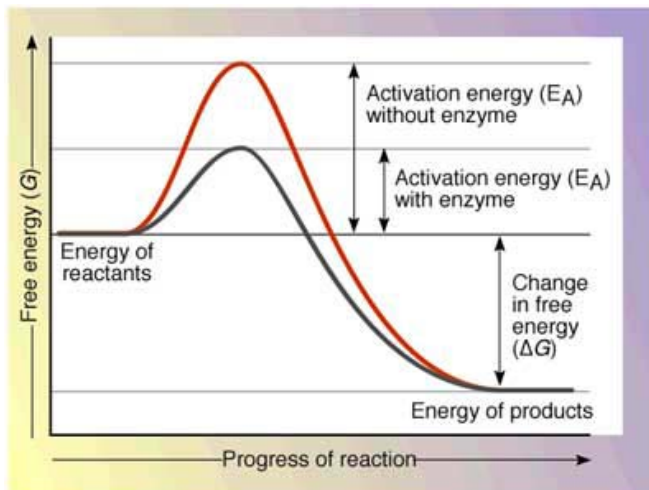


Figure 2: Graph depicting the activation energy required to start a reaction with an enzyme versus without one. (From: <http://163.16.28.248/bio/activelearner/06/images/ch06c1.jpg>)

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 3).

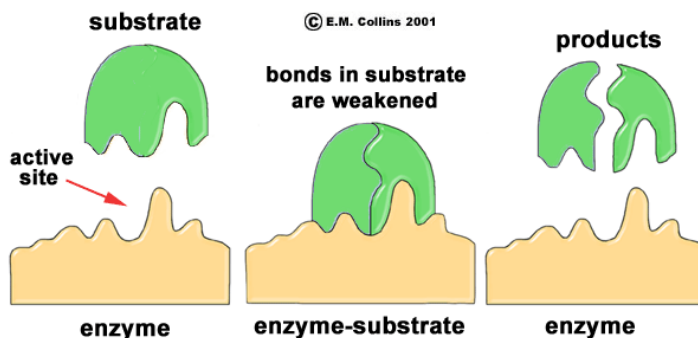


Figure 3: Diagram depicting how an enzyme combines with a substrate to form a product or products. (From: waynesword.palomar.edu)

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.

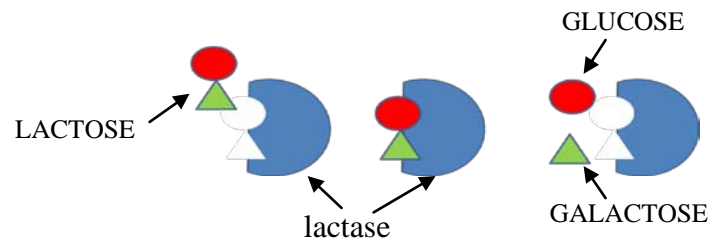
What if the shape changes? When an enzyme is subjected to high temperatures or pH values outside of its range of activity, the enzyme ceases to function. This is due to the denaturing of the enzyme (unfolding of its 3-D structure). At lower temperatures, the structure stays intact, but there may not be enough energy for a reaction to occur. In this case, the enzyme is inactivated, but not permanently denatured.

Student Laboratory Protocol - Part 1

You are a research scientist at a company called Pharmex. We need to verify the activity of lactase found in our newly developed drug called Lactex. The drug must be tested on different types of milk that contain different sugars to verify that it only works on 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 determine which milk sample is which and to prove that Lactex breaks down only lactose.

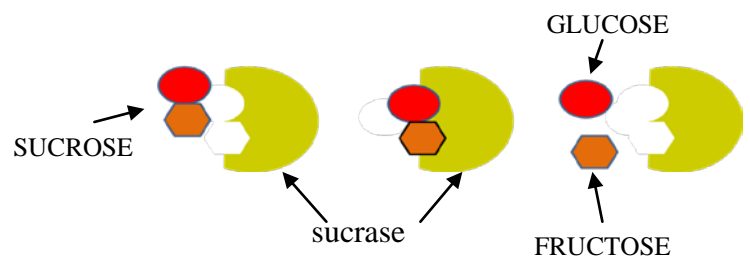
Cow milk contains the sugar LACTOSE.

- 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.
Why is there no enzyme that breaks it down?



Student Laboratory Protocol - Part 1 (continued)

1. Identify the independent and dependent variables:

Independent Variable: _____

Dependent Variable: _____

2. 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.

3. Dip glucose test strip #1 into test tube # 1 and lay it down on the paper towel.
4. Dip glucose test strip #2 into test tube # 2 and lay it down on the paper towel.
5. Dip glucose test strip #3 into test tube # 3 and lay it down on the paper towel.
6. Wait 30 seconds for each test strip.
7. Record the color of the test strips in Table 1.
8. 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.
9. Gently mix the Lactex solution
10. Using the pipette labeled “Lactex”, add 1 ml of the solution to tubes #1, #2, and #3.
11. Cap tubes #1, #2, #3 and invert to mix a few times, but do not shake vigorously. **Hold cap on tube while inverting to avoid leakage.**
12. Wait 3 minutes.

Student Laboratory Protocol - Part 1 (continued)

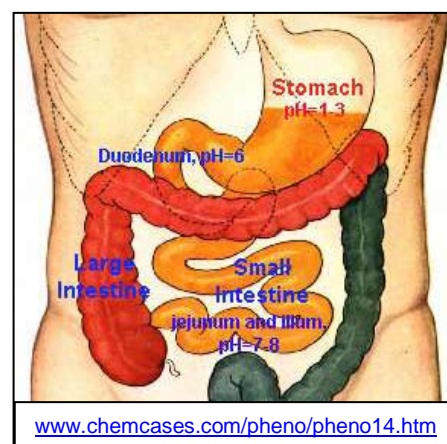
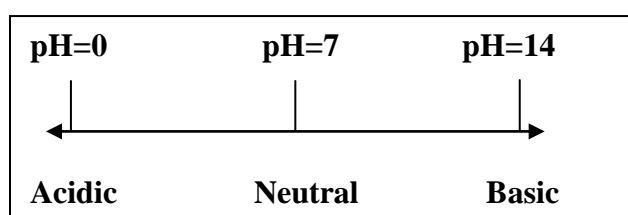
13. Remove 3 new glucose test strips. Label as #1, #2, and #3.
14. Dip glucose test strip #1 into test tube # 1 and lay it down on the paper towel.
15. Dip glucose test strip # 2 into test tube # 2 and place on the paper towel.
16. Dip glucose test strip # 3 into test tube # 3 and place on the paper towel.
17. Wait 30 seconds for each test strip and then record the color of the test strips in Table 1.
18. 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.
19. Identify the milk type based on your results in Table 1. Complete the last column of Table 1.

Table 1: Glucose Results Before and After Adding Lactex

Sample Number	Color of test strip BEFORE adding Lactex (Step 7)	Concentration of glucose (mg/dl) BEFORE adding Lactex (Step 8)	Color of test strip AFTER adding Lactex (Step 17)	Concentration of glucose (mg/dl) AFTER adding Lactex (Step 18)	Milk Type
1					
2					
3					

Student Laboratory Protocol - Part 2

As the research scientists, you have been asked to determine under what conditions the Lactex solution will work. Since the solution is ingested, it must be determined whether or not the Lactex solution can remain effective throughout a wide range of pH values that it may encounter with different foods and liquids while entering the human digestive tract. Using cow milk, your team will conduct an experiment to test the activity of the Lactex solution across a range of pH values.



1. Identify the independent and dependent variables:

Independent Variable:

Dependent Variable:

2. Gently mix the tube labeled “Lactex”.
3. 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 dH₂O.
4. Cap all the tubes and invert to mix a few times, but do not shake vigorously. **Hold cap on tube while inverting to avoid leakage.**
5. Wait 5 minutes.
6. Using the pipette labeled “Cow Milk”, add 2 ml of cow milk to all 6 test tubes.

Student Laboratory Protocol - Part 2 (continued)

7. Cap all the tubes and invert to mix a few times, but do not shake vigorously. **Hold cap on tube while inverting to avoid leakage.**
8. Wait 3 minutes.
9. Label six new glucose test strips with the labels found on the test tubes: 2, 4, 7, 10, 12, and dH₂O.
10. Take glucose test strip labeled “2” and dip it into the tube with pH solution “2” and lay it down on the paper towel.
11. Repeat step 10 for the other samples (pH 4, pH 7, pH 10, pH 12, and dH₂O). Keep the strips separated on the paper towels.
12. Wait 30 seconds for each test strip.
13. Record the color of the test strips in Table 2.
14. 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.

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 ₂ O control		

Student Laboratory Protocol - Part 2 (continued)

15. In the space below, graph the dependence of enzyme activity on pH. Be sure to put a title on the graph and label the axes.



Analysis Questions – Part 2

1. Why do we use the water sample?
2. What is the optimum pH range for Lactex? How do you know?
3. What effect does changing the environment from an alkaline (basic) environment to an acidic environment have on the activity of the lactase enzyme?
4. Why would pH affect the enzyme's ability to function?

Student Laboratory Protocol - Part 3

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.

1. Identify the independent and dependent variables:

Independent Variable: _____

Dependent Variable: _____

2. Gently mix the tube labeled “Lactex”.
3. Using the pipette labeled “Lactex”, add 1 ml of Lactex solution to the three test tubes labeled: HL, RTL, and CL.
4. Using the pipette labeled “Cow Milk”, add 2 ml of cow milk to the test tubes labeled: HM, RTM, CM.
5. Cap all test tubes EXCEPT for the hot test tubes. Leave those uncapped.

3D structure of Lactase



Student Laboratory Protocol - Part 3 (continued)

6. Place the test tubes at 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
7. Record the hot water temperature, the ice temperature, and room temperature in degrees Celsius in Table 3.
8. 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.
9. Carefully pour the contents of the CM tube into the CL tube.
10. Carefully pour the contents of the RTM tube into the RTL tube.
11. Cap all the tubes and gently mix a few times. **Hold cap on tube while mixing to avoid leakage.**
12. Place each of the three full tubes back into its appropriate temperature for 5 minutes (No caps for hot tubes).
13. Label 3 new glucose strips “H” for Hot, “C” for Cold, and “RT” for Room Temperature.
14. Dip glucose test strip “H” into the HOT test tube and lay it down on the paper towel.
15. Dip glucose test strip “C” into the cold test tube and lay it down on the paper towel.
16. Dip glucose test strip “RT” into the room temperature test tube and lay it down on the paper towel.
17. Wait 30 seconds for each test strip.
18. Record the color of the test strips in Table 3.

Student Laboratory Protocol - Part 3 (continued)

19. Record 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.

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			

20. Draw a line graph that shows the dependence of enzyme activity on temperature. Be sure to put a title on the graph and label the axes.



Analysis Questions– Part 3

1. Which temperature represents the control? Why?
2. What is the optimum temperature for Lactex? How do you know?
3. Explain the difference in enzyme activity at varying temperatures. What happened to the enzyme?
4. Do you think it possible to recover the enzyme activity from the ones treated to extreme temperature conditions? Why or why not?

