Name _____ **Instructor** _____ **Lab Section**_____

Objectives: To gain an understanding of:

- Leaf structure
- The nature of light and pigments (*eg*, chlorophyll)
- How the wavelength and intensity of light affect the rate of photosynthesis

Background material may be found in

- Chapter 7.1-7.7, 7.9

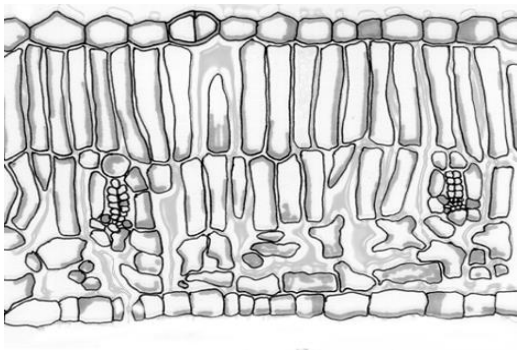
Biology: Concepts & Connections, 8th ed.

Photosynthesis uses light energy to convert the gas, carbon dioxide (CO_2), and water (H_2O) into sugar. Plants contain subcellular organelles called **chloroplasts** in which photosynthesis occurs. The chloroplast, in turn, contains pigments which absorb light so that it can drive the chemical reactions involved in the production of sugar. The most important pigment is **chlorophyll**. It is the green color of chlorophyll that colors the leaves of plants.

In the exercises that follow, you will investigate the structure of leaves and the wavelengths of light that are absorbed by chlorophyll and other leaf pigments, which wavelengths are important to photosynthesis, and what influence light intensity has on the rate of photosynthesis.

$$6\,CO_2 + 6\,H_2O \xrightarrow{\text{LIGHT + CHLOROPLAST}} C_6H_{12}O_6 + 6\,O_2$$

carbon dioxide + water in the presence of light and a chloroplast yields sugar + oxygen

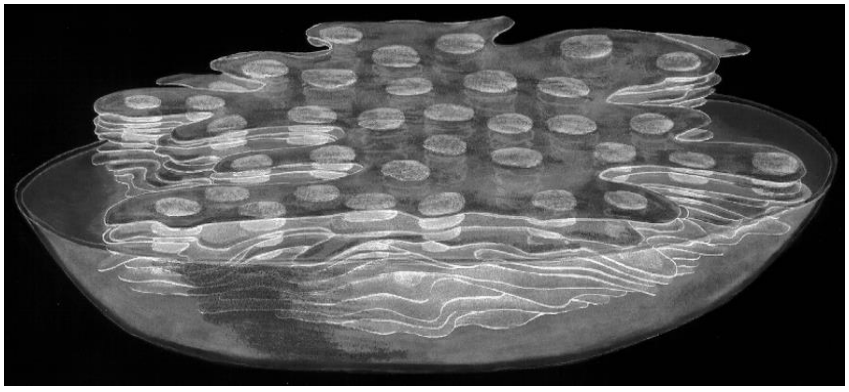


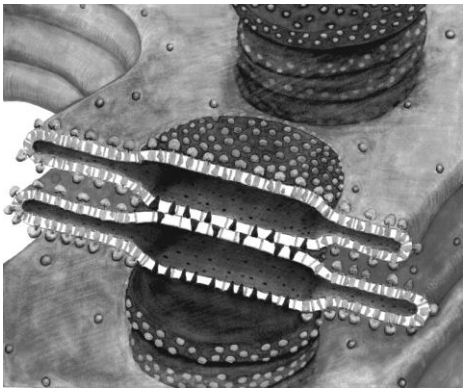
ABOVE, LEFT: LEAVES ARE SOLAR PANELS THAT CONVERT SUNLIGHT ENERGY INTO CHEMICAL ENERGY.

ABOVE, RIGHT: DRAWING OF A LEAF IN CROSS-SECTION.

BELOW, LEFT: DRAWING OF A CHLOROPLAST CUT IN CROSS-SECTION. STACKS OF THYLAKOIDS, CALLED GRANA, CONTAIN THE PHOTOSYSTEMS.

BELOW, RIGHT: CLOSE-UP OF STACKED THYLAKOIDS (GRANA). PHOTOSYSTEMS ARE SEEN AS SMALL WHITE PATCHES ON THE THYLAKOID MEMBRANES.





ISOLATION OF CHLOROPLASTS FOR USE IN THE HILL REACTION:
YOUR INSTRUCTOR WILL DEMONSTRATE THIS PROCEDURE

1. Weigh out 4.0 g of fresh spinach or chard leaves from which the major veins have been removed. Weigh the spinach in a clean weigh boat on a digital balance.

2. Cut the leaves into small pieces with scissors and place in a chilled mortar with 15.0 ml ice cold NaCl buffer and a sprinkling of chilled purified sand. Grind the tissue with the chilled pestle for 2 minutes.

3. Filter the suspension through four layers of cheesecloth into a chilled 15 ml conical centrifuge tube. Wring out the juice from the cheesecloth to obtain all the chloroplasts.

4. Centrifuge the filtrate at 22g (#4 on centrifuge) for 1 min. Be sure the centrifuge is balanced.

5. Decant ("pour off") the supernatant (solution above the pellet) into a clean chilled centrifuge tube and spin at 1300g (#6 on centrifuge) for 5 min.

6. Decant ("pour off") and discard the supernatant and then add 10 ml of ice cold NaCl buffer to the pellet ("the leftover solid") at the bottom of the centrifuge tube. With a pasteur pipette, gently resuspend the pellet into the buffer. If necessary cap the tube and invert several times.

7. Transfer 4.0 ml of the suspension to a clean chilled tube and dilute with 6.0 ml of NaCl buffer. This is the diluted chloroplast suspension you will use in your experiments today. KEEP THIS - Put it in the ice bath on your lab bench.

PREPARATION OF A "BLANK" FOR SPECTROPHOTOMETER CALIBRATION:

1. Your instructor will demonstrate use of micropipettors, which are used to accurately measure and distribute small volumes of liquids. You will then use a micropipettor to prepare your "blank".

CAUTIONS WITH REGARD TO THE USE OF THE MICROPIPETTORS

- **You will be using a p1000 micropipettor, and it should be set to 050. This will allow you to accurately measure 0.5 mL of liquid. Check that your micropippetor is set correctly.**

- **When using a micropipettor, first apply a tip**. Forgetting to do this would cause liquid leakage into the barrel. Since a micropipettor works by air displacement, its internal mechanism must remain dry.

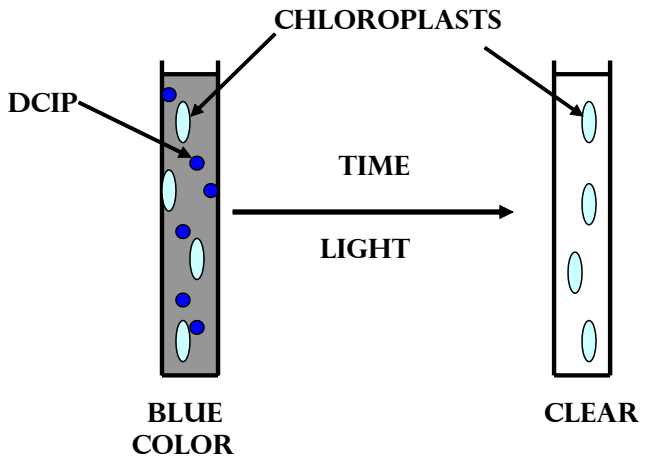
- **Always keep a micropipettor in a vertical position when there is fluid in the tip.** Do not allow liquid to accidentally run back into the barrel of the micropipettor.

- **Use your thumb to control the speed at which the plunger rises after taking up or ejecting fluid. Release the plunger slowly**. Releasing the plunger too quickly will cause liquid to be sucked into the barrel.

2. Produce a "blank" as described by the instructor, by adding 0.5 mL of chloroplast suspension to the buffer already present in the test tube labeled "blank". Your instructor will demonstrate how to mix the contents of the tube by covering the top with parafilm and inverting.

THE HILL REACTION: MEASURING THE RATE OF PHOTOSYNTHESIS

The Hill reaction involves the use of an indicator dye, DCIP, to measure the rate at which photosynthesis occurs in a suspension of chloroplasts. In the presence of actively photosynthesizing chloroplasts, the blue DCIP becomes colorless. The faster the rate of photosynthesis, the faster the dye becomes colorless. Thus DCIP is an indicator of the rate of photosynthesis.

