Chapter 4

Electron Flow in Photosynthesis

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Leaf Structure and Pigmentation

Objectives

1. To make leaf cross-sections and become familiar with leaf structure.

2. To separate and identify the pigments present in leaves.

3. To determine the absorption spectra of a plant and an alga.

Pre-lab Preparation

READ the lab outline before coming to class. Background information can be found in the following references in Keeton and Gould.

Leaf structure:	pp. 213-214,274-278
Chromatography:	p. 62
Absorption spectra:	pp. 199-202

INTRODUCTION

In our study of plants, we have yet to consider their most obvious characteristic, that being their ability to harness the sun's energy to build complex molecules from simple ones - something that animals are unable to do. Before examining this process, called photosynthesis, in detail, we want to look at some features of plants which enable this process to occur.

The leaf is the plant's organ of photosynthesis. It is the structure which captures sunlight and makes it available for the chemical reactions of photosynthesis. Leaves come in all shapes and sizes depending on the particular species of plant. Because the leaf is also the site of water loss for the plant, each plant has evolved a compromise between maximizing the capture of sunlight and yet minimizing its loss of moisture. In some environments, such as the desert, the need for water is so great that the leaves have been reduced to non-photosynthetic spines and the stem has taken over the role of capturing sunlight. In the conifers, the leaves take the form of needles which have a lower rate of water loss compared to the broad-leaved trees which occur more frequently in areas of moderate to heavy rainfall.

EXERCISES

A. LEAF STRUCTURE (work in pairs)

We will study the leaves of *Photinia*, an evergreen shrub found locally on campus. Its general leaf structure is typical of broad-leaved trees and shrubs.

1. Make a cross-section of the *Photinia* leaf (do both a green and a red leaf) by placing it on a glass slide and, while using another slide as a 'ruler', making very thin slices using a sharp razor blade. Float the sections on water in a watch glass. To be able to see the structure of the leaf, VERY THIN SECTIONS will have to be made. (If you are not able to make them thin enough, obtain some sections from someone else.) Once you have succeeded in making good sections, place two or three of them on a glass slide and make a wet mount. Observe the cross-section with your compound microscope.
In *Photinia*, when new leaves are produced in the spring they are reddish-purple in color, rather than green.

   Make a sketch of the cross-section of each leaf below.

Label the diagrams with the following structures/tissues and state their function below (Keeton & Gould: p. 213):

Cuticle
Upper epidermis
Palisade mesophyll
Spongy mesophyll
Vascular bundle
Lower epidermis

How is the leaf well-adapted to its photosynthetic role?

Is there any difference between the older green leaf and the younger red leaf with respect to:

cuticle thickness?

size and number of chloroplasts?

Where is the red pigment mainly concentrated in the younger leaves? (This water-soluble pigment is called **anthocyanin** and is contained within the vacuoles of the cells.)

3. As the leaves of this plant age, the walls of certain cells near the vascular bundles become impregnated with lignin, a material which makes the cell walls thick and rigid. This results in a stiffened leaf, giving it more structural support. This effect is especially noticeable along the central vein of the leaf. These lignified cells are called **sclerenchyma** (sclare-rank-ke-ma).

   Make some more cross-sections of both leaf types and stain these sections with **phloroglucinol.** This stain is specific to lignin which is only found in xylem cells within the vascular bundles, and in sclerenchyma. Xylem cells can be identified by their large circular spaces - these are the passageways through which water moves.

   CAUTION: Phloroglucinol is made up in strong acid. Do not touch the stain or your slide. Also, make sure phloroglucinol does not get on the microscope lenses as it is very corrosive.

   Leave the phloroglucinol on the sections for about two minutes, then use an eye dropper to wash the stain off the slide. Make a wet mount of the stained sections and observe under the compound microscope.

   Where in your preparation do you find sclerenchyma tissue? Can you give a reason why it might be situated as it is?

   Compare your preparation of the green leaf with the younger leaf that has been stained with phloroglucinol. Does the younger leaf contain any sclerenchyma?

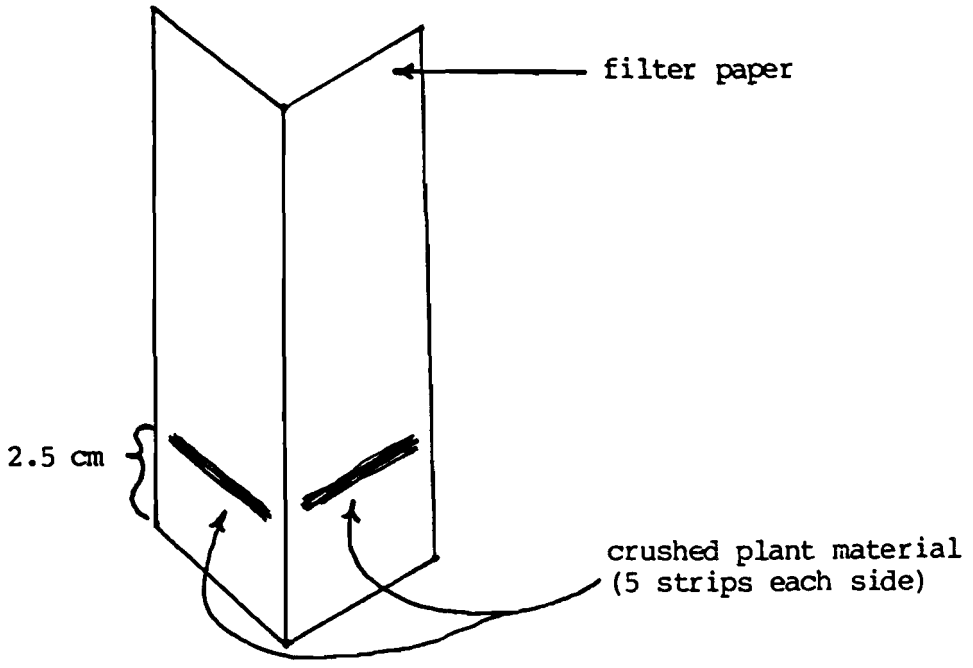
   If you were a herbivore, on which leaf would you prefer to browse? What might the purple anthocyanin pigment have to do with this?

B. LEAF PIGMENTS

Based on your background reading you should be aware that chloroplasts contain a variety of different pigments. Chlorophyll a, chlorophyll b, and the carotenoids are the major pigments associated with photosynthesis. Each pigment is responsible for capturing light of specific wavelengths and making it available for further photosynthetic reactions.

When we look at a leaf, our eyes can distinguish only a general green color; however, by using the process of chromatography, we can separate the pigments using filter paper and a solvent. We will use *Coleus* leaves since they contain ample quantities of the photosynthetic pigments, as well as the purple anthocyanin pigment.

1. Cut the *Coleus* leaf into thin strips which are equal in length to half the width of the filter paper and approximately 2 mm in width. Place the strip *2.5* cm from one end of the filter paper and roll the handle of your scissors over the strip, crushing the leaf material and allowing the pigments to be absorbed by the filter paper (see figure below). Remove and discard the leaf strip. Repeat this procedure five times on each side of the folded filter paper, using 10 fresh strips of *Coleus* leaf, making sure to place each strip directly on top of where the last one was crushed. Put your name on the top right hand corner of the filter paper and place it in one of the beakers containing solvent on the side bench. **Do not touch the solvent!**



At this point you may begin the last exercise of the lab.

2. After 30 minutes remove your chromatogram from the solvent beaker and immediately proceed to distinguish the different pigments, as the color of the pigments will fade rapidly when exposed to light. Holding the filter paper up to the light will aid you in discriminating among the pigments; you may wish to outline the bands of pigment using a pencil. Identify the pigments by their colors:

Chlorophyll a	-	blue-green
Chlorophyll b	-	grass-green
Carotenoids	-	yellow
Anthocyanins	-	purple (this pigment does not dissolve in the solvent and so will not move from its original spot)

What is the order of pigments on the filter paper? What does this indicate about their relative solubility in this solvent? What are the roles of the pigments in photosynthesis?

C. THE ABSORPTION SPECTRA OF A SPINACH CHLOROPLAST SUSPENSION AND A RED ALGA SUSPENSION

In the photosynthesis lab you will be carrying out an experiment to determine how effective light of different wavelengths is at enabling a spinach chloroplast suspension to carry out photosynthesis. Before doing that experiment, we want to formulate an hypothesis concerning its results. One clue regarding the effectiveness of different wavelengths would be the absorption spectrum of the suspension. **What does an absorption spectrum tell you?** In this lab we will generate the absorption spectrum of a spinach chloroplast suspension and compare it to the absorption spectrum of a red alga suspension. Before generating the spectra make a rough sketch below outlining your prediction of the results. The color of the spinach and the alga should help.

1. Zeroing of the Spectrophotometer (work in groups of 6). See guide by the spectrophotometer. Why is zeroing a type of control?

2. Once you have zeroed the spectrophotometer, determine the absorption spectrum as follows:

   a. Fill one spectrophotometer tube with 5 ml of chloroplast suspension and a second one with 5 ml of red alga suspension.

   b. Measure the absorbance of the suspensions at each of the wavelengths in Table 1 on the next page (you MUST zero the spectrophotometer with the blank at each wavelength BEFORE measuring the suspension - work as a team on this).

Graph your results in class. Do the absorption spectra correspond with your predictions? What is the major difference between the two spectra? What does this suggest to you about the pigments contained in the red alga? Might there be a reason for the different absorption spectrum of the red alga? Where do red alga commonly grow? (Caution - not a hard & fast rule.) What is the difference (if any) between an absorption spectrum and an action spectrum? Think about this before next week's lab on Photosynthesis.

SUMMARY

By the end of this exercise you should be able to:

1. Make a cross section of a leaf and identify its basic structural components, relating each to its function.

2. Make and analyze a chromatogram of the pigments found in a leaf.

3. Use the spectrophotometer to determine the absorption spectrum of plant or algal species.

Table 1. Absorbance of the chloroplast and red alga suspension recorded at wavelengths in the visible spectrum.

	Wavelength	Absorbance	
		Spinach	Red Alga
BLUE BULB SPEC	400 nm		
	430 nm		
	460 nm		
	490 nm		
	520 nm		
	550 nm		
	580 nm		
RED BULB SPEC	620 nm		
	640 nm		
	660 nm		
	680 nm		
	700 nm		

PRE-LAB ASSIGNMENT FOR PHOTOSYNTHESIS EXERCISE

Before corning to class, you must design your experiment, including which controls and replicates you will use. The following questions will help you do this:

1. What is the specific question this experiment is trying to answer?

2. List the different factors involved in this experiment.

3. The most obvious control in an experiment is the one where the experimental variable has been removed. What would be the control in this experiment?

4. When you measure absorbance change in your experimental tubes, you assume it is due to the DCPIP absorbing electrons from the photosynthetic process. How can you be sure that the dye or the chloroplast suspension do not change in absorbance all by themselves? What tests or further controls could you carry out to show that the results of your experiment are not due to changes in the dye or chloroplast suspension?

5. If the latter tests (controls) do show changes in absorbance, how could you take this into account when reporting your experimental results?

6. What does it mean to oxidize a substance? To reduce a substance?

Photosynthesis

Objective

To determine the effect of light quality on the energy-capturing reactions of photosynthesis.

Prelab Preparation

READ the lab outline. You must understand not only the procedures but also the theory behind the exercise. You can find useful information in the following texts:

Keeton & Gould: pp. 195-210
Curtis 4: pp. 210-224

Complete the pre-lab assignment found at the end of the previous exercise prior to coming to lab.

New terms to learn from this lab exercise:

Light energy
Pigments
Energy-capturing reactions
Oxidized
Reduced
Light quality
Buffer
Nanometer (nm)

INTRODUCTION

Photosynthesis is the major process by which external energy (derived from the sun) is made available to the living world. **Light energy** striking **pigments** in the chloroplast is transformed first to **electrical energy** (excited electrons) and then to chemical energy bonds in the molecules ATP and NADPH2. Some of these bonds are subsequently broken down and in the process energy is released which is used to drive the enzymatic reactions which change atmospheric carbon dioxide, a low energy molecule, into sugars. Although photosynthesis is restricted to chlorophyll-containing organisms (plants and some protists) and some bacteria, the sugars they produce can be used by all living organisms, via glycolysis and respiration, to provide chemical energy for living processes.

The overall photosynthetic reaction is:

$$6C0_2 + 12H_20 + \underset{\text{energy}}{\text{light}} + \underset{\text{pigments}}{\text{photosynthetic}} \text{--------} > \underset{\text{(sugar)}}{C_6H_{12}0_6} + 6O_2 + 6H_2O$$

In this exercise you will be dealing only with the **energy-capturing** reactions. These reactions occur only in the presence of light. Energy is released as electrons move along electron transport chains (series of molecules held in membranes) after being 'energized' by photons of

light. You will measure photosynthetic activity by determining **the extent of color loss of the dye DCPIP. This dye intercepts the flow of electrons** in the process of photosynthesis. When it accepts electrons, it becomes reduced and changes color, **from blue (oxidized form) to colorless (reduced form)** (see Figure 1**).** This color change can be measured with a **spectrophotometer.** The amount of color lost is proportional to the number of electrons activated during photosynthesis, which is proportional to photosynthetic activity.

The white light emitted from the sun consists of a range of wavelengths (colors). In this exercise you will design an experiment to determine the effect of **light quality (wavelength)** on the photosynthetic (energy-capturing) activity of a chloroplast suspension isolated from spinach leaves.

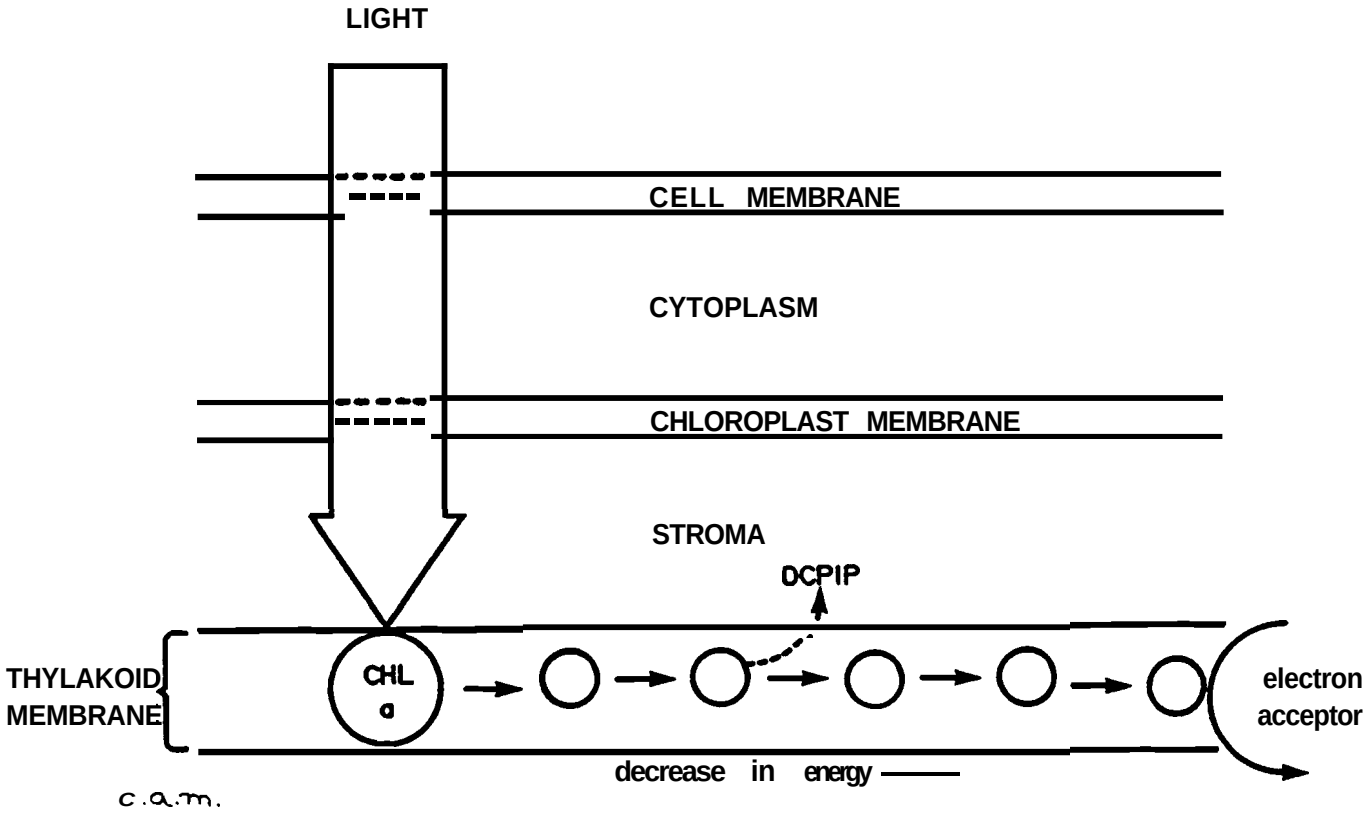


Figure 1. DCPIP interrupts the flow of electrons in Photosystem II and turns from blue to colorless as it is reduced.

EXERCISES

A. PREPARATION OF SPINACH CHLOROPLASTS

Preparation of the spinach chloroplasts will be done for you before the lab begins. The chloroplasts are obtained as follows:

1. Blend spinach leaves and sorbitol (a pH-buffered sugar solution). Why is a **buffer** used?

2. Filter mixture through cheese cloth, discard residue. What does the residue contain?

3. Dilute filtrate to appropriate concentration and keep on ice. Why keep it cool?

B. REDUCTION OF DCPIP - a measure of photosynthetic rate.

In the previous lab you determined the absorption spectrum for spinach chloroplasts, so that you now know which wavelengths of light are absorbed most effectively by the chloroplast suspension. In this lab we want to determine which wavelengths cause the **highest rate of photosynthesis:** an action spectrum. (Would you expect a relationship between the two?) As explained in the introduction, we will measure rate of photosynthesis by change in color of DCPIP The spectrophotometer will be used to monitor color change (change in absorbance) of DCPIP.

The peak absorbance of DCPIP occurs at 600 nm (see Figure 2); however, since this wavelength lies on the border between the red and blue light spectrophotometers, we will record absorbance by DCPIP at 620 nm using the "red light" spectrophotometer (what would be the outcome if we set the spectrophotometer at 420nm & use the blue bulb?). This will allow each of the four groups to do the following experiment.

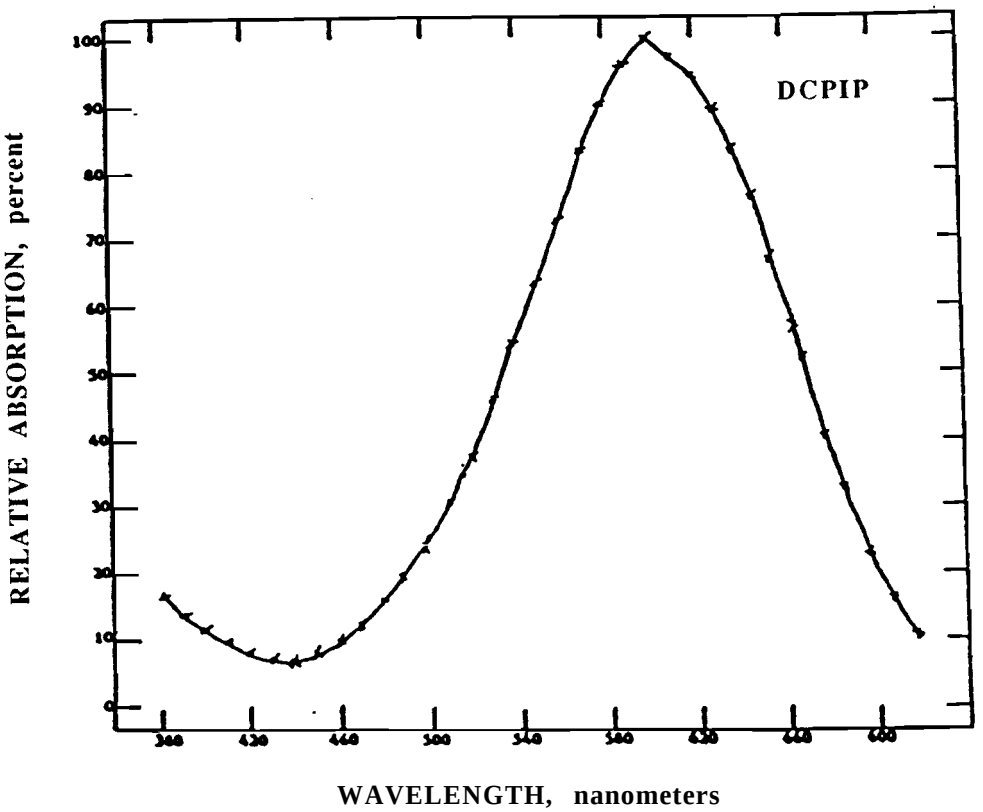


Figure 2. Relative absorbance spectrum of DCPIP in sorbitol solution.

Before doing this section turn off the lights in the lab room and close the blinds. Why are these procedures necessary?

Procedure

1. To a spectrophotometer tube add 5 ml of chloroplast suspension
2. Then add 2 drops of DCPIP
3. Cover with a piece of parafilm and invert to mix
4. Record the absorbance of the mixture at 620 nm (this is time = 0 reading)

Place this tube in a 250 ml beaker 68 cm from a 150 watt desk lamp. The lamp should be resting on the bench top. After 5 minutes (exactly) in the light, record the absorbance of the chloroplast suspension in Table 1 below (remember to zero the machine before measuring the absorbance).

TABLE 1. Use of the dye DCPIP to measure rate of photosynthesis of a chloroplast suspension. (Is this a control?)

Time (min)	Absorbance
t = 0	_____
t = 5	_____
Change in absorbance	_____

How do you account for the change in absorbance? Discuss this within your group.

You should now have established that the chloroplast suspension can reduce the dye and that the amount of reduction can be measured quantitatively. The oxygen produced during photosynthesis can reoxidize the dye very rapidly after the dye has been reduced (see previous equation). What precautions should you take when measuring the reduction of the dye?

C. AN EXPERIMENT TO DETERMINE THE EFFECT OF LIGHT QUALITY (WAVELENGTH) ON THE RATE OF PHOTOSYNTHESIS

Work in the same four groups as before.

Materials

Each group of students is provided with the following lab materials:

150 watt light bulb
meter stick
spectrophotometer
six 250 ml beakers 24 Spec-20 tubes
150 ml diluted chloroplast suspension (kept cold)
DCPIP in dropper bottle
50 ml of .4M sorbitol solution
2,5 m graduated cylinders

Acetate filters	Wavelengths transmitted	Constant Intensity Distance (determined by a photometer)
Blue	440 nm	16 cm
Green	520 nm	12 cm
Red	620 nm & longer	38 cm

* At these distances the intensity of light passing through the different filters is constant (is this a control?)

Note: A blue filter transmits blue light unlike a blue sweater which reflects blue light.

Based upon your absorption spectrum of isolated chloroplasts, formulate an hypothesis regarding the possible effects of light quality on the energy-capturing reactions of photosynthesis. Decide which steps your group will take to test this hypothesis.

Once you have decided which experimental tubes you will use you will have to deal with the following questions:

1. What controls do you need?

2. How will you make sure that there is no change in absorption occurring in the chloroplast solution or in the dye itself, which are not caused by photosynthesis?

3. If your controls show change in absorbance, how will you take this into account when reporting your experimental values?

4. Which tubes should be replicated and how many replicates should there be?

Once your group has answered the questions, proceed with the experiment. You should have time to repeat the entire procedure. If you come up with new ideas, these can be incorporated in a second run.

Analysis of the Results of this Experiment

Discuss in your groups what this experiment demonstrated about the following: which portion of photosynthetic process was observed in this experiment; experimental procedure; action spectra of different wavelengths of light; the controls used; the purpose of replicates. Then discuss your results with another group comparing your outcome with theirs. Then each student should independently write up the results, discussion, and conclusion (see assignment sheet) and hand this in before leaving the lab.

SUMMARY

By the end of this exercise you should be able to:

1. Describe the relationship between photosynthetic (energy-capturing) activity and light quality.

2. Use the spectrophotometer to measure the absorbance of a sample.

3. Design, carry out, and write up an experiment to test the effects of isolated factors on photosynthetic activity.

IN CLASS ASSIGNMENT
(Photosynthesis Exercise)

(Turn in at end of period)

NAME: _____

LAB SECTION: _____

RESULTS: (append tables/figures)

DISCUSSION:

CONCLUSION: