

Name _____

Pre-lab 1–Cellular Energetics: Photosynthesis and Respiration

NOTE: *Bring into lab a leaf from 1 plant.*

1. True or False: All heterotrophs rely on autotrophs for food.
2. Fill in the blanks with the appropriate chemical equations.
Photosynthesis = _____
Respiration = _____
3. For each experiment below, write out the variables that could be tested, how they are measured, and what control is used.
 - A. Photosynthesis experiment
 - B. Cellular respiration experiment

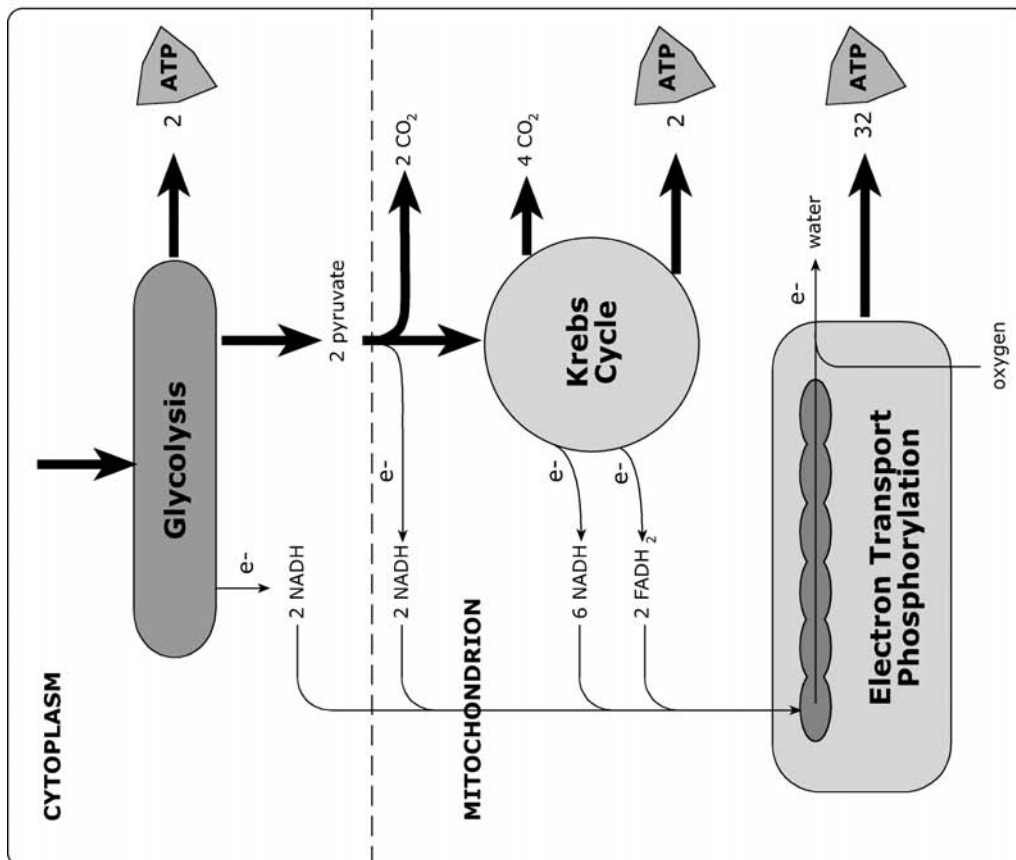
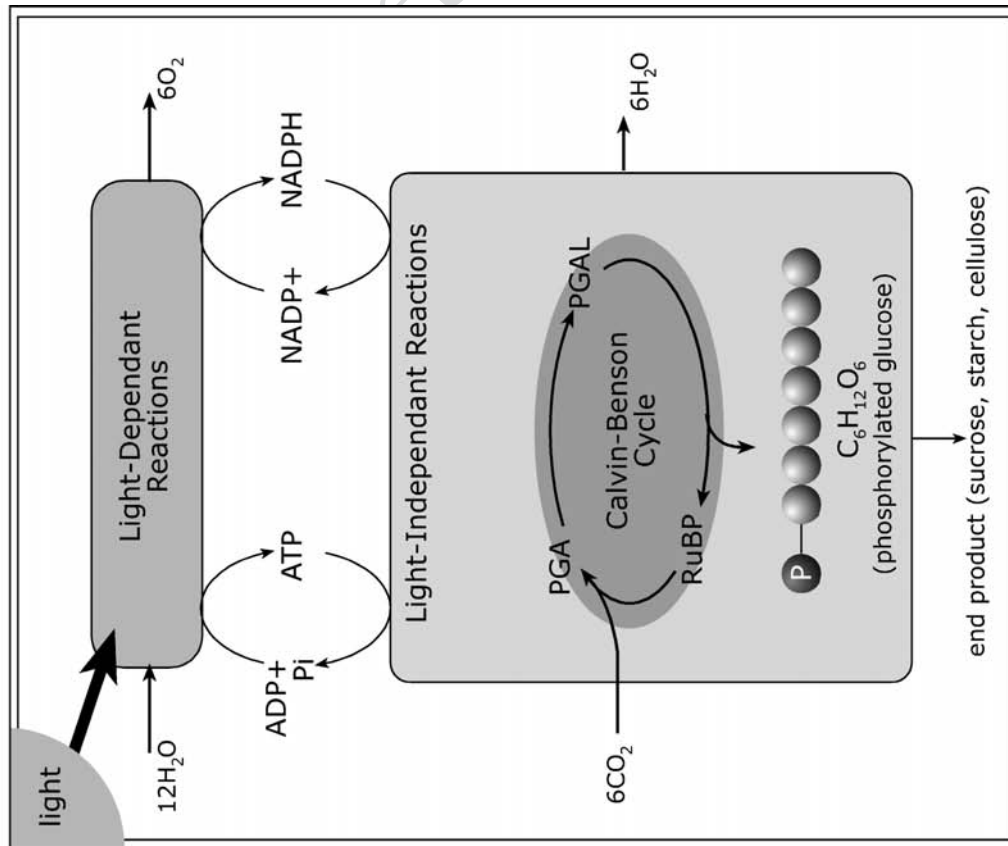


Figure 1.1 Reaction Schemes for Glucose Catabolism and for Photosynthesis

Cellular Energetics

INTRODUCTION

I. Cellular Respiration

If all the potential energy contained within every molecule of glucose inside a living cell were released suddenly, the cell would be unable to trap this energy to drive other chemical reactions. In fact, the heat produced would probably kill the cell. Instead, the cell breaks down glucose stepwise, through a series of reactions, and the energy from the broken glucose bonds can then be utilized to perform cellular work such as making new chemical bonds or maintaining a concentration gradient. The first series of reactions involved in breaking down glucose is known as glycolysis and occurs in the cytoplasm. This process is anaerobic (requires no molecular oxygen) and the end product is pyruvic acid. Pyruvic acid can be used for fermentation under anaerobic conditions, or it can be further broken down (yielding even more energy) using the citric acid (Krebs) cycle under aerobic conditions.

Aerobic respiration begins with the oxidation of pyruvic acid to acetyl-Coenzyme A. In the citric acid cycle, acetyl-CoA is oxidized further to release carbon dioxide. During these oxidation reactions, electrons that are removed are transferred to the electron carrier molecules NAD^+ or FAD which then transfer the electrons to the electron transport system. The energy released during electron transport is used to form ATP. These low energy electrons are then transferred to oxygen, along with two protons, forming water.

Many organisms are anaerobic and do not need oxygen as the final electron acceptor molecule. They use organic molecules for this purpose and function by the process of fermentation. This also can occur in some tissues of aerobic organisms for a short term under certain physiological conditions. In this process the electrons from NADH are transferred back to pyruvic acid, which is converted to ethanol in yeast and some bacteria (and in water-logged plant roots), or lactic acid in muscle cells and some bacteria. The reoxidation of NADH to produce NAD^+ is necessary to perpetuate glycolysis.