Assessment Questions

Multiple Choice Questions

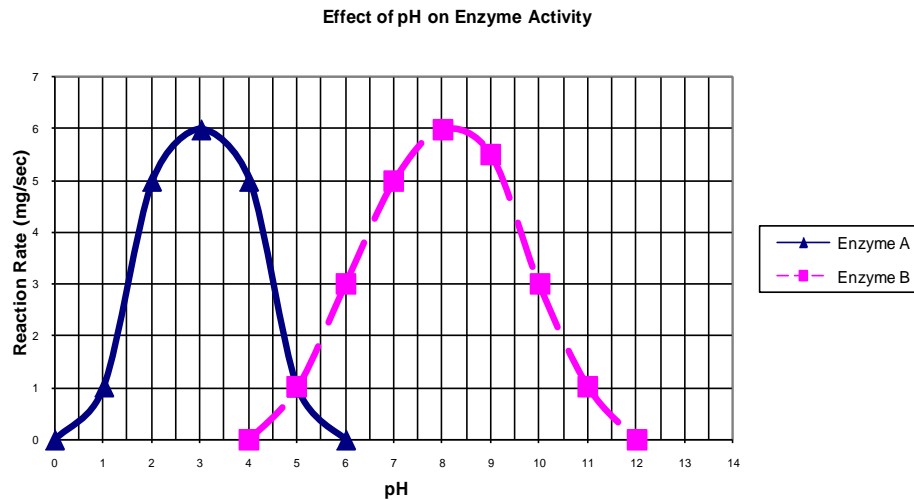
1. The enzyme _____ can break down _____ into _____ and galactose.
 - a. lactose, glucose, lactase
 - b. lactase, lactose, glucose
 - c. glucase, lactase, lactose
 - d. lactase, glucose, lactose
2. Enzymes are _____ molecules which _____ specific chemical reactions.
 - a. carbohydrate, inhibit
 - b. 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. At which temperature did the Lactex solutions work best to break down the lactose in the milk—freezing, room temperature, or hot? What does this suggest about how effective the Lactex solutions would be for someone who likes ice cream compared with someone who drinks coffee?

Assessment Questions

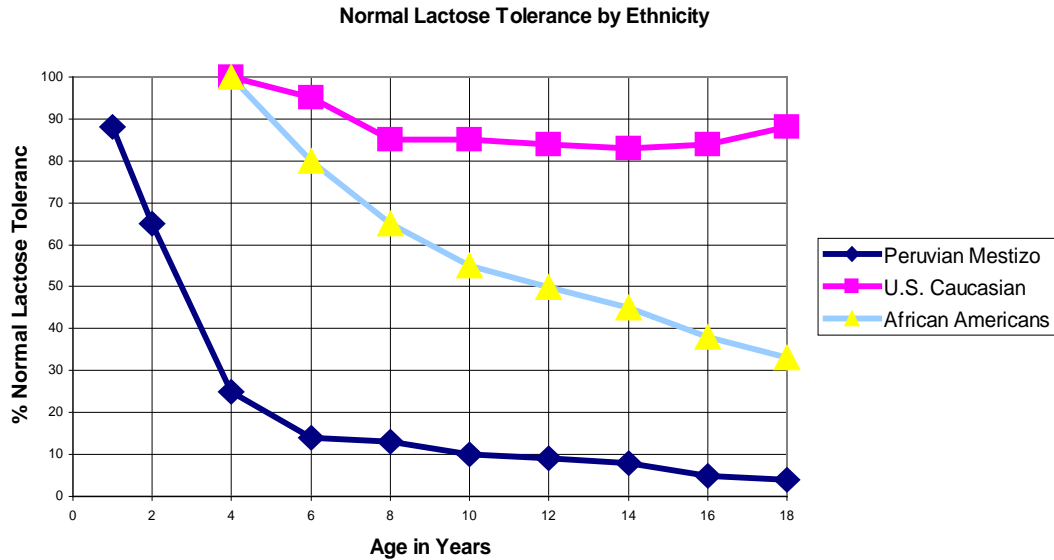
7. Look at the following graph showing the reaction rates of two enzymes.



- A. Which enzyme works better in an alkaline environment? How do you know?
- B. At which pH do the two enzymes work equally well? How do you know?
- C. What is the optimum pH for Enzyme A? How do you know?
- D. Describe the overall reaction rate pattern shown by both enzymes.

Suggested Extension Activities

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



- 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

- 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.

Suggested Extension Activities

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 product which 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).