This teacher's guide is designed for use with the *Photosynthesis* series of programs produced by TVOntario, the television service of The Ontario Educational Communications Authority. The series is available on videotape to educational institutions and nonprofit organizations.

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**Contents**

*Introduction* ................................................................. 1

*Seeing the Light* .............................................................. 2

*Absorbing the Light* ........................................................ 6

*The Light Reaction* .......................................................... 10

*The Dark Reaction* .......................................................... 13

*C3 and C4 Plants* ........................................................... 16

*The Fluid-Transport System* ............................................. 19

*Bibliography* ................................................................. 23

*Ordering Information* ....................................................... 24

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Introduction

The *Photosynthesis* series of six 10-minute programs is intended to convey a basic understanding of biological principles to senior highschool students.

This teacher's guide gives a more detailed account of the material in each program. It also suggests related before-viewing activities, which are directed at teachers. After-viewing activities are directed at students, and can be photocopied and distributed. These activities include laboratory exercises, model building, research assignments, and even the dramatic simulation of the events of photosynthesis. A bibliography at the end provides highschool and college-level references for further study.

The series begins with a historical survey of early ideas and experiments in the area of photosynthesis; many of the latter can be duplicated in a highschool lab. The first program, "Seeing the Light," develops the chemical equation that represents photosynthesis, and introduces the light and dark reactions and the morphological features of photosynthetic organs.

"Absorbing the Light," builds on the morphological theme at the molecular level, exploring the various pigments involved in photosynthesis. It encourages students to study the absorption spectra of plant pigments and devise means of separating and identifying them.

"The Light Reaction" traces the pathways of electrons and protons through the thylakoids, introducing students to the major electron carriers. In "The Dark Reaction," students gain an understanding of the Calvin cycle's complex series of reactions at the molecular level.

The two final programs follow up some interesting related concepts. "C3 and C4 Plants" investigates the C4 plants to discover why they have evolved in some tropical areas, but have failed to displace C3 plants anywhere. "The Fluid-Transport System" explains the position of plants in the ecosystem. The problem of getting water to the photosynthetic machinery at the tops of tall trees is considered.

The series should convey the complexity and sophistication of plants, and spark students' curiosity to pursue further studies.
Objectives

After viewing this program, students should be able to do the following:

1. Identify the reactants and products of photosynthesis.
2. Write a balanced chemical equation for photosynthesis.
3. Describe, in a general way, the contributions of the light-dependent and light-independent (dark) reactions.
4. Name the site of photosynthesis in plants.
5. Describe a use of isotopes of elements in the study of biology.

Program Description

The earliest experiments in photosynthesis established the identities of reactants and products and the need for light to drive the process. In 1771, Joseph Priestley found that "something" in air supported the burning of candles and the breathing of animals was restored by plants. A few years later, Jan Ingen-Housz learned that this restoration of air occurred only in light and that only the green parts of the plant were able to do it. Later Jean Senebier found that carbon dioxide was used up in the process and assumed that this was the source of the oxygen released. In later years, water was recognized as both a reactant and product of photosynthesis, and chlorophyll as a necessary participant.

Photosynthesis is represented by this chemical equation:

\[ 6CO_2 + 12H_2O \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O \]

The overall reaction of photosynthesis has been broken up into two complex series of reactions: the light-dependent reactions use the "old" water and give off oxygen; the light-independent (dark) reactions combine products of light-dependent reactions with carbon dioxide to form organic matter and release "new" water as a waste product.

Anatomically, the reactions of photosynthesis occur in the parts of a plant that contain chloroplasts. These are most abundant in the leaves, particularly in the palisade cells located near the upper surface of the leaf. Within the chloroplast, elaborate systems of membranes, the lamellae, organize chlorophyll molecules so they present an immense surface area to the light.

Before-Viewing Activities

1. Discuss the general nature of photosynthesis and its importance in gathering and storing energy in a form that can be used by other members of the biological community.

2. Look at photosynthesis from a human point of view and discuss its importance in concentrating energy as biomass and fossil fuels for space heating, transportation, and powering industry, as well as its role as feedstock for the organic chemicals industry.

After-Viewing Activities

**ACTIVITY 1. Examining a Chloroplast**

Locate an electron micrograph of a chloroplast and trace or photocopy it. Identify the outer membrane, stroma, lamellae, thylakoids, and grana. While it is a two-dimensional structure, calculate the approximate surface area to volume ratio (ratio of total length of membranes vs. cross-sectional area) of the organelle as it appears and as it would, if there were no internal membranes.
Method (see Fig. 1.1)

1. Fill the jar about 3/4 full with sodium bicarbonate solution.
2. Invert the funnel (stem upwards) with the wire mesh screen inside.
3. Place the Elodea plants around the wire mesh, but inside the funnel wall. The base of the Elodea stems should point upwards.
4. Place the funnel, wire mesh, and Elodea in the container.
5. Fill the test tube with sodium bicarbonate solution and invert it over the stem of the funnel.
6. Place the apparatus in the light for one or two days, or until enough gas has collected in the tube to be tested.
7. Test the gas collected in the test tube by plunging a glowing splint into the gas.
8. Record all observations and draw appropriate conclusions.

Discussion Questions

1. Why was sodium bicarbonate supplied to the plants? Why was the solution aerated?
2. What gases might have collected in the test tube? For each gas, explain where it may have come from.
3. How is this experiment similar to Priestley’s experiment?

ACTIVITY 3. Are Light, Carbon Dioxide, and Chlorophyll Necessary for Photosynthesis?

The following supplies and procedures are common to Parts A, B, and C of this activity.

Apparatus for testing leaves

Beaker
Large test tube
Forceps
Watch glass or Petri dish
Light source or sunny window
Safety goggles
Wet towel to act as emergency fire extinguisher
Boiling water (use electric kettle or coffee urn if possible)
Denatured ethanol (CAUTION: INFLAMMABLE)
Lugol's iodine solution

Method for Testing Leaves for the Presence of Starch

Caution: Wear safety goggles while working with chemicals and flames and work only from a standing position!

Do not use an open flame to boil ethanol nor to boil water while ethanol is being heated in the room.

1. Remove the test leaf from the plant.
2. Using forceps, immerse the leaf for 30 seconds in boiling water in the beaker.
3. Add about 30 mL of ethanol to the test tube and immerse the leaf in it.
4. Put the test tube into the beaker of boiling water.
5. Leave the leaf in boiling ethanol for four minutes, replacing the water in the beaker as necessary to maintain boiling.
6. Remove the leaf from the ethanol with forceps and dip it in boiling water for 30 seconds.
7. Flatten the leaf in a watch glass or Petri dish and cover it with Lugol's iodine solution. After two minutes, examine the leaf for black patches that indicate the presence of starch in the treated leaf.

Discussion

1. Account for the distribution of starch in the leaf as seen with the naked eye and with the microscope.
2. Why was the plant kept in the dark for 24 hours prior to the experiment?
3. Was there a "control" in this experiment? Explain.

PART B: Is Carbon Dioxide Necessary for Photosynthesis?

Apparatus

Supplies to test leaves for starch (described previously)
Potted geranium plant (kept in the dark for 24 hours)
Paperclip
Cross (about 2 cm x 2 cm) cut from black cardboard
Compound microscope

Blank microscope slide
Cover slip for microscope slide
Glycerine (in dropper bottle)

Method

1. Remove the geranium plant from storage and test one of its leaves for starch. Immediately fasten the cross to the upper surface of one of its leaves.
2. Place the plant in the light for a day.
3. Remove the treated leaf and test it for the presence of starch.
4. Tear a small piece of leaf from an area that tested positive for starch and mount it (bottom surface upwards) in glycerine on a microscope slide. After two minutes, examine the section with medium and high magnifications and describe the distribution of black color in the leaf.

PART A: Is Light Needed for Photosynthesis?

Apparatus

Supplies for testing leaves (outlined previously)
Potted geranium plant (kept in the dark for 24 hours)
Paperclip
Cross (about 2 cm x 2 cm) cut from black cardboard
Compound microscope
Method

Caution: Wear safety goggles while handling sodium hydroxide. If you get any chemical on your hands, wash them thoroughly.

1. Remove the geranium plant from storage.
2. Place about 10 pellets of sodium hydroxide into the flask.
3. Place the rubber stopper over the petiole of one leaf, with the broad end toward the stem of the plant.
4. Seal the blade of the leaf in the flask, using vaseline to complete the seal.
5. Support the flask so the leaf will not be damaged.
6. Expose the plant to light for a day.
7. Disassemble the apparatus and test the leaf from the flask and one other leaf from the plant for the presence of starch (method for testing for the presence of starch described previously).
8. Explain your observations.

ACTIVITY 4. Review

1. Write a balanced chemical equation for the overall reaction of photosynthesis.
2. Explain why early conclusions about photosynthesis were drawn by physicians and clergymen rather than by scientists.
3. If the hydrogen of water supplied to a plant during photosynthesis were labelled in some way, where would it be found following photosynthesis? Explain.
4. Sketch the appearance of a chloroplast as it would be seen in cross-section with the transmission electron microscope and label its parts.

PART C. Is Chlorophyll Necessary for Photosynthesis?

Apparatus

Supplies to test leaves for starch (described previously)
Potted variegated coleus plant (white/green)

Method

1. Place a variegated coleus plant in the light for a day.
2. Perform a starch test on one of its leaves (method for testing for the presence of starch described previously).
3. Explain your observations.
PROGRAM 2 / Absorbing the Light

Objectives

After viewing this program, students should be able to do the following:

1. Describe the internal organization of a chloroplast.
2. Associate the light reactions with thylakoids.
3. Explain the concept of "spectrum" and apply it to absorption, reflection, and transmission of light by leaves.
4. Identify the pigments found in chloroplasts.
5. Distinguish between the roles of antenna pigments and reaction centre pigments.
6. Explain how organic molecules absorb light in the visible range.
7. Identify the two photosystems and account for their names.

Program Description

Chlorophyll is located in membranes within chloroplasts. The membranes are called lamellae and, at points along their length, form disk-like expansions, the thylakoids. Thylakoids form stacks within the chloroplast. These stacks are the grana. The lamellae, and especially the thylakoids, are the location of the light-dependent reactions of photosynthesis.

If visible light is separated into its component colors, a spectrum results. Different spectra can be obtained for leaves, depending on whether the light examined has been reflected, absorbed, or transmitted through the leaf.

The wavelengths of the absorption spectrum are most important because they represent the portion of the sun's light that may be available for the synthesis of carbohydrates.

The major pigments of photosynthesis are the chlorophylls. Chlorophyll a and chlorophyll b are very similar chemically, with a methyl group on chlorophyll a being replaced by a carbonyl group on chlorophyll b. Accessory pigments, such as carotenoids, enable the plant to absorb additional wavelengths of light and may increase the efficiency of photosynthesis. They are also responsible for the brilliant colors of foliage every autumn. It is common to refer to these pigments as "antenna" pigments, since they gather energy and direct it to a reaction centre where it is utilized. The reaction centre always contains chlorophyll a.

Although the various pigments have different roles and chemical structures, they share one feature. All possess a series of alternating single and double bonds within the molecule. These confer the ability to absorb light in the visible range, but each molecule absorbs only a few well-defined wavelengths. Thus, the existence of many different pigments in slightly different chemical environments extends the range of wavelengths (photons) that can be absorbed by the chloroplast and increases the efficiency of photosynthesis.

The groupings of antenna pigments and their associated reaction centres constitute a photosystem. There are two photosystems spread through the lamellae. These are named on the basis of the wavelength absorbed by their reaction centres as P 680 and P 700. Each plays a different role in the light reactions and both are vital to photosynthesis.

Before-Viewing Activities

1. Discuss how a prism or diffraction grating separates different wavelengths or colors of light; follow up with an examination of why chemicals such as dyes and food coloring appear colored when examined in "white" light.
2. Introduce the units (nanometres) used to measure wavelengths of light and develop in students some feeling for the values, in nanometres, of visible light ranging in color from violet to red.
After-Viewing Activities

ACTIVITY 1. Light Sources

Apparatus

Hand spectroscope, student grade (e.g., Science Kit Inc.)
Fluorescent light source (e.g., classroom lights)
Incandescent light source (or several with different wattages)
Fresh leaf

Method

1. If using a "Science Kit" hand spectroscope, note the square hole at the narrow end. This is the viewing port. At the wide end, there is a narrow slit through which light to be analyzed enters the instrument. There is also a long rectangular opening that provides background illumination for a numerical scale that is part of the instrument. Each division of the scale corresponds to 10 nm. The numbers on the scale should be multiplied by 100 to convert them to nm.

2. Aim the instrument so that light from a fluorescent light fixture passes through the slit and look through the viewing port. You will see multiple colored images of the slit superimposed over the scale. If you wear glasses for near-sightedness, removing them may enable you to read the scale with greater ease. Note the bright bands in the spectrum. These correspond to the major emission bands for the fluorescent bulb. Sketch the appearance of the spectrum, including only the part that is over the scale.

3. Repeat the observations and sketches, using each of the following as light sources:
   a) incandescent bulb
   b) sunlight
   c) sunlight reflected from the surface of a leaf
   d) sunlight passed through a leaf

4. Compare the four spectra and comment on the significance of any differences.

Discussion

Plant growth chambers often use a combination of fluorescent and incandescent light bulbs to grow plants artificially. The incandescent bulbs are of low power output (e.g., 40 watts). Explain why using this combination of lights is a good practice.

ACTIVITY 2. Absorption Spectrum of Spinach

Apparatus

Hand spectroscope
Focused light source (e.g., fibre-optics microscope illuminator or slide projector with hole cut by a paper punch in the middle of a cardboard square, sized to fit the projector)
Two 30 mL plastic disposable tissue culture flasks with caps
Mortar and pestle
Small funnel
Beaker
Large test tube
Forceps
Safety goggles
Wet towel (for emergency fire extinguisher)
Fresh spinach leaves
Clean sand
Source of hot water (e.g., electric kettle or coffee urn)
Denatured ethanol
Sucrose solution, 0.25 mol/L

Method

1. Noting the safety precautions, follow the procedures of Activity 3 in the previous chapter (see page 4) to extract chlorophyll from one or two spinach leaves, but do not dip the leaves in boiling water a second time. The leaves may be discarded and need not be tested for the presence of starch. Collect the extract of chlorophyll into one of the tissue culture flasks and cap the flask.
2. Prepare a suspension of spinach chloroplasts by grinding a spinach leaf (with a pinch of sand) with 30 mL of sucrose solution in the mortar. When the suspension is as fine as you can get it, decant it into the second tissue culture flask and cap the flask.

3. For each flask, observe the spectrum of a light beam passed through the contents from top to bottom of the flask (shortest dimension) and observe, with the unaided eye, any light scattered at right angles to the beam. Record all observations.

4. Save the chlorophyll extract in ethanol for use in Activity 3. (Your teacher may wish you to use the chloroplast suspension in sucrose for the Hill reaction in the next chapter, if this is to be performed during the same laboratory period. Check with the teacher before discarding it.)

Discussion

1. Compare the absorption of light wavelengths of each preparation with that of the leaf as a whole (Activity 1).

2. The scattered light you observed with the unaided eye is a demonstration of "fluorescence". Discuss the significance of this light and comment on the different fluorescent properties (if any) between extracted chlorophyll and extracted chloroplasts.

**ACTIVITY 3. Separation of Pigments**

**Apparatus**

- 100 mL graduated cylinder
- Rubber stopper, #9
- Small beaker
- Pair of scissors
- Ruler
- Thumbtack
- Hair dryer (optional)
- Filter paper (Whatman #1) strip, 25 cm x 2.5 cm
- Extract of chlorophyll in ethanol (see Activity 2)
- Chromatography solvent consisting of 12 mL of 90% acetone mixed with 100 mL of petroleum ether (100-120 degrees)

**Method**

Caution: Perform this step in a fume hood. Acetone fumes can cause liver damage and/or cancers

1. Place 10 mL of chromatography solvent into the cylinder and insert the stopper firmly in the top of the cylinder. This must be left standing for at least half an hour to allow the solvent vapors to saturate the air inside, before it is used in step 6.

2. Transfer the chlorophyll extract into the beaker.

3. Fold the filter paper transversely at a point about 5 cm from one end.

4. Build up a dark band of chlorophyll extract along the fold by dipping the fold into the extract in the beaker until it just wets the paper, withdrawing the paper, and drying the extract. Repeat the process until there is a dark band of chlorophyll extract along the fold. This requires great patience, since each application must be dried before the next can be applied. However, the darker the chlorophyll band, the easier it will be to carry out the last step.

5. Trim the paper so that the chlorophyll band is about 3 cm from the end. Cut the width of the strip to 1.5 cm, choosing that portion of the paper having the most uniform band of chlorophyll extract. Trim the last 2.5 cm off the end near the extract of chlorophyll to a uniform, symmetrical point.

6. Working in the fume hood, set up the filter paper strip in the apparatus so that the point, but not the chlorophyll extract, is immersed in the solvent at the bottom. The strip will have to be trimmed to fit your cylinder (about 22 cm long) and may be gently folded so that it will hang straight in the cylinder. Use the thumbtack to fasten the filter paper to the rubber stopper. See Fig. 2.1 for help in setting up the apparatus.

7. Allow the solvent to be absorbed and move up the paper. It will take about an hour for the solvent to reach the top gradation on the
8. At that time, remove the paper and, working in the fume hood, mark the position of the solvent front and circle each colored spot on the paper. This is best done with pencil before the paper is completely dry.

After the paper has dried, take it to your desk and, for each spot, calculate the ratio of the distance travelled by the colored compound (to the centre of density of its spot) to the distance travelled by the solvent. This should be a constant (the $R_F$ = ratio of fronts) for each substance.

5. How did you distinguish between chlorophyll $a$ and chlorophyll $b$ in this experiment?

**ACTIVITY 4. Research**

Association of a particular function with a specific pigment often requires study of an action spectrum in which the dependence of the function on light wavelengths is compared with the absorption spectrum of the pigment suspected of mediating the function. A classic study of the action spectrum of photosynthesis was performed by T.W. Engelmann. In the library, do research on Engelmann's experiment and describe it in a short written report (about 300 words).

**ACTIVITY 5. Review**

1. Name three major pigments involved in photosynthesis and discuss the role played by each.

2. Explain why leaves turn yellow, orange, or red in autumn, or when they are diseased.

3. Evaluate chromatography as a means of identifying pigments in a complex mixture of pigments.

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**Discussion**

1. How many pigments were in your leaf extract?
2. What is the value of your "ratio of fronts" for the pigments you think are probably chlorophyll $a$, chlorophyll $b$, and carotenoids?
3. Compare your "constants" with those obtained by other students for the same pigments. Account for any differences.
4. Why do pigments travel different distances? What property distinguishes a pigment that travels a long distance from one that travels a short distance?
Objectives

After viewing this program, students should be able to do the following:

1. Cite the importance of double bonds in determining a molecule's ability to absorb light.
2. Explain the concepts of oxidation and reduction.
3. Identify water as the source of electrons, and oxygen gas as a waste product.
4. Define photolysis.
5. Identify and explain the role of the two photosystems in the light reaction.
6. Explain the concept of a proton pump with specific reference to plastoquinone.
7. Relate phosphorylation of ADP to a flow of protons down a proton gradient.

Program Description

Within the chloroplasts of green plants are abundant thylakoids. Here reside the essential components of the light reactions of photosynthesis. Within every stack of thylakoids are about 200 photosynthetic units, each containing about 300 pigment molecules.

These chlorophyll and carotenoid molecules act together as an "antenna," absorbing photons and channelling their energy to a single reaction centre. The reaction centre contains a single molecule of chlorophyll a near an electron acceptor molecule. The reaction centres P 680 and P 700 are named on the basis of the longest photon wavelength (measured in nanometres) that they can utilize.

The existence of conjugated double bonds (i.e., alternating single and double bonds) within each molecule determines the wavelengths of light absorbed by that molecule. In fact, when a chlorophyll molecule absorbs a photon, the molecule becomes "excited" and places an electron from a double bond into one of its outer orbitals. An electron acceptor molecule near the reaction centre can collect such a "loose" electron from the chlorophyll molecule. Any electron lost by the chlorophyll must be replaced, if the process is to continue and perform useful work. For P 680, replacement electrons are drawn from water in a process known as photolysis.

The electron transport system, a series of molecules in the thylakoid membrane, carries electrons to P 680 and P 700 and from P 700 to NADP. NADP is the coenzyme that supplies electrons and hydrogen nuclei to reduce carbon compounds in the dark reactions of photosynthesis.

Near the beginning of the electron transport system is a special carrier, plastoquinone (PQ), which is fairly mobile within the thylakoid membrane. Oxidized PQ is at the outer edge of the membrane. As it receives electrons from P 680 it combines them with protons from the stroma (fluid surrounding the thylakoid) to complete its reduction. The reduced plastoquinone, PQH2, migrates to the inner edge of the membrane before becoming oxidized again. There it passes its electrons to the next carrier in the sequence but the protons are released into the thylakoid interior. Then the oxidized plastoquinone returns to the outer edge of the membrane to repeat the process.

During the process of photolysis, protons are also released into the thylakoid interior. This leads to an increased concentration of protons within the thylakoid (lower pH) and a decreased concentration in the stroma (higher pH). The result is a proton gradient across the thylakoid membrane. A channel through the thylakoid membrane permits the spontaneous flow of protons back into the stroma. This flow of positive charge constitutes an electrical current which can be made to do work.

This is accomplished by a complex of enzymes, the CF1 particle, which traps the protons and harnesses their energy for the phosphorylation of ADP to form ATP, used in the dark reactions of photosynthesis.
Before-Viewing Activities

1. Review the overall equation for photosynthesis, the ultrastructure of the chloroplast, and the identification of pigments. As students' background knowledge may vary, it may also be necessary to review (or teach) the concepts of oxidation, reduction, and oxidation/reduction potential.

2. It may be helpful to compare the behavior of biological systems to that of an electrochemical cell.

ACTIVITY 1. Discussion of Cyclic Photophosphorylation

The electron transport system carries electrons from water to NADP. Additional ATP can be generated by "cyclic photophosphorylation," and class discussion of the process would be useful. Electrons are pumped to a high-energy state by P700 then shunted back to the beginning of the electron transport system, generating more phosphorylating capacity.

ACTIVITY 2. The Hill Reaction

This exercise mimics experiments done in the late 1930s and early 1940s by Robert Hill and his associates. They attempted to find out whether isolated chloroplasts, exposed to iron ions as electron acceptors, would give off oxygen in the presence of light. Since oxygen is difficult to analyze, you will attempt the experiment using DCPIP (2,6 dichlorophenolindophenol) as the electron acceptor. The E° value of DCPIP places it at about the midpoint of the electron transport system. Reduction of the DCPIP in the presence of chloroplasts will suggest that electron flow is taking place. Note any color changes that occur as DCPIP oscillates between oxidized and reduced states.

Apparatus

- Fresh spinach
- Two 30-mL disposable plastic culture flasks
- Mortar and pestle
- Two tubes suitable for centrifuging
- Centrifuge
- Microscope slide and cover slip
- Compound microscope
- Overhead projector

* Spinach from a cellophane package may have been stored too long for this experiment to achieve its desired results.
Eyedropper calibrated at 1.0 mL
Fine wire screen
50-mL calibrated beaker
Aluminum foil
Clean sand
3.5% sucrose solution
0.02% DCPIP solution

Method

1. Place a little sand in the bottom of the mortar.
2. Add part of a leaf (about 4 g) of fresh spinach.
3. Add about 25 mL of sucrose solution (use beaker to measure).
4. Grind the spinach thoroughly with the pestle.
5. Strain the ground spinach into the beaker through the screen. If the volume of spinach extract is less than 20 mL, add sucrose solution to bring the volume to 20 mL.
6. Divide the extract equally between the two tubes.
7. Wrap one culture flask in aluminum foil.
8. Centrifuge each tube of the extract for two minutes at 3000 r/min.
9. Into each flask, pour the contents of one tube.
10. Add 1.0 mL of DCPIP solution to each flask and swirl contents.
11. Place the unwrapped flask on the stage of the lighted overhead projector for 5 to 10 minutes.
12. While you are waiting, withdraw a drop of the contents from the foil-wrapped flask, prepare a wet mount, and record observations with the compound microscope at high power.
13. When the time is up, remove the unwrapped flask from the overhead stage, unwrap the foil-covered flask, and compare the colors of the contents of the two flasks.

Discussion

1. DCPIP is often used in titrations to determine the vitamin C content of fruit and vegetable juices. How would you counter the suggestion that the experiment's results were caused by vitamin C in the spinach extract?
2. Do you think that oxygen was being created during the experiment? Why or why not?
3. Is it likely that ADP was being phosphorylated to ATP during the experiment? Explain.
4. Is it likely that NADP was being reduced during the experiment? Explain.
5. Design a possible procedure to measure the rate of color change over time in the experiment.

ACTIVITY 3. Library Research

Recent specialized research has given scientists a clearer understanding of the photosynthetic process. Use your library's most recent books and articles to find answers to the following questions:

1. How do we know there are two photosystems (P 680 and P 700)?
2. How was it decided where, in the sequence of reactions, to place the various electron carrier molecules?
3. How do we know that the light reactions of photosynthesis proceed much faster than the dark reactions?
4. What evidence supports the idea that a proton gradient drives the phosphorylation of ADP?
OBJECTIVES

After viewing this program, students should be able to do the following:

1. Explain how the availability of radioactive isotopes was necessary before the dark reactions could be understood.
2. Explain the role of ribulose diphosphate as an acceptor of carbon dioxide.
3. Account specifically for the requirement of ATP and reduced NADP in photosynthesis.
4. Explain why both light reactions and dark reactions take place only in light.
5. Account, generally, for the fact that chloroplasts never run short of ribulose diphosphate as photosynthesis proceeds.

PROGRAM DESCRIPTION

The dark reactions of photosynthesis occur in the stroma of the chloroplasts and use the ATP and reduced NADP generated by the light reactions to join and reduce carbon dioxide molecules. The direct products are carbohydrates such as glucose, cellulose, and starch, but fats, oils, and the carbon skeletons of amino acids (proteins) are also generated. Since light is not directly used during these chemical alterations of carbon dioxide, the reactions are said to be light-independent (or dark). However, since neither ATP nor reduced NADP is stored to an appreciable extent, the dark reactions take place only while light is supplied to the plant.

The complex series of reactions is called the Calvin cycle after Melvin Calvin who discovered most of the steps, using pulse-labelling techniques with the green alga, chlorella.

The first product to appear after radioactive carbon dioxide has been used is phosphoglycERIC acid. This contains three carbon atoms. Plants that form this compound are, therefore, named C3 plants. The actual fixing of carbon dioxide involves its attachment to a 5-carbon compound, ribulose diphosphate, forming a 6-carbon compound that is broken immediately into two molecules of phosphoglyceric acid. Ribulose diphosphate is formed from ribulose phosphate, using energy and a phosphoryl group contributed by ATP from the light reactions. Its energy lost, ADP returns to the light reactions to acquire additional energy.

Products of the light reaction are required again as each phosphoglyceric acid molecule is phosphorylated (using ATP) and reduced (using reduced NADP). During the reduction, inorganic phosphate is split off so two molecules of phosphoglyceraldehyde are produced in this central series of reactions. These can, after one of these molecules is slightly changed, form glucose phosphate or, ultimately, starch. These are usually considered the end products of the dark reactions. Most of the phosphoglyceraldehyde, however, is used to regenerate ribulose phosphate by way of a complex series of interactions that completes the Calvin cycle. It takes six turns of the cycle to lead to a net gain in the cell of one glucose molecule.

BEFORE-VIEWING ACTIVITIES

Summarize for the students the light reactions, specifically the output by them of reduced NADP and ATP. Recall the importance of these compounds as suppliers of energy to reduce carbon skeletons of molecules and to join carbon skeletons in non-redox reactions respectively. Note the large increase in potential energy and decrease in entropy as carbon dioxide is fixed into simple sugars, then into polysaccharides. Also note the need of free energy to effect such reactions.
After-Viewin Activities

ACTIVITY 1. Discussion of the Calvin Cycle

Discuss with the students the importance of cyclical sequences of reactions and compare the Calvin cycle with the citric acid (Krebs) cycle if the latter has already been studied. To encourage some in-depth study of the reactions of the Calvin cycle, encourage students to predict which carbon atom(s) of glucose would become tagged with radioactivity following exposure of leaf cells to radioactive carbon dioxide. They should, of course, be able to explain how they arrived at their answers. If styrofoam spheres and paint are available, have small groups of students prepare models of the different compounds of the Calvin cycle and link the models into a mobile to hang in the classroom.

ACTIVITY 2. Dramatizing Photosynthesis

In groups of six to eight students, write a play to depict the events of photosynthesis. It must be possible to perform the play in ten minutes or less. Using a designated portion of the classroom for the set, arrange the furniture to represent the interior of a leaf cell or a portion of it. A drawing or map of your proposed stage set must accompany your script.

Prepare a cast of "chemical characters" and assign each member of your group to play one or more of these characters. A person may be assigned many roles but can only appear on stage in one role at a time. Any chemical that is actively involved in photosynthesis is a valid character, but compounds that are essential but passive should be part of the set. The more valid "characters" you include in your play, the more credit you will get.

The script should include each character's dialogue and directions as to positional changes, body motions, or gestures to accompany the lines. The more frequently key scientific words denoting processes, structures, or principles (e.g., reduction, thylakoid) appear in your script, the more credit you will receive.

Each group is responsible for submitting a stage set and script to the teacher by a deadline the teacher will announce. You will also be told if and when you are to perform your play for the rest of the class and whether or not to wear special costumes.

ACTIVITY 3. Starch Formation in the Dark

Apparatus

Supplies to test leaves for starch (see Activity 3, Program 1 on page 3)
50 mL or 100 mL beaker
Watch glass or other beaker cover
Marking pencil
50-mL graduated cylinder
Cork borer
Potted geranium plant stored 24 hours in the dark
5% glucose solution

Method

1. Mark the beaker so you can identify if later.
2. Place 20 mL of glucose solution into the beaker.
3. Obtain a fresh geranium leaf. Cut eight discs from the leaf with a cork borer. Avoid areas where there are major veins.
4. Float the discs in the glucose solution. Four should float right side up and four upside down.
5. Cover the beaker and store it in a dark place for a minimum of 24 hours.
6. After 24 hours, recover the discs, being careful to separate those that were floated upside down from those that were floated right side up.
7. Test each disc for the presence of starch, following the instructions and observing the precautions outlined for Activity 3, Program 1 on page 4.
8. Record and interpret all observations.

Discussion

1. What "control" should have been performed? Why?
2. Why are leaf discs a better choice for this experiment than intact plants?
3. ATP is required to combine glucose molecules into starch molecules. How could leaf cells generate ATP in the absence of light?

ACTIVITY 4. Research

In the library, research the experiments of Calvin that established the intermediates of the dark reactions of photosynthesis. Report on these experiments in an essay of about 300 words. Include sketches of illustrative chromatograms and a discussion of the variety of techniques and apparatus used by Calvin or others working the same field of research.

ACTIVITY 5. Review

1. Discuss the roles of ribulose, carbon dioxide, ATP, and reduced NADP in the dark reactions of photosynthesis.
2. When a molecule changes chemically but its formula is unchanged, the reaction is called an isomerization. Discuss the importance of isomerization in the dark reactions of photosynthesis.
3. Explain why the determination of events in the dark reactions had to wait until the 1940s.
4. Experiments have revealed that the rate of incorporation of radioactive carbon dioxide into glucose by a plant is unchanged if a rapidly blinking light source replaces a steady light source of the same intensity and quality. This substitution reduces the rate at which energy is supplied. Explain this observation.
Objectives

After viewing this program, students should be able to do the following:

1. Explain the low efficiency of photosynthesis in C3 plants.
2. Outline the differences in arrangements of cells within the leaves of C3 and C4 plants.
3. Explain the biochemical basis for defining plants as C3 or C4.
4. Compare the chloroplasts of mesophyll cells and bundle-sheath cells in C4 plants.
5. Explain the role of oxaloacetate, malate, and pyruvate in C4 plants.
6. Explain why C4 metabolism is advantageous in tropical, desert, or saline environments.

Program Description

The biochemical difference is accompanied by an anatomical difference in the arrangement of photosynthetic cells within the leaves. The interior of leaves is called the mesophyll. In most C3 plants the mesophyll cells are arranged into an upper palisade layer and a lower spongy layer. Cells in both layers contain similar chloroplasts, although these are more numerous in cells of the palisade layer. Within the spongy layer lie numerous small veins (vascular bundles) surrounded by nonphotosynthetic bundle-sheath cells. The mesophyll cells of C4 plants form rings around the bundle-sheath cells, and the bundle-sheath cells contain chloroplasts. However, the chloroplasts of bundle-sheath cells have few, poorly developed grana and cannot participate in the light reactions. The light reactions of such plants are carried out, as you would expect, in the thylakoids of mesophyll cells.

Comparing the energy stored by plants using the Calvin cycle with the quantity of energy absorbed in the light reactions reveals an impressive theoretical efficiency of 38%. In reality, however, the plants operate at an efficiency of less than 1%. This is because of the nature of the enzyme that is supposed to join carbon dioxide to ribulose diphosphate. This enzyme also has an affinity for molecular oxygen, and if oxygen is combined with ribulose diphosphate rather than carbon dioxide, the ribulose diphosphate enters a series of reactions that effectively removes it from the Calvin cycle. Since oxygen is much more abundant in air than carbon dioxide (21% vs. 0.035%), a substantial loss of ribulose diphosphate occurs in plants relying on the Calvin cycle to fix carbon dioxide.

The problem has been avoided by some plants in tropical or desert areas in which radioactive carbon supplied to the plant in carbon dioxide appears first in four-carbon compounds rather than in the three-carbon compound, phosphoglyceraldehyde. Such plants are called C4 plants.
C4 strategy of using mesophyll cells to effectively "pump" carbon dioxide into the oxygen-deficient chloroplasts of the bundle-sheath cells is of great adaptive value.

**Before-Viewing Activities**

1. Discuss enzyme specificity and the possibility of there being two equivalent substrates for the same site. Following logically is a discussion and predictions about competitive inhibition of enzymes (interaction of O2 or CO2 with ribulose diphosphate carboxylase).

2. Review with students the concepts of the previous two programs, especially the release of molecular oxygen by the light reactions (photolysis) and the entry of carbon dioxide into the dark reactions via combination with ribulose diphosphate.

**After-Viewing Activities**

**ACTIVITY 1. Further Discussion**

Crop plants using C4 metabolism (e.g., corn and rice) are very efficient energy converters. Could ethanol from corn have an increased use as a source of energy for transportation? Why has such a high-efficiency process that has been around long enough for many plant families to adopt it not displaced C3 metabolism through evolution? Seek answers by reading further in the area of photorespiration.

**ACTIVITY 2. Microscope Study**

**Apparatus**

- Compound microscope
- Prepared slide: cross-section of Syringia leaf (lilac)
- Prepared slide: cross-section of Zea leaf (corn)

**Method**

1. Draw a strip from the top to the bottom of each leaf, including a small vessel in each drawing. Be sure each drawing is the same scale and has a title accompanying it.
2. In each epidermal layer, look for a site where gases and vapors could be exchanged with the environment. If you find such a site, include it in the appropriate location of your drawing, even if it was not visible in the strip you originally chose.
3. Look carefully for chloroplasts within each cell type in your drawings. Note that the slides are stained artificially so cell parts will not necessarily have the color you intuitively expect. For example, chloroplasts are often stained red in commercial preparations. Add chloroplasts of appropriate size, shape, and location to the cells of your drawing that are found to contain chloroplasts.
4. Label the leaf parts with which you are familiar on each drawing.
5. On each drawing, superimpose color-coded arrows to indicate the following pathways within the leaf:
   a) Carbon dioxide from air to site of incorporation in glucose.
   b) Oxygen from release point to air.
   c) Liquid water from plant reserves to photolysis site.
   d) Water vapor being transpired.
   Include a legend of color codes on the same sheet(s) as your drawings.

**Discussion**

Which plant(s) in the study is/are C3? Which is/are C4? Justify your answers.

**ACTIVITY 3. Research**

A third type of adaptation to extreme habitats is that of "Crassulacean metabolism." Research this type of adaptation and write a 250-word report on it.
ACTIVITY 4. Dramatizing Photosynthesis (Act 2)

Using the same guidelines as in the chapter on Program 4 (see page 14), write a second act to your photosynthesis play, entitled "Variations on a Theme." Keep it short - no longer than five minutes (excluding time spent in shifting scenery). Act 2 should address both the C4 and Crassulacean adaptations.

ACTIVITY 5. Review

1. Explain why photosynthesis fails to operate at its theoretical efficiency.
2. In C4 metabolism, why are cytoplasmic bridges (plasmodesmata) between mesophyll and bundle-sheath cells important?
3. Why is C4 metabolism more useful in arid than in moist habitats?
4. If a palaeontologist were to find the fossil leaf of an extinct plant, how might he or she determine if the plant had been C3 or C4?
5. Anthropologists can use the ratio between carbon-13 (an isotope formed in the atmosphere by cosmic rays) and carbon-12 (normal isotope) in ancient bones to discover if, when alive, those people had based their agriculture on C3 or C4 plants. Suggest a reason that such determinations may be possible.
Objectives

After viewing this program, students should be able to do the following:

1. Define transpiration and relate it to xylem transport.
2. Discuss the role of stomata in the exchange of gases and vapors between the leaf's interior and the environment.
3. Appreciate the difficulty of raising water to the top of a tree against the pull of gravity.
4. Relate the structure of a water molecule to the cohesive and adhesive properties of water.
5. Explain how water enters the roots of a plant.
6. Outline the active processes upon which phloem transport depends.

Program Description

As plants evolved, some grew to great heights and gained a significant advantage in competing with other plants for light. However, the great separation between the leaves and the source of minerals and water (soil) necessitated the evolution of efficient transport systems.

The exchange of gases between the leaf interior and the surrounding atmosphere that is necessary for photosynthesis is inevitably accompanied by loss of water vapor to the air. This process, called *transpiration*, is also useful to the plant, as it provides the energy to lift water from the roots to the highest branch. Some minerals accompany the water and become available for maintenance of leaf tissue. Water and some minerals travel through columns of interconnected, dead cells that make up the xylem tissue. This tissue also imparts tensile strength to the aerial parts of the plant.

Within the xylem tissues, as in all areas of the plant, water molecules interact in important ways. Water molecules are polar - each possesses a permanent, asymmetric separation of positive and negative charge. This means that water molecules can cling firmly together, a process called *cohesion*. Also, they may cling to other polar molecules or the polar hydroxyl groups of macromolecules such as the cellulose that comprises the cell wall. This process is called *adhesion*. Cohesion and adhesion enable water to be lifted to great heights through the xylem tissue. Adhesion serves to "anchor" the top of a water column in the leaf, and cohesion binds the water column together with a strength approaching that of a steel wire of the same diameter. As water evaporates in the leaf, adhesion of water to cellulose fibres in the leaf cell serves to pull the column upwards to replace the evaporated water.

Initially, water enters roots by osmosis. The conditions for osmosis are established by the active uptake of mineral ions from the soil. The supply of minerals is ensured because the plant is constantly exploring new areas of soil by growing and forming new root hairs. The ions are often associated with soil colloids, but can be dislodged by replacing them, on the colloids, with hydrogen ions generated by the intermediary metabolism of root cells.

Similar processes are involved in phloem transport. Active transport of organic molecules (or certain inorganic ions) into phloem cells causes water to enter these phloem cells by osmosis, with a consequent increase in phloem cell turgor. Elsewhere, in the same column of phloem cells, active unloading of solutes leads to a decrease in turgor. The result is a pressure gradient within the column of phloem cells from the point of solute uptake to the point of solute release. This causes mass flow of phloem cell contents along the gradient. The flow is facilitated by perforations in the walls between neighboring cells in the column. The phloem cells even contain a protein that causes "clotting," if the column should be injured.
Before-Viewing Activities

1. Review with the students the concepts of active transport and osmosis, and the structure of the leaf (Program 5, Activity 2).
2. Discuss the expected rates of diffusion of carbon dioxide and water into and out of the leaf. Highlight the fact that water can be expected to diffuse out of a leaf much more rapidly than carbon dioxide will diffuse into the leaf, under most circumstances (steeper concentration gradient, less massive molecules). As a result, some water loss inevitably accompanies photosynthesis.
3. Discuss the importance of the plant's conducting and gas exchange systems in enabling the leaves to photosynthesize effectively.

After-Viewing Activities

ACTIVITY 1. Investigating Water Transport in Plants

Apparatus

Two microscope slides
Motor oil
Cover glass
Stewed rhubarb
Compound microscope
Glycerol
Phloroglucinol

Method

PART A: Comparing the Cohesive and Adhesive Power of Water with that of a Nonpolar Substance
Using a pair of plain microscope slides, place a drop of water on one slide and press the other on top. Note how difficult it is to separate the slides unless you slide one over the other. Now put a drop of motor oil (nonpolar) on one slide instead of water and note how easily the slides are separated.

PART B: Observing Plant Vessels

Prepare a dichotomous key that could be used to classify the cell types found in stewed rhubarb. A few drops of glycerol should be added to each preparation, and staining of lignin with phloroglucinol could be attempted.

Note to the teacher: You might want to set up a potometer, using the top portion of the plant decapitated in Activity 2. However, to demonstrate the lifting power of the aerial portions of plants, mercury has to be used as the working fluid. Mercury fumes are toxic and many jurisdictions prohibit the use of mercury in schools. If you decide to demonstrate this activity, be sure the mercury is contained in such a way that it cannot vaporize into the air. Also, be certain that the water used between the plant and the mercury has been boiled to free it of dissolved air. Otherwise, air bubbles may develop in the water column under tension and break its cohesive-ness.

ACTIVITY 2. Locating Plant Vessels

Apparatus

250-mL beaker
Fresh celery
Water darkly colored with red ink or eosin
Scalpel or single-edged razor blade
Fine forceps
Compound microscope
Two microscope slides with cover glasses

Method

1. Twenty-four hours before the investigation, place a fresh celery stalk into a beaker of darkly colored water.
2. Note the distribution of red stain in the stalk. Describe its apparent distribution.
3. Cut a thin section of the stalk (petiole) from an area to which color has extended. Prepare a wet mount of the section and examine it with the microscope. Sketch the cross-section and indicate the location(s) of vessels transporting water.
4. Dissect from the stalk, a longitudinal section of the vascular area. Prepare a wet mount of the vessels, examine it with the microscope, and sketch the arrangement of the cells within a vessel carrying water.

**ACTIVITY 3. Can Roots Push Water Upwards?**

**Apparatus**

- Healthy potted plant (e.g., tomato)
- 1-metre capillary tubing
- Petroleum jelly
- 3-cm rubber tubing (approximately)
- Hose clamp and screwdriver
- Retort stand with burette clamp

**Method**

1. Cut aerial portions from plant, leaving one to two cm of stem projecting above the soil.
2. Coat the stem lightly with petroleum jelly and fasten one end of the rubber tubing over it, using a hose clamp to secure it firmly.
3. Insert the capillary tubing into the other end of the rubber tubing.

4. Secure the capillary tubing in an upward position, using the burette clamp and place the entire apparatus aside in a plant-growing area for several days.
5. Note and account for any changes in the level of fluid within the capillary tubing. How would the level be affected if the plant were 'watered' with 1 percent salt solution?

**ACTIVITY 4. How Rapidly Do Plants Transpire?**

**Apparatus**

- Volumeter consisting of side-arm test tube, 1 mL pipette (or graduated capillary tube), and syringe as shown in Fig. 6-1 (syringe needle should be sealed in place in the first hole of the two-holed stopper, using epoxy cement)
- Plasticene
- Stopwatch
- Plant cutting, ideally with woody stem (e.g., top of plant from Activity 2, white pine cutting, or leather fern from florist)

**Method**

1. Prepare the plant cutting for the experiment by snipping the terminal inch or two of stem from it, while holding the stem end immersed in water.
2. Secure the cutting in the second hole of the rubber stopper using plasticene.
3. Fill the side-arm tube with tap water and insert stopper firmly. Excess water should be forced out around the stopper and the end of the pipette.
4. The syringe should be filled halfway with water and attached to the delivery needle. This can be used to adjust the level of water in the pipette.
5. Use the gradations on the pipette and a stopwatch to calculate the rate of transpiration of the cutting. Several environmental conditions can be tried, such as different light exposures, humidities, temperatures, or wind speeds.
Duplicate determinations should be made under each environmental condition, if time permits.

6. Account for any variations noted in transpiration rates.

**ACTIVITY 5. Leaf Impressions**

Caution: This experiment should only be performed in well-ventilated areas or outdoors. Avoid breathing ethyl acetate fumes.

**Apparatus**

- Ethyl acetate in drop-dispenser bottle
- Plastic cover slips or 2-cm squares cut from overhead transparency acetate
- Small paper envelopes e.g., philatelists's envelopes
- 15-cm ruler
- Compound microscope

**Method**

Choose various plants growing in different habitats or the same plant at different times of day as your subject of investigation. Examine the density of stomata and degree of stomatal opening by making replicas of their surfaces. For each of the surfaces of the leaf in turn, place one drop of ethyl acetate on the surface and press a plastic square firmly against the leaf surface over the drop for one minute. Then peel away the square and store the square in an appropriately labelled envelope. This should be done outdoors, with the leaf remaining attached to the plant throughout the procedure.

1. Store each replica in its own envelope and keep clear records of the plant/leaf surface/habitat/time.
2. In the laboratory, determine the density of stomata in each replica, using the ruler and compound microscope. Indicate the degree to which the stomata are opened by sketching a typical stoma/guard cell complex, as revealed in the replica.

4. Draw conclusions based on your data. Depending on the nature of your replica collection, you could compare plant species from exposed habitats with those from shaded habitats, the same species from exposed and shaded habitats, or the same plant at different times of day.

**ACTIVITY 6. Review**

1. Why do horticulturalists recommend that some branches be pruned from a plant following transplantation?
2. Explain how a plant can be killed either by overwatering or by overfertilizing.
3. Why should you cut 2 cm or so of stalk away from cut flowers, while holding the cut ends immersed in water, before you place the flowers in a vase for display?
4. Split the cut end of a stem of a white carnation in half longitudinally and place one-half in water stained with a blue dye and the other half in water stained red. Predict how the blossom will be colored after a few hours. Justify your prediction.
5. Is there any practical limit to how tall a tree can grow? Explain your reasoning.
6. Does xylem transport in a plant stop when the air around the plant has a relative humidity of 100 percent? Explain the experimental procedure you would use to try to answer this question.


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<table>
<thead>
<tr>
<th>Videotapes</th>
<th>BPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Program 1: Seeing the Light</td>
<td>279901</td>
</tr>
<tr>
<td>Program 2: Absorbing the Light</td>
<td>279902</td>
</tr>
<tr>
<td>Program 3: The Light Reaction</td>
<td>279903</td>
</tr>
<tr>
<td>Program 4: The Dark Reaction</td>
<td>279904</td>
</tr>
<tr>
<td>Program 5: C3 and C4 Plants</td>
<td>279905</td>
</tr>
<tr>
<td>Program 6: The Fluid-Transport System</td>
<td>279906</td>
</tr>
</tbody>
</table>