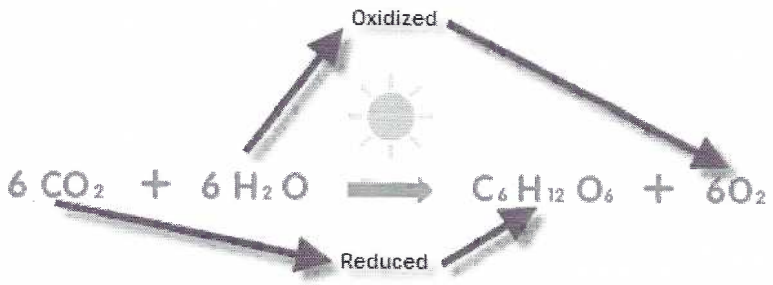
OBJECTIVES

- Design a plan for collecting data to show that all biological systems are affected by complex biotic and abiotic interactions.
- Use models to predict and justify that changes in the subcomponents of a biological polymer affect the functionality of the molecule.
- Analyze data to identify how molecular interactions affect structure and function.

BACKGROUND

Growth and reproduction are heavily energy-dependent processes that have driven organisms to evolve strategies, structures, and processes that enable them to capture, utilize, and store free energy. Free energy is available in the environment in a multitude of forms, and autotrophs and heterotrophs employ different approaches to harvest the energy they need to live. Photosynthesis and chemosynthesis enable autotrophs (or primary producers) to obtain free energy directly from their surroundings, whereas heterotrophs employ cellular respiration to produce energy. They must seek sources of food, and utilize the energy stored in carbon compounds produced by other organisms.

Autotrophs are "self-feeders". This name is derived from the Greek words *auto* (meaning "self") and *trophos* (meaning "feeder"). Multicellular plants are examples of photoautotrophs, organisms that produce organic molecules from light energy. Photosynthesis is the name of the process whereby photoautotrophs capture light energy present in the environment for use in growth, reproduction, and maintaining homeostasis.



The set of chemical reactions involved in photosynthesis transforms the substrates carbon dioxide and water, into glucose (a simple carbohydrate) and oxygen. The chemical bonds of the glucose molecule serve to store the transformed light energy until it is harvested in the process of respiration. Measuring the oxygen produced in this reaction is one way to measure the rate of photosynthesis.

In plants, chloroplasts are required to capture the energy that drives the reaction. Chloroplasts are membrane-bound organelles that contain a variety of photoreactive pigments, including the primary photosynthetic pigments - chlorophylls. Chlorophyll molecules absorb light energy in the red and blue portions of the spectrum, and reflect green wavelengths, making the chlorophyll, and thus plants, appear green to us. Accessory pigments in chloroplasts and leaves have

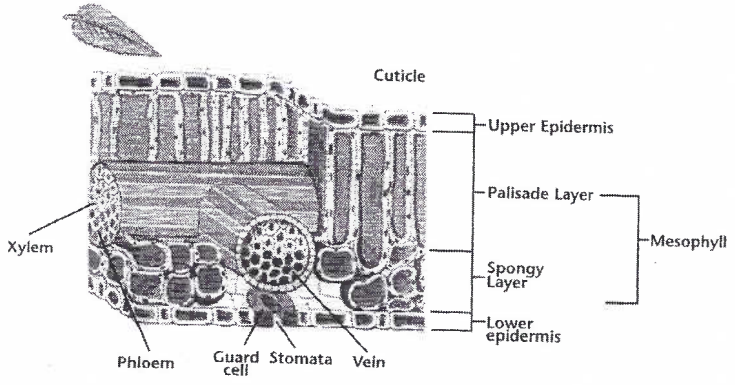
(continued on next page)

NOTES

BACKGROUND (CONTINUED)

other roles in regulating light energy. For example, yellow/orange/red carotenoids absorb high frequency ultraviolet light that can damage DNA. The pigments in chloroplasts are embedded in stacks of membrane and are hydrophobic. Some of the common pigments found in leaves are listed in order from most polar (hydrophilic) to least polar (hydrophobic) as follows: chlorophyll b, chlorophyll a, phaeophytin b, phaeophytin a, xanthophylls, carotene.

The terrestrial plant cells that are specialized for photosynthesis (and thus contain most of the chloroplasts and chlorophyll) are in the leaves. The structure of the leaf has evolved to regulate the photosynthetic reactions by regulating how the substrates of carbon dioxide and water are brought together with light to form carbohydrates and release oxygen. Guard cells in the lower epidermis of the leaf form pores called stomata that can be opened or closed to regulate gas exchange in the leaf under different environmental situations. One of the substrates for the photosynthetic reaction, CO_2, enters the leaf through the open stomata. The other substrate, H_2O, enters the leaf mostly through the vascular system of the plant, the xylem tubules. The palisade cell layer of a leaf has access to both substrates and consists of cylindrical cells that contain large numbers of chloroplasts. These are the cells primarily responsible for photosynthesis and thus energy capture in the plant. Carbohydrates (products of photosynthesis) can be transported out to other parts of the plant through the phloem of the vascular system. Oxygen (another product of photosynthesis) passes into the spongy mesophyll layer. This layer contains air chambers that expand as the oxygen is produced and then released through the stomata into the environment. Alternatively, the carbohydrates and oxygen can be used as substrates for respiration in the leaf cells.



NOTES

SAFETY PRECAUTIONS

- **When working with the chromatography solvent, use a chemical fume hood or proper ventilation.**
- As general safe laboratory practice, it is recommended that you wear proper protective equipment, such as gloves, safety goggles, and a lab apron.
- As general lab practice, read the lab through completely before starting, including any Material Safety Data Sheets (MSDSs) and live materials care sheets at the end of this booklet as well as any appropriate MSDSs for any additional substances you would like to test. One of the best sources for the material is the vendor. For example, when purchased at Wards, searching for the chemical on the Ward's website will direct you to a link for the MSDS. *(Note: There are no live materials care sheets included in this particular lab.)*

At the end of the lab:

- All laboratory bench tops should be wiped down with a 10% bleach solution or disinfectant to ensure cleanliness.
- Wash your hands thoroughly with soap and water before leaving the laboratory.

PROCEDURE TIPS

- When performing this lab activity, all data should be recorded in a lab notebook. You will need to construct your own data tables, where appropriate, in order to accurately capture the data from the investigation.
- If directed to do so by your teacher, this part of the lab may be done at the same time as Part 2 of the lab.

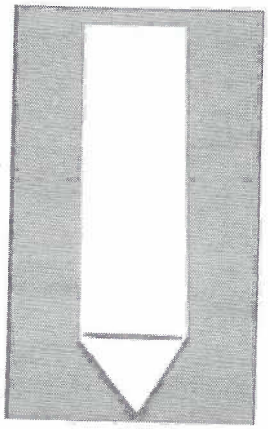


Figure 1

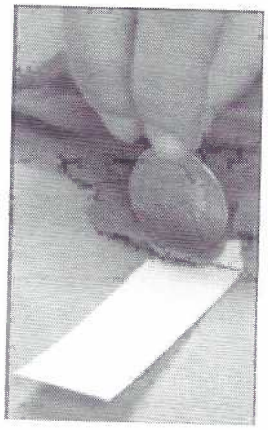


Figure 2

PART 1 – STRUCTURED INQUIRY: PLANT PIGMENTS AND CHROMATOGRAPHY

MATERIALS NEEDED PER LAB GROUP

- ❑ 1 Chromatography vial, with cap (provided)
- ❑ 1 Wax pencil
- ❑ 1 Disposable transfer pipet (provided)
- ❑ 1 mL Chromatography solvent (acetone:ethyl alcohol::1:1. (provided)
- ❑ 1 Chromatography paper strip (provided)
- ❑ 1 Sharp pencil
- ❑ 1 Ruler (metric)
- ❑ 1 Pair of scissors
- ❑ 1 Piece of fresh (pre-soaked) spinach
- ❑ 1 Coin (a quarter works well)
- ❑ 1 Pair of forceps

PART 1 – PROCEDURE: STRUCTURED INQUIRY

1. Obtain a chromatography paper strip.
   - NOTE: Be sure to handle the chromatography paper by the edges. Do not touch the surface of the strip with your fingertips as the oils from your fingers will interfere with the chromatogram.
2. Measure 1.5 cm from one end of the chromatography strip and lightly draw a pencil line across the width of the strip. This is the point of application (see Step 4).
3. Use a pair of scissors to cut off two small pieces below the pencil line to form a pointed end (see Figure 1). The pointed end will be referred to as the bottom end of the chromatogram.
4. Obtain a well-hydrated leaf of spinach which, has been pre-soaked to jump-start the process of photosynthesis. Place it over the point of application on the chromatography strip. Rub or roll the ribbed edge of a coin over the spinach leaf to extract the pigments. Repeat 5-10 times with different portions of the spinach leaf, making sure you are rubbing the coin over the pencil line (see Figure 2).

(continued on next page)

NOTES

PROCEDURE – PART 1: STRUCTURED INQUIRY (CONTINUED)

5. Obtain a chromatography vial from your teacher and label it with your initials using a permanent marker or wax pencil.

   ➲ **CAUTION: Steps 6-11 should be performed under a fume hood or in a well ventilated area.**

6. Go to a fume hood or a well ventilated area and remove the cap from the chromatography vial. Using a disposable pipet, add 1 mL of chromatography solvent to the vial. The solvent is a volatile organic compound (hydrophobic) and a fume hood is required to capture the volatile fumes.

7. Carefully place the chromatography paper strip into the vial so that the pointed end is barely immersed in the solvent. Do not immerse the pigments into the solvent.

8. Cap the vial and leave it undisturbed. Observe as the solvent is drawn up the chromatography paper strip by capillary action.

9. Record the different colors you observe as they separate along the strip.

10. When the solvent reaches a level approximately 1 cm from the top of the strip, remove the cap from the vial. Using forceps, remove the strip from the vial. This is your chromatogram.

11. The solvent will evaporate quickly; immediately use a pencil to mark the location of the solvent at the top end of the chromatography paper strip. This is the solvent front.

    ➲ NOTE: You will need this location to identify the distance the chromatography solvent traveled.

12. List and record the pigment colors or names. Once the strip has completely dried, mark the middle of each pigment band on the chromatography paper strip with a pencil.

13. Using a metric ruler, measure the distance from the original pencil line with the spinach extract to the solvent front and each mark you made on the pigment bands (see Figure 3). Record these distances in millimeters (mm).

14. Calculate the R_f value for each pigment on your chromatogram.

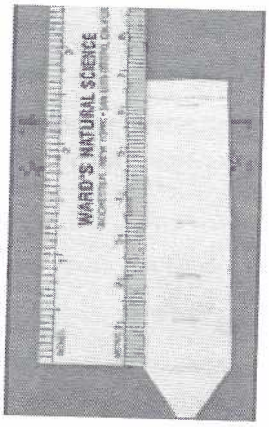


Figure 3

$$R_f = \frac{\text{Distance traveled by component from point of application}}{\text{Distance traveled by solvent from point of application}}$$

PROCEDURE TIPS

- When performing this lab activity, all data should be recorded in a lab notebook. You will need to construct your own data tables, where appropriate, in order to accurately capture the data from the investigation.
- If directed to do so by your teacher, this part of the lab may be done at the same time as Part 1 of the lab.

PART 2 – GUIDED INQUIRY: FLOATING DISC ASSAY

MATERIALS NEEDED PER LAB GROUP

❑	10 mL	0.2% sodium bicarbonate solution with dish soap added
❑	100 mL	0.2% sodium bicarbonate solution
❑	1	Disposable beaker 100 mL (or plastic cup)
❑	1	One-hole punch
❑	1	Timer
❑	1	Light source
❑	1	10 mL syringe
❑	1	Piece of fresh (pre-soaked) spinach

PART 2 – PROCEDURE: GUIDED INQUIRY

1. Obtain a leaf of spinach that is well hydrated, and has been pre-soaked to jump-start the process of photosynthesis.
2. Use a one-hole punch to cut discs from the leaf (at least 10 discs per trial).
   - Each trial requires at least 10 leaf discs. When cutting discs, avoid major veins, aberrant tissue, and leaf edges. Make an effort to obtain consistent discs.
3. Remove the plunger from the barrel of a 10 mL syringe. Place the leaf discs inside the barrel, and tap to get them down to the bottom of the syringe barrel. Replace the plunger at the top of the syringe barrel and depress it. Push the plunger in enough to expel most of the air from the syringe.
4. Fill the barrel of the plunger half full with the sodium-bicarbonate/dish soap mixture. Invert the syringe (air bubble floats to syringe tip) and depress the plunger farther to remove air from the syringe.
   - **NOTE:** The liquid soap in the sodium bicarbonate aids in wetting the normally hydrophobic surface of the leaf. The surface tension of the water is broken by the addition of the soap, and the leaf surface can become wet with the solution in order to allow it to infiltrate the pores on the leaf surface, and fill the intercellular spaces in the spongy mesophyll.

(continued on next page)

NOTES

PROCEDURE – PART 2: GUIDED INQUIRY (CONTINUED)

5. Hold your finger over the hole at the end of the syringe and draw back on the plunger to form a vacuum within the chamber of the syringe. Hold this for 10 seconds while swirling the syringe to wet the leaf discs. Repeat until all of the leaf discs sink under normal pressure. This means that the solution has infiltrated the spongy layer of the leaf.
   - Do not use leaf discs that do not sink.
   - Additional soap (up to double the concentration) may be added if leaves will not sink.
   - A negative control may be set up with water and soap, but no bicarbonate.
6. Remove the plunger from the syringe and pour the discs and solution into a clear plastic cup containing 0.2% sodium bicarbonate (or plain water for a negative control) at a depth of about 3 cm. Be sure to place all of the discs in the bottom of the cup. The sodium bicarbonate serves as the source of CO_2 necessary for photosynthesis to occur. Keep the depth of the solution in the cup consistent throughout the trials.
7. Place the reaction vessels under a bright light source. Start the timer immediately.
   - Hint: Put the light source as close to the experimental beakers as possible.
8. Record the number of discs that are floating at 30 second intervals.
9. Graph your results over time for bicarbonate and water-only conditions.
10. Explain your results.

Name: _____

ASSESSMENT QUESTIONS (FOR PARTS 1 AND 2)

1. Which pigment migrated the farthest on the chromatogram? Explain how this migration occurred.

2. What does the R_f value represent? If you were to perform your experiment on a chromatography paper twice the length of the one used, would your R_f values still be the same?

3. How do plant pigments and the absorption spectrum relate to photosynthesis?

4. Name at least four parameters that will affect the rate of photosynthesis as measured by this investigation. How does each parameter have bearing on the reactions of photosynthesis?

5. What might happen if you were to remove all light from the setup after the discs have all become buoyant? Describe what you would see. Explain why this would occur with relation to cellular processes like respiration.

EXPERIMENT DESIGN TIPS

The College Board encourages peer review of student investigations through both formal and informal presentation with feedback and discussion. Assessment questions similar to those on the AP exam might resemble the following questions, which also might arise in peer review:

- Explain the purpose of a procedural step.
- Identify the independent variables and the dependent variables in an experiment.
- What results would you expect to see in the control group? The experimental group?
- How does a specific concept (XXXX) account for described findings (YYYY)?
- Describe a method that could be used to determine a given concept/observation (XXXX).

PART 3 – OPEN INQUIRY: DESIGN AN EXPERIMENT

What questions occurred to you as you performed the investigations? Now that you are familiar with photosynthetic pigments, chromatography, and a photosynthesis rate assay, design an experiment to investigate one of your questions.

Questions may include:

How is the rate of photosynthesis in leaves related to pigments in the leaf? How does the amount (intensity) or wavelength of light affect the rate of photosynthesis? Does temperature or pH affect the rate of photosynthesis? Is there a variation in chloroplast density in different leaves that affects photosynthesis? Does the age of the leaf or plant that the leaf came from affect the rate of photosynthesis? Does the habitat in which the plant evolved affect pigments or rates of photosynthesis? Does the time of year that the leaf was collected affect pigments or photosynthetic rate? What other aspects of the leaf or plant environment might affect photosynthetic rates?

Before starting your experiment, plan your investigation in your lab notebook. Have your teacher check over and initial your experiment design. Once your design is approved, investigate your hypothesis. Be sure to record all observations and data in your laboratory sheet or notebook.

Use the following steps when designing your experiment.

1. Define the question or testable hypothesis.
2. Describe the background information. Include previous experiments.
3. Describe the experiment design with controls, variables, and observations.
4. Describe the possible results and how they would be interpreted.
5. List the materials and methods to be used.
6. Note potential safety issues.

After the plan is approved by your teacher:

7. The step by step procedure should be documented in the lab notebook. This includes recording the calculations of concentrations, etc., as well as the weights and volumes used.

(continued on next page)

NOTES

PART 3: OPEN INQUIRY (CONTINUED)

8. The results should be recorded (including drawings, photos, data print-outs).
9. The analysis of results should be recorded.
10. Draw conclusions based on how the results compared to the predictions.
11. Limitations of the conclusions should be discussed, including thoughts about improving the experiment design, statistical significance and uncontrolled variables.
12. Further study direction should be considered.