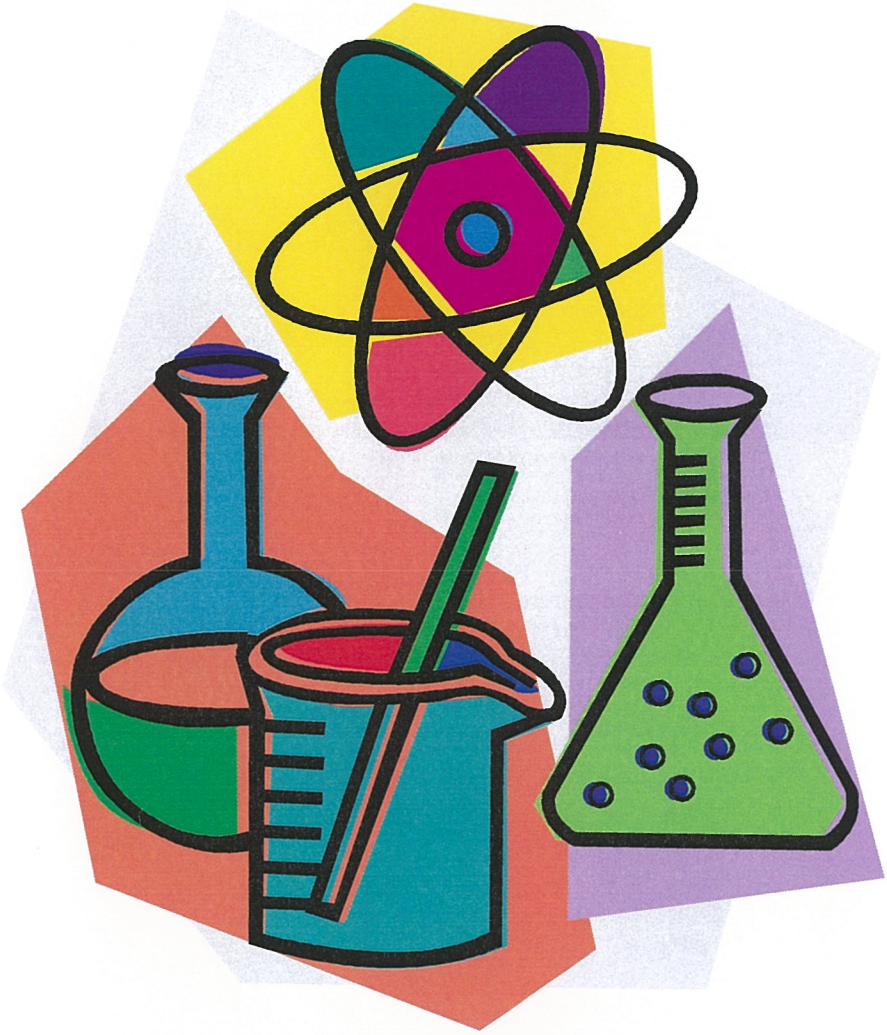
Unit Three:



Biochemistry

Name: _____ Lab # ____

Determining the pH of Common Substances

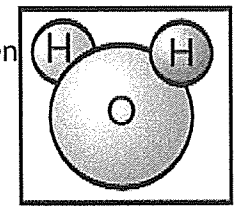
PURPOSE: In this lab you will learn how to use several methods of determining the pH level of a substance, and you will estimate and measure the pH of some common household substances.

BACKGROUND INFORMATION:

What is pH?

You may have heard people use the words acid, neutral, or basic, or maybe alkali. Advertisements on television talk about how a product "neutralizes excess stomach acid." So, what exactly does pH mean?

How is water connected to pH?



You may have heard people talk about water as "H2O." That refers to the chemical makeup of water. Water is made up of two hydrogen ions (the"H2" in "H2O") and one oxygen ion (the "O" in "H2O"). Water is constantly falling apart into its ionic parts and then coming back together. When it falls apart, it breaks into one "H", a hydrogen ion, and one "OH", a hydroxide ion.

Many other substances contain hydrogen and hydroxide ions, but not in equal amounts. When a substance has more hydrogen ions (H+), it is called an acid. Usually, acids have a sour taste. Some good examples of acids you may have around your house are vinegar and lemon juice.

When a substance has more hydroxide ions, (OH^-), it is called alkaline or basic. Alkaline substances are slippery to the touch and have a bitter taste. Good examples of bases around your house are baking soda, antacid medicines, and liquid drain cleaner.

WARNING: Most alkaline substances are poisonous and should not be tasted.

pH is a way to measure how acidic or alkaline a solution is. The pH scale goes from 0 to 14. If there are an equal number of hydrogen and hydroxide ions, the solution is said to be "neutral" and has a pH of 7. Because a molecule of water is made up of one of each ion, pure water is neutral and has a pH at, or near, 7. If the pH of a solution is less than 7, the solution is an acid; and if the pH is greater than 7, the solution is a base (or alkali). Each step on the pH scale represents a factor of 10. For example, a liter of a solution with a pH of 4 has 10 times as many H^+ ions as a liter of a solution with a pH of 5. A liter of a solution with a pH of 9 has 100 times as many OH^- ions as a liter of a solution with a pH of 7.

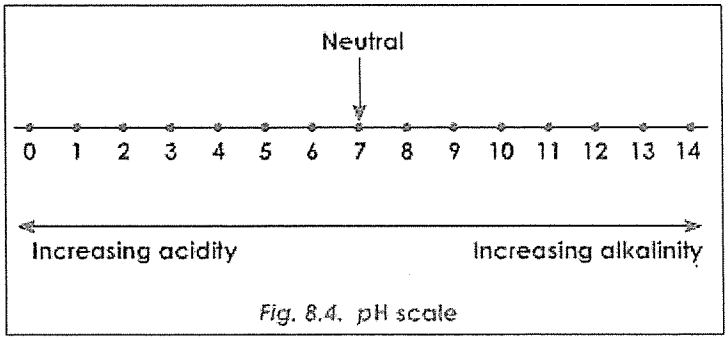


Fig. 8.4. pH scale

MATERIALS:

Red and blue litmus paper
pH hydrion paper
5 common household substances to use as samples
5 Petri dishes or paper towel sheet

PROCEDURE and DATA COLLECTION:

1) Predict whether each substance to be tested will be acidic, basic, or neutral. Construct and record your predictions in a data chart like the one below.

2) Follow these steps for each sample being tested.
   1 - In a Petri dish or on paper towel sheets, lay one small piece each of the following:
   - Red litmus paper
   - Blue litmus paper
   - pH paper

   2 - Place a drop (or rub a piece) of the sample on the indicator papers.

   3 - Write the results of each in your data table and conclude if each substance is an acid or a base.

IMPORTANT NOTE: ** Both litmus papers will be RED when exposed to an acid and BLUE when exposed to a base, they each remain their original color when exposed to a neutral substance**

Sample #	Name of Sample	Prediction: Acid or Base?	Red litmus paper turns...	Blue litmus paper turns...	pH paper color / pH level	Results: Acid or Base?
1						
2						
3						
4						
5						

3) Create a **BAR GRAPH** illustrating the various pH levels of the household materials sampled. Remember to label both the x-axis and y-axis and give your graph an appropriate title.

POST LAB QUESTIONS: Please write out each question and answer in complete sentences.

1) Advertisements on television talk about how a product "neutralizes excess stomach acid." Explain how a product might be able to do this.

2) Differentiate between the litmus paper tests and the test using pH paper. Why perform both?

3) Infer what might happen to your results if you placed your samples and pH papers to be tested in used Petri dishes that were washed and not dried thoroughly before the experiment.

4) If a certain type of vinegar has a pH of 2.5 while coffee has a pH near 5, how much stronger of an acid is the vinegar? Show your calculations and explain your answer.

Name: _____ Lab # _____

The Effects of Environmental Conditions on Enzyme Activity

PURPOSE: To observe the effects of different environmental conditions (acid/base/boiled/frozen) on the activity of the enzyme catalase in potatoes.

BACKGROUND INFORMATION:

What would happen to your cells if they made a poisonous chemical? You might think that they would die. In fact, your cells are always making poisonous chemicals. They do not die because your cells use enzymes to break down these toxic wastes into harmless substances. Enzymes are proteins that speed up the rate of reactions that would otherwise happen slowly. The enzyme is not altered by the reaction. You have hundreds of different enzymes in each of your cells. Each of these enzymes is responsible for one particular reaction that occurs in the cell.

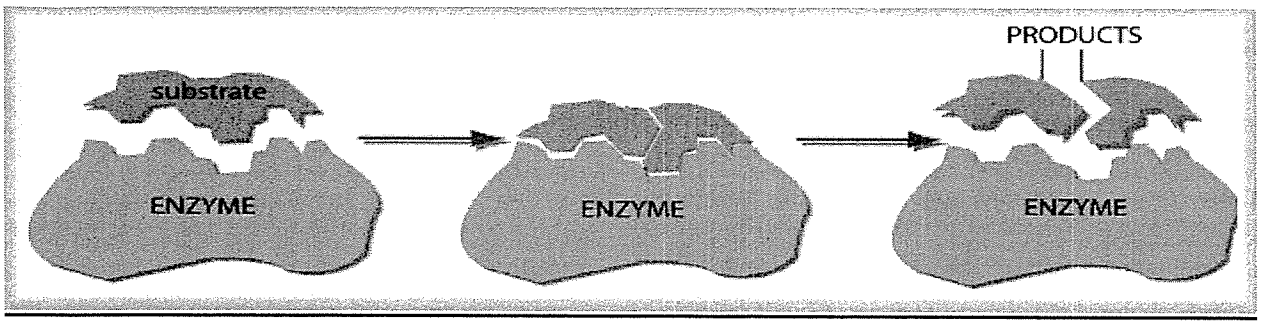
In this lab, you will study an enzyme that is found in the cells of many living tissues. The name of the enzyme is catalase; it speeds up a reaction which breaks down hydrogen peroxide, a toxic chemical, into two harmless substances, water and oxygen. The source of this enzyme in lab today is the cells of a potato plant. The reaction being observed is:

$$2H_2O_2 \longrightarrow 2H_2O + 0_2$$

This reaction is important to cells because hydrogen peroxide (H_2O_2) is produced as a byproduct of many normal cellular reactions. The enzyme is called catalase and is found in both animals and plants. In animal cells, catalase is found in organelles called peroxisomes. An example of where catalase comes into play in animal physiology is during the final stages of aerobic respiration. During the Electron Transport Chain, Hydrogen Peroxide is formed as a toxic by-product which is quickly broken down by catalase. In human beings, the largest source of catalase is the liver. In plants, catalase is used to break down the Hydrogen Peroxide produced by the oxidation of fatty acids and photorespiration. Although some of the chemical can be useful to organisms, too much can be toxic and must be broken down and eliminated.

Address the following questions in your "Background":

- What are enzymes? What is catalase? How does catalase function in the human body? In the potato plant? What effect does catalase have on hydrogen peroxide? Under what pH and temperature range do potatoes grow best? (look up the answers online!)



PROCEDURE and DATA COLLECTION:

1. Obtain a test tube rack/beaker with 5 small and 5 large test tubes
2. Calculate the area of the circle created by the mouth of the one **small test tube** using πr^2 _____(mm)
3. Obtain goggles and gloves
4. **Label the small test tubes** (**BA**-base, **BO**-boiled, **F**-frozen, **A**-acid, **C**-control) with a wax pencil
5. Obtain a small sample of cored potato for each condition and place it inside each small test tube
6. Fill up the large test tubes almost (about 1½ inches) from the top with 3% hydrogen peroxide
7. Quickly and simultaneously, invert the small test tubes into the large ones, cover the top tightly with a rubber cork, and invert a few times until the small test tube is void of air and the resting firmly on the bottom of the large test tube (potato piece under mouth of small test tube)
8. Take note of any initial activity (bubbling)
9. Record your data table into your lab notebook.
10. Allow reactions to catalyze for an agreed upon time frame (5-10 minutes)
11. Using a ruler, measure the gas produced in mm (from bottom of bubble mass to the top of the small test tube). Record your data in a table like the one below. Calculate the Volume of liquid that has been displaced by oxygen gas. Record.