II. Photosynthesis

Photosynthesis is unquestionably the most important series of chemical reactions that occur on earth. Most life is totally dependent on this process for food (and oxygen), with the exception of chemoautotrophs. This includes all **heterotrophs**. Photosynthesis is a complex series of reactions that use radiant (light) energy and inorganic carbon to form chemical bonds. This complex set of reactions is present in prokaryotic as well as eukaryotic life forms. Glucose is readily formed from the products of the carbon-fixing light-independent reactions.

Photosynthesis can be divided into two sets of reactions. Some characteristics of these reactions are compared below. In this laboratory we will be dealing with some properties of the photochemical reactions.

Photochemical Reactions	Biochemical Reactions (Calvin Cycle)
Light Dependent	Light Independent
Fast (practically instantaneous)	Slower (but still extremely fast)
Captures light energy and converts it into usable chemical energy. Electrons excited by light go through an electron transport system which results in the production of ATP and NADPH. Oxygen is given off.	Adds electrons and protons from NADPH to reduce carbon dioxide and uses ATP energy to form new bonds to make carbohydrates.

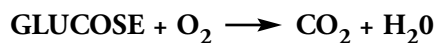
Although ATP is produced during photosynthesis, plant cells also contain mitochondria and can use newly synthesized carbohydrates for energy as needed (especially at night and in nonphotosynthetic tissues).

NOTE: BOTH EXPERIMENT A AND EXPERIMENT B SHOULD BE SET UP SIMULTANEOUSLY; EACH GROUP OF STUDENTS WILL NEED TO SET UP BOTH EARLY IN CLASS.

EXPERIMENT A: MEASURING THE RATE OF RESPIRATION

Background

Consider the following reaction:



This is the summary reaction for Respiration. Notice the reactants include glucose (or a sugar that can serve as a substitute) and oxygen; the products are carbon dioxide and water. Which of these components would you consider would make a good **indicator** of the rate of respiration? _____

This experiment is designed to measure the rate of respiration in yeast cells fed glucose (or other sugars) by measuring the rate of carbon dioxide release. Some sugars are used more readily by yeast than others. This is an important observation and illustrates the concept of enzyme regulation called **induction**. This process occurs when yeast cells, usually grown in the presence of one type of sugar, experience the absence of the food it usually encounters. If it is fed an alternative energy source for which the cells maintain no enzymes, it takes some time for the yeast to induce expression of genes for the new enzymes it needs. You can tell this is happening if you observe a lag time in respiration while the yeast is involved in producing sufficient enzyme for there to be a noticeable effect (increase in respiration rate).

We will be conducting an experiment to determine: What is the effect of different carbohydrates on the rate of respiration in yeast?

Write hypotheses regarding the amount of time required for respiration to begin. Consider conditions to which yeast would be likely to be adapted, the structures of the different carbohydrates, and that honey is a mixture of many sugars and other compounds.

Materials

graduated centrifuge tubes 50 mL beakers
various sugar solutions (see Table 1.1) active yeast culture

Procedure

1. Stir the stock yeast culture before using it. Pipette 10 mL yeast suspension into each of 8 graduated centrifuge tubes. Label the tubes A–G.
2. As you are ready, add a volume of each different sugar solution to one of the six centrifuge tubes (A–F) sufficient to fill the tube. Fill tube G with distilled water (**WHY?**) Record the solution with its corresponding label in Table 1.1.
3. Cap each tube with a 50 mL beaker and quickly invert them together. Add a few mL of water to the inside of the beaker to prevent air leaks. This set-up will be demonstrated.
4. Record the volume of the air bubble in each tube at time 0 and at 10 minute intervals and record in Table 1.1. This is accomplished by marking the initial volume (time 0) of fluid in the inverted centrifuge tube with a grease pencil. Continue to mark the tube with the grease pencil at each 10 minute interval if the volume of the fluid decreases. The amount of fluid decrease represents the rate at which sugar in the solution is being converted to carbon dioxide gas.

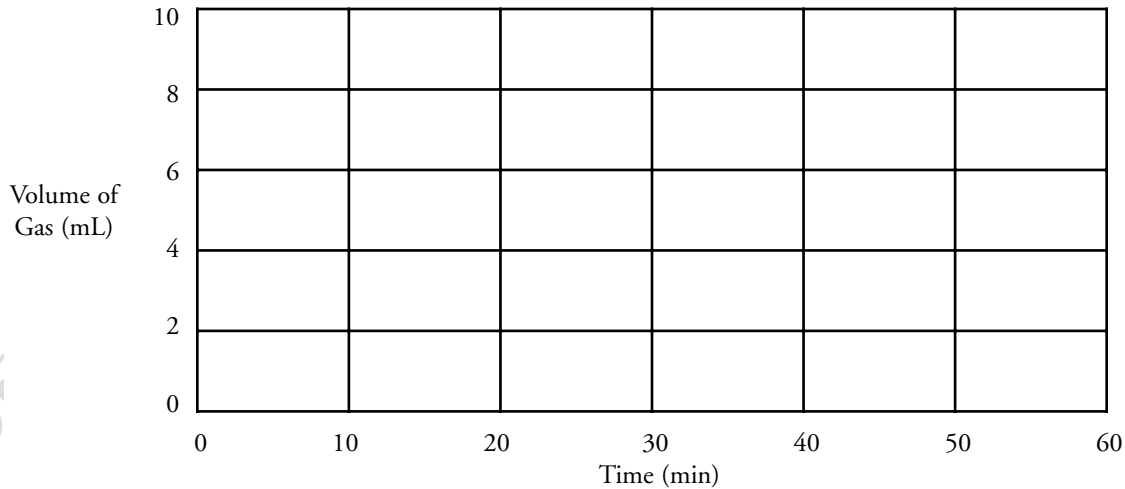
Results

Table 1.1 Carbon Dioxide Production from Different Sugars by Yeast Cells.

Sugars	0 min	10 min	20 min	30 min	40 min	50 min
A: glucose						
B: fructose						
C: lactose						
D: galactose						
E: sucrose						
F: honey						
G: water						

Plot the volumes of carbon dioxide production with respect to reaction time for all reactions in Graph 1.1. Use your colored pencils so different solutions will be easily identified.

Graph 1.1 Differential use of Sugars by Yeast.



END OF CLASS DISCUSSION OF RESULTS FROM THIS EXPERIMENT

What gas is being produced in this experiment?

What was the purpose of using water instead of sugar solution in reaction G?

Which sugar was used most rapidly in this experiment?

Which sugar was used most slowly?

What is the reason for the differences in the rates of use of these sugars?

EXPERIMENT B: MEASURING THE RATE OF PHOTOSYNTHESIS

Background

Consider the following reaction:



This is the summary reaction for photosynthesis. Note that $\text{C}(\text{H}_2\text{O})$ is a general formula for a carbohydrate. The actual products of the Calvin Cycle are two three-carbon compounds that can be used to synthesize carbohydrates. Also remember that LIGHT is required.

Which of the products of this reaction would you use to monitor photosynthesis? _____
Notice that the reaction requires CO_2 —we will supply a source of CO_2 using a solution of sodium bicarbonate (NaHCO_3).

As a class, we will be conducting an experiment to determine: What is the effect of light intensity on the rate of photosynthesis in *Elodea*? **We will also attempt to answer the question:** What is the effect of different colors of light on the rate of photosynthesis in *Elodea*?

Write hypotheses regarding how you think the rate of photosynthesis may or may not be affected by these conditions. Consider requirements plants have for photosynthesis, and look in your textbook for ideas on how or if light color may have an effect.

Materials

graduated centrifuge tubes	50 mL beakers
10 mm sodium bicarbonate (pH 7)	<i>Elodea</i> stalks
small box	cylindrical colored filters with lids
light source (strong)	(red, blue, green, yellow)

Procedure

Check with your instructor to see if every group should do the light intensity experiment plus one-two color filter(s) (a different one for each group) OR if part of the class should do the light intensity experiment and part of the class should do the color filter experiment (3-4 different filters for each group). What should be used as a **control** for the light intensity experiment? What should be used as a **control** for the color filter experiment?

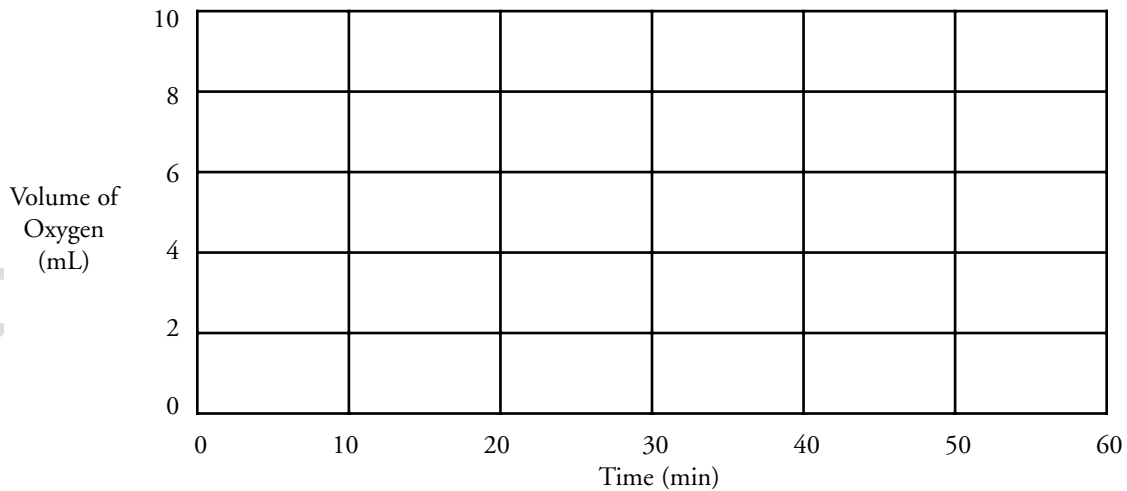
1. Pipette 10 mL of sodium bicarbonate solution into 5 tubes. Label the tubes A, B, C, D, & E.
2. Add 1 stalk of *Elodea* to each tube and fill the tube with more bicarbonate solution.
3. Cap each tube with a 50 mL beaker and quickly invert them together. Add a few mL of sodium bicarbonate to the inside of the beaker to prevent air leaks. This set-up will be demonstrated.
4. Record the initial volume of the air bubble in each tube. This is accomplished by marking the initial volume of fluid in the inverted centrifuge tube with a grease pencil.
5. Place a small box over tube A, put tube B 15-20 cm in front of a direct light source, and leave tube C out on the bench in diffuse room light. Tube D is placed the same distance from a direct light source as tube B but with a colored filter between the light and the tube. Your filter color(s) is/are _____. NOTE: if you are doing an experiment testing different filter colors, put all samples 15-20 cm in front of a direct light source with the filter cylinder over the tubes.
6. Every 10 minutes, check the height of the air bubble in each tube, marking it with a grease pencil as in Experiment A (with the one in the box, simply lift one side of the box and glance at the tube). Record your data in Table 1.2.

Table 1.2 Volume of Oxygen Production.

Time	Dark	Direct Light	Diffuse Light	Colored Filter
0 min				
10 min				
20 min				
30 min				
40 min				
50 min				
60 min				

Plot the volumes of gas produced for all reactions in Graph 1.2. Use your colored pencils so different reactions can easily be identified.

Graph 1.2 Oxygen Production by Elodea.



END OF CLASS DISCUSSION OF RESULTS FROM THIS EXPERIMENT(S)

What was the control for the light intensity experiment? for the color filter experiment?

What effect did the light intensity have on the rate of O_2 production?

What would this tell you about photosynthetic rates in plants in different levels of a forest canopy?

What effect did the filter color have on the rate of O₂ production?

Can you explain why certain filters would affect photosynthesis rates?

INVESTIGATION C: PAPER CHROMATOGRAPHY OF PHOTOSYNTHETIC PIGMENTS

Light must be absorbed before its energy can be utilized. A substance that absorbs light is called a pigment. Some of the pigments in plants that absorb light are called chlorophylls. Accessory pigments such as carotenes and xanthophyll also absorb light that may be used in photosynthesis. The light energy absorbed by accessory pigments is transmitted to chlorophyll, the primary photosynthetic pigment.

Paper chromatography is a technique for separating a mixture of compounds such as chlorophyll, carotene, and xanthophyll. When a solution of these pigments is applied to strips of paper, the pigments are adsorbed into the cellulose fibers of the paper. When the tip of the paper is immersed into a solvent, the solvent moves through and up the paper by capillary action. As the solvent moves through the spot where the mixture of pigments was applied, the pigments will dissolve in the moving solvent and move with it. However, all of the pigments cannot keep up with the solvent. Some pigments will move almost as fast as the solvent, while others will move more slowly. This differential movement of pigments is the result of each pigment having a characteristic tendency to stick (i.e., be adsorbed) to the cellulose fibers of the paper and a result of how soluble the pigment is in the solvent being used. A pigment's molecular size, polarity, and solubility in the solvent determine the strength of this tendency. Pigments adsorbed the strongest, and least soluble in the solvent, will move slowly, while those weakly adsorbed and highly soluble in the solvent will move fastest. Thus, each pigment has a characteristic rate of movement, and the pigments can be separated from each other. The relationship of distance moved by a pigment to the distance moved by the solvent is specific for a given set of conditions. This relationship is the R_f.

$R_f = \text{distance moved by pigment} / \text{distance moved by solvent}$

Thus paper chromatography can be used to help identify each pigment by its characteristic R_f value. **As a class, we will be conducting an investigation to determine:** How does the pigment composition of leaves change during autumn? **If the lab session does not happen during the autumn season, we will answer the question:** Do different plants contain the same proportions of photosynthetic pigments? OR do leaves of plants of the same type but grown under different light conditions contain the same proportions of photosynthetic pigments?

Write hypotheses related to the above questions. Consider the roles of different photosynthetic pigments (check your text).

Materials

fresh leaf material	
chromatography chamber	solvent (pet. ether: acetone; 95:5)
chromatography paper	mortar and pestle
acetone	toothpick

PROCEDURE

1. Pour 2-3 mL of chromatography solvent (composed of 95 parts petroleum ether and 5 parts acetone) into a large diameter test tube. Stopper the tube and set it in a rack to let the vapors reach equilibrium. **DO NOT INHALE THE VAPORS! DO NOT USE ANY FLAMES DURING THIS ACTIVITY!**
2. Examine the fresh leaf material. If you will be examining leaves in autumn, your instructor should have samples of leaves from the same tree that are in varying states of change from very green to just starting to turn to leaves that have achieved their full fall coloration. If you will be examining different plants, your instructor may have directed you to bring in your own fresh leaf material.
3. Take a leaf, cut it into small pieces, and grind it in a small volume (about 1 mL) of acetone with a mortar and pestle as demonstrated by your instructor.
4. Obtain a piece of chromatography paper (Whatman #1 paper, 1.5 x 12.5 cm). You can leave it straight on the end or you may cut a point (symmetrically) on one end. Make sure your hands are clean and handle the paper by the edges so that oil on your fingers does not contaminate the paper!
5. Make a pencil line 3 cm from the one end (the pointed end if you are using this method). **DO NOT USE A PEN (Why not?).**
6. With a toothpick, apply a small amount of sample to the pencil line on the chromatography paper. Blow dry the applied amount and repeat for 10-15x until a nice band of leaf extract is applied to the paper in a thin, dark line. If using a blunt end paper, gently crease the paper long-ways up the middle. This will help it stand up in the chromatography chamber (large test-tube with cork).
7. Place the paper strip in the tube containing solvent. Make sure the tip of the paper is well immersed in the solvent, but the solvent level is well below the pigment spot. Cork the tube right away!!!
8. Immediately begin observing the pigment bands that separate as the solvent moves up the paper. Remove the paper from the chamber as soon as the solvent gets within 2-3 cm from the top of the paper. Let it dry.
9. Draw the results of your paper chromatography experiment on the next page (see example on next page). Label each colored band with its name and R_f value. (KEY: chlorophyll b—yellow-green, chlorophyll a—bluegreen, xanthophyll—greenish yellow, carotenes—orangeish yellow).

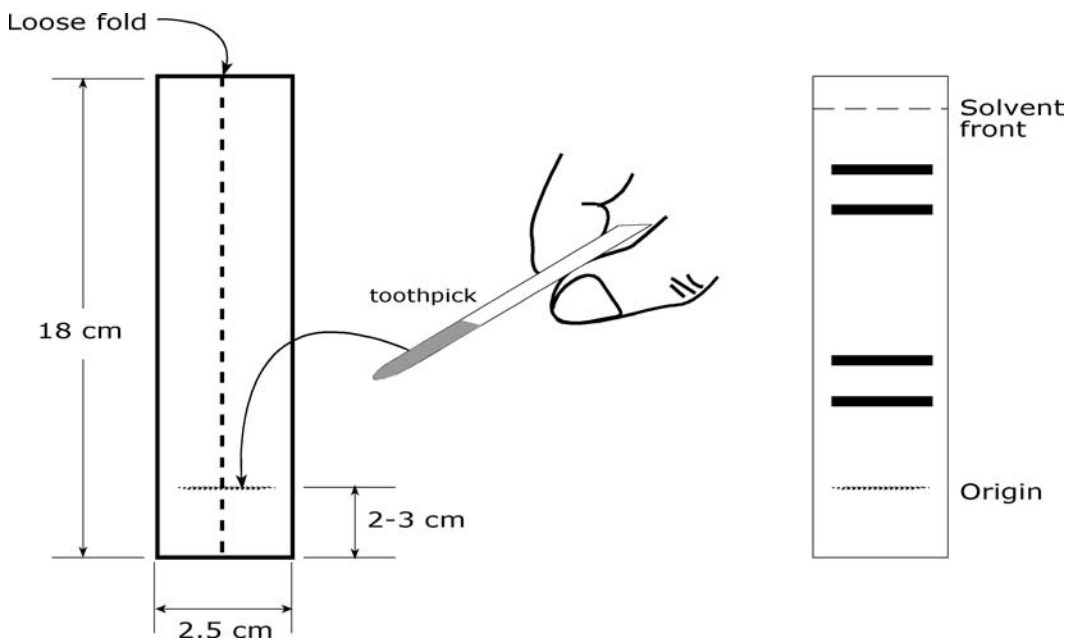


Diagram of chromatography procedure and results.

Table 1.3 Rf Calculations.

	Plant 1	Plant 2	Plant 3
Rf band 1			
Rf band 2			
Rf band 3			
Rf band 4			

END OF CLASS DISCUSSION FOR INVESTIGATION C

What does a small Rf indicate about the molecule?

Would you expect the Rf to change if we used a different solvent? Explain.

Which is more soluble in this solvent: xanthophyll or chlorophyll a?

Were there any other pigments visible in the sample in addition to primary photosynthetic pigments?

What causes leaves to turn yellow or red in autumn?

Is it possible to obtain R_fs of greater than 1? Why or why not?

During fluorescence, why is light emitted at a longer wavelength than was absorbed?

REVIEWING

Understand the method for measuring cellular respiration and rates of photosynthesis.

Understand why cells may use some sugars more readily than others.

Understand the effect of light intensity on photosynthesis.

Understand the chemical equations describing photosynthesis and respiration.

Understand how chromatography works. What things may influence levels of photosynthetic pigments found in leaves?