Potato Treatment	**Air Space (in mm)**	$V = \pi r^2 h$ V represents Volume, h represents height(air space) in mm, and πr2 represents the area of the circular base.	**Ranking (1 – 5)** 1 = least activity, 5 = most activity
Control (pH=)			
Acid (pH=)			
Base (pH=)			
Boiled			
Frozen			

11. Create a **BAR GRAPH** illustrating the effect of varying environmental conditions on enzyme action. Remember to label both the x-axis and y-axis and give your graph an appropriate title.

ANALYSIS QUESTIONS:

1. How does observing and rating the release of gas bubbles allow you to determine the rate of catalase enzyme activity?

2. Based on the collected data, what are the optimal conditions for the catalase enzyme to function at the fastest rate?

3. What is meant by the term "denature" in regard to enzyme activity?

4. Draw a picture representing the action of the enzyme catalase. Use the formula from the introduction to identify the substrate, the products, and the catalyst.

5. Not all enzymes operate under the same optimal conditions. Use the article provided by your teacher to provide specific examples that support this statement.

Applications: Read this article and relate to the Enzyme Lab. Refer to this article in your Analysis questions and Conclusion.

Questions to keep in mind when reading this article:

1) How are the enzymes of extremophiles different from most other organisms?
2) Why are the current methods we use to treat hydrogen peroxide waste not efficient?
3) Why is *Thermus brockianus* of interest to researchers?
4) What are some hurdles to obtaining and utilizing this enzyme on a large scale?

Enzyme From Extremophile Holds Promise For Industrial Applications

For centuries chemists have looked for ways to synthesize or alter molecules. The countless reactions that were discovered have been essential to industrial development. But in four billion years, living organisms have found far more ways to alter their chemical environments. Enzymes, proteins produced by all living organisms, catalyze an incredible array of reactions under all manner of challenging circumstances.

One of nature's most amazing creations is extremophiles. These tiny organisms thrive in some of the harshest conditions on the planet. They survive in places devoid of nutrients other organisms rely on, or in environments poisonous to nearly any other life form. To survive, extremophiles must produce enzymes that function normally at extremes of temperature, pH, and salinity. This makes extremophile enzymes an interesting and potentially lucrative target for research.

One extremophile enzyme currently under scrutiny recently won researchers at the Idaho National Laboratory (INL) a 2004 R&D 100 Award, given by *R&D Magazine*. Vicki Thompson, a staff scientist at INL, and colleagues have found a potential use for an enzyme from an extremophile called *Thermus brockianus*, found in a geyser in Yellowstone National Park. The enzyme degrades hydrogen peroxide and could find use in treating waste from bleaching processes. "What's really special about this enzyme is that it loves high temperature and pH," says Thompson.

Hydrogen peroxide is increasingly being used as a less environmentally harmful alternative to chlorine bleach, primarily in paper and fabric bleaching. There are several methods for treating hydrogen peroxide waste, including commercially available catalase products. These catalases don't come from extremophiles, though, so they are rendered useless in just seconds by high temperature or pH. Manufacturers using catalase must choose to either pretreat their waste or purchase large amounts of catalase. Either option is an effective way to treat hydrogen peroxide waste, but both are quite expensive.

There are several major hurdles to overcome in working with extremophiles. The first is finding them. The very definition of an extremophile means one must search strange and often secluded environments to get them. Hot springs, deserts, salt lakes, and even Antarctica are some of the places one must visit to look for these incredible organisms.

Finally, once an enzyme has been selected and rigorously tested to show its marketable function, it must be produced on a large scale so that it can be sold. Simply harvesting extremophiles from nature and extracting enzymes is impractical, if not impossible.

However, most enzymes can be easily expressed in a lab in other bacteria, often *E. coli*, which is easy to grow and can be made to produce quantities of enzyme much greater than the original organism produces. INL has yet to do this with the catalase, but it is the next step once they find the funding, Thompson says. Soon the catalase will join the ever growing list of extremophile enzymes making chemistry cheaper and cleaner.

By Chris Wentz, a senior in the biochemistry department at the University of Washington. Excerpt from: *Northwest Science and Technology*, University of Washington

Name: _____ Date: _____

Graphing Rates of Enzyme Action

PURPOSE: to graph the effect of various environmental factors such as temperature and pH on the rate of enzyme action.

BACKGROUND INFORMATION: Enzymes are functional proteins that are specifically shaped to catalyze or speed up the rate of chemical reactions. To do so, they must fit with the shape of their substrate, the molecule(s) they are acting upon. Factors such as temperature and pH can slow down the rate of enzyme action or even stop it all together by changing the enzyme's functional shape.

PRE-LAB QUESIONS:

1) Why are enzymes also called organic / biological catalysts?

2) Explain enzyme denaturation including what happens and the factors that may cause it.

MATERIALS: graph paper, pencil with eraser

PROCEDURE & DATA COLLECTION:

Part A – The Effect of Temperature on the Rate of Enzyme Action

Graph the following information. Be sure to properly label and set up a consistent scale on each axis. Provide an appropriate title at the top of the graph. Then complete the accompanying questions.

Temperature (Celsius)	Rate of enzyme action (grams per minute)
0	0.00
10	0.40
20	0.60
30	0.80
40	1.00
50	0.40
60	0.20
70	0.00

Questions

1) At what temperature does this enzyme work best? How do you know?

2) At what temperature does this enzyme become denatured? How do you know?

3) What were the independent and dependent variables measured in the experiment that provided this data?

Part B – The Effect of pH on the Rate of Enzyme Action

Graph the following information. Be sure to properly label and set up a consistent scale on each axis. Provide an appropriate title at the top of the graph. Then complete the accompanying questions. On your graph, label which line represents the enzyme trypsin, which works best in an alkaline (base) environment, and label which line represents pepsin, which works best in an acidic environment.

	Rate of enzyme action (grams per minute)	
pH Level	Enzyme 1	Enzyme 2
0	0.00	0.00
1	0.20	0.00
2	0.90	0.00
3	0.70	0.00
4	0.50	0.00
5	0.30	0.10
6	0.00	0.20
7	0.00	0.50
8	0.00	0.80
9	0.00	1.00
10	0.00	0.80
11	0.00	0.50
12	0.00	0.30
13	0.00	0.10
14	0.00	0.00

Questions

1) What is the optimal pH for pepsin?
2) What is the optimal pH for trypsin?
3) Your stomach has a very low pH level, which enzyme will be most effective there?
4) At what pH does pepsin become denatured?
5) At what pH does trypsin become denatured?
6) What were the independent and dependent variables measured in the experiment that provided this data?

Name: _____ Lab # _____

Enzyme Salad!

Problem: To determine the effects temperature on enzyme activity.

Background: Have you ever wondered why there is a warning label on Jell-O gelatin dessert boxes warning you not to use fresh or frozen pineapple in your Jell-O? The enzymes in some tropical fruits catalyze (speed up) protein digestion. Pineapples contain a digestive enzyme called bromelin. Bromelin digests the gelatin protein in Jell-O (the stuff that makes it "gel"). However, the enzymes of canned pineapples have been denatured, meaning the enzymes lost their functional shape and catalyzing abilities during the canning process, due to extreme temperature or pH levels.

Materials:
3 small cups
1 piece of fresh pineapple
1 piece of canned pineapple
Jell-O gelatin dessert mix (any flavor)
Hot plate
Beaker
Markers
Heat resistant glove / beaker tongs

Hypothesis: Write a hypothesis predicting the effect of the presence of various pineapple types (canned, fresh, or none present) on the final consistency of Jell-O gelatin. Remember, this may NOT be in the form of a question

Procedure:

1. Label each of your 3 small cups (fresh, canned, and no pineapple). Be sure to include your group members' initials on the cups so you can identify your samples later.
2. Obtain 1 piece of fresh pineapple and 1 piece of canned pineapple and place the pieces in the appropriately labeled cups. One cup should have no pineapple in it to serve as the control group.
3. Pour a small amount of the prepared Jell-O liquid into each of the 3 beakers.
4. Make sure the pineapple and Jell-O are well mixed by gently swirling the cups.
5. Follow your teacher's directions on where to place your cups. Make sure they are appropriately labeled.
6. Begin answering the analysis questions.

Day 2

7. Obtain your group's samples. Record your observations on a data table like the one below.
8. Answer the remaining analysis questions.

Observations and Data

Beaker containing:	Observations	Time to Gel
No Pineapple		
Fresh Pineapple		
Canned Pineapple		

Analysis Questions:

1. Why do you think one of the pineapple samples caused the Jell-O to gel, but the other did not?

2. Why did one cup contain only Jell-O and no pineapple pieces?

3. What can you infer about the canning process that might affect the enzyme bromelin?

4. Describe what you could do to fresh pineapple to get the Jell-O to gel. Why will that work?

Conclusion:

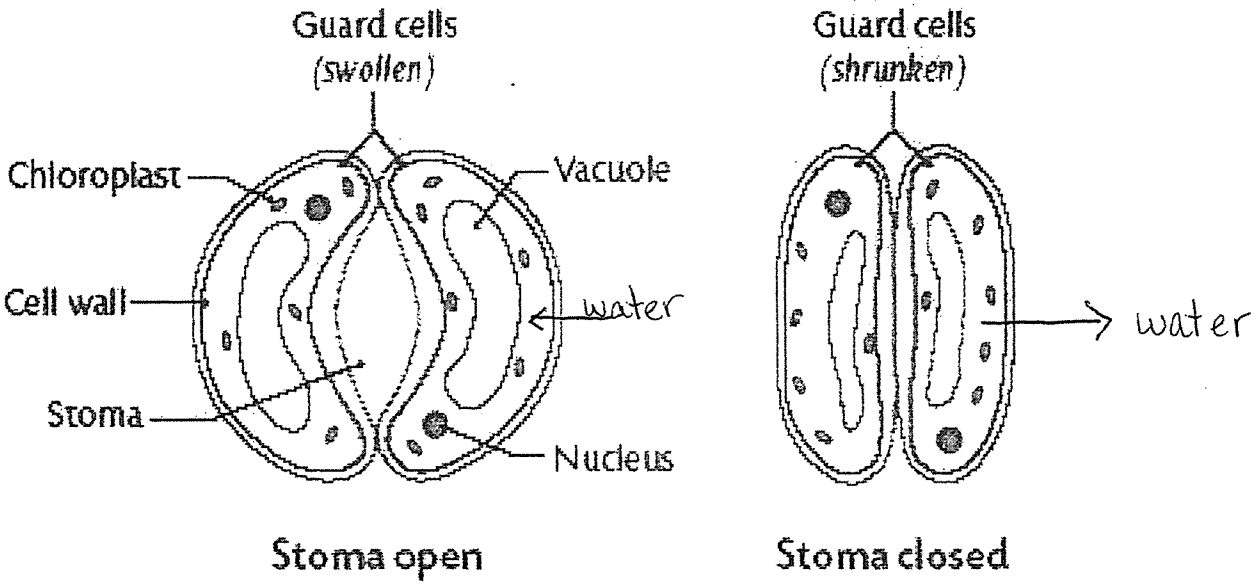
Write a minimum of 4 sentences describing what you learned from this lab. You should reflect on your own hypothesis, state whether it was supported or rejected based on your observations. You may also include a discussion of possible sources of error, suggestions for additional data that should be collected to further support your findings, suggestions for other investigations that may me done on the same topic, how the information you learned can be applied to the "real world" or be connected to your own life experiences.

Observing Leaf Structure

Background Information:

Plants and animals both have a layer of tissue called the epidermal layer. Plants have special pores called stomata/stoma/stomates to allow passage of certain substances. The stomates are surrounded on both sides by jellybean shaped cells called guard cells. Unlike other plant epidermal cells, the guard cells contain chlorophyll to do photosynthesis. These cells expand and contract by osmosis to open or close the stomata. When the leaf is dehydrated, the guard cells become shriveled and close up in order to prevent excess water loss, allowing the plant to maintain homeostasis.

Most stomata are on the lower epidermis of the leaves on plants (underside of leaf). The number of stomata on the epidermal surface can tell you a lot about a plant. Usually, a high concentration of stomata indicates fast growth and wet climate. Lower concentrations of stomata indicate lower rates of photosynthesis and growth or adaptations for dry weather.



Microscopic Observations of a Leaf

I. Prepared Slide of a Leaf Cross Section

(a) Sketch a leaf cross section viewed on high power (use pencil)
(b) Label the following structures on high power:
Waxy cuticle, upper epidermis, palisade layer, spongy layer, vascular bundle, lower epidermis, and any visible chloroplasts.

II. Prepared Slide of a Leaf Epidermis

(a) Sketch the leaf epidermis viewed on high power (use a pencil)
(b) Label the following structures on high power:
Guard cells, stomata, and lower epidermal cells

Analysis Questions:

1. How would salt water affect the guard cells and stomata? Explain what causes this to occur.

2. Explain how stomates are an important adaptation for plants to maintain homeostasis in warm, dry weather.

3. In which layer of the leaf does most photosynthesis occur? Why is this so?

4. Explain how each of the following parts of the leaf function in photosynthesis.

   a) guard cells

   b) stomata

   c) vein (vascular bundle of xylem and phloem)

   d) upper epidermis

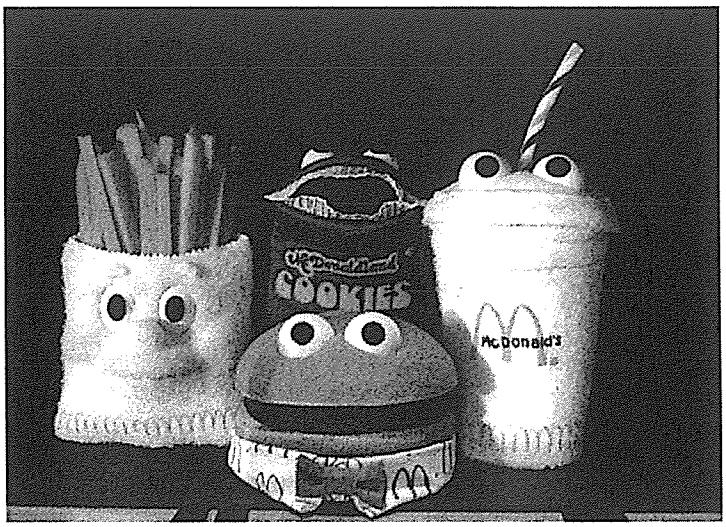
   e) air spaces

Name: Date:

Mc Mush

A Study of Biochemistry

Introduction



Carbohydrates, fats, proteins, vitamins, minerals and water are all nutrients that provide your body with energy and raw materials. These compounds are present in the plants and animals you use as food. In this lab, you will test for specific organic compounds and then determine if those compounds are present in a McDonalds Happy Meal. (Not Including the Toy!)

Materials

Test Tubes	Mc Mush	Benedict's Solution
Test Tube Racks	Glucose Solution	Biuret's reagent
Test Tube Holders	Starch Solution	Lugol's solution
Hot Water Bath	Pepsin	HCL
Graduated Cylinder		

Procedure

Testing for Fats

1. Pour 150 mL of McMush into a 500 mL beaker.
2. Add 150 mL of water and stir to mix.
3. Pour into a graduated cylinder
4. Refrigerate to cool.
5. Measure and record the fat accumulated near the top of the graduated cylinder and calculate the percent fat.

Testing for Simple Sugars

1. Label three test tubes and add the following:
    a. 1 mL of glucose solution
    b. 1 mL of water
    c. 1 mL of Mc Mush
2. Add enough water to each of the test tubes to cover the samples. Swirl to mix.
3. Place the test tubes in a hot water bath for three minutes.
4. Record the color of the solution in each test tube.

Testing for Starch

1. Label three test tubes and add the following:
    a. 1 mL of starch solution
    b. 1 mL of water
    c. 1 mL of Mc Mush
2. Add 5 drops of Lugol's solution to each test tube.
3. Record the color of each of the solutions in each tube.

Testing for proteins

1. Label three test tubes and add the following:
    d. 1 mL of albumin (protein solution)
    e. 1 mL of water
    f. 1 mL of Mc Mush
2. Add 1 mL of pepsin and 1 mL of HCL to each of the test tubes.
3. Add one dropper full of Biuret's reagent to each test tube. Swirl to mix.
4. Record the color of the solution in each test tube.

DATA

Fats

Total Volume	
Volume of Fat	
Percent Volume	

Simple Sugars

Test Tube	Initial Color	Final Color	Positive or Negative
A. Glucose			
B. Water			
C. McMush			

Starch

Test Tube	Initial Color	Final Color	Positive or Negative
A. Starch Solution			
B. Water			
C. McMush			

Proteins

Test Tube	Initial Color	Final Color	Positive or Negative
A. Albumin			
B. Water			
C. McMush			

QUESTIONS

1. What reagents or test method is used to detect the presence of the following:
    a. Fats: _____
    b. Simple Sugars: _____
    c. Starch: _____
    d. Protein: _____
2. In each of the tests performed, how do you know when a positive reaction took place?
3. In each case, we tested substances known to have sugar, starch and protein in them. Why was this done?
4. Interpret that data in your tables. What compounds are found in a Happy Meal? How do you know they are present? What foods are these compounds in?

Name _____ Class _____ Period _____

19–1 What Do the Inside Parts of Leaves Look Like?

A leaf is one of the organs of a plant. The main function of a leaf is to make food for the rest of the plant. The food is carried to other plant parts. What parts of a leaf make and carry food?

Since leaves are organs, they are made of many different kinds of tissues. In leaves these tissues appear as layers of cells. Each tissue has a specific function and the shape of the cells in the tissue is related to its function. Some cells look like small boxes stacked side by side, while others are long and balloon-shaped. Certain cells are round and loosely packed while others look like small tubes stacked together.

EXPLORATION

OBJECTIVES

In this exercise, you will:

a. build a model of the structure of a leaf.

MATERIALS

scissors
1 sheet white paper
prepared slide of leaf
colored pencils: red, blue, purple, yellow, tan, light green, dark green
microscope
transparent tape

PROCEDURE

Part A. Making a Model of a Leaf

1. Figure 1 shows the different cell layers of a leaf. Your teacher will provide you with a copy to color as follows:
   a. waxy layer—purple
   b. upper epidermis layer—yellow
   c. lower epidermis layer— orange
   d. spongy layer— pink
   e. palisade layer— green
   f. xylem—red
   g. phloem—blue
   h. guard cells— green

Questions

1. List the function of each of the following leaf parts:
    a. Waxy layer:
    b. Upper and lower epidermis:
    c. Guard cell:
    d. Spongy layer:
    e. Palisade layer:
    f. Xylem:
    g. Phloem:
    h. Stoma:
2. Many houseplants have very thick, waxy leaves. They do not wilt as quickly as houseplants with thinner leaves. Explain why.
3. Where does the phloem get the food that it carries to the stems and the roots?
4. What would happen to the action of the guard cells and the size of the stomata on a hot sunny day?
5. What is the evolutionary advantage of the position of the stomata and guard cells being located on the underside of the leaf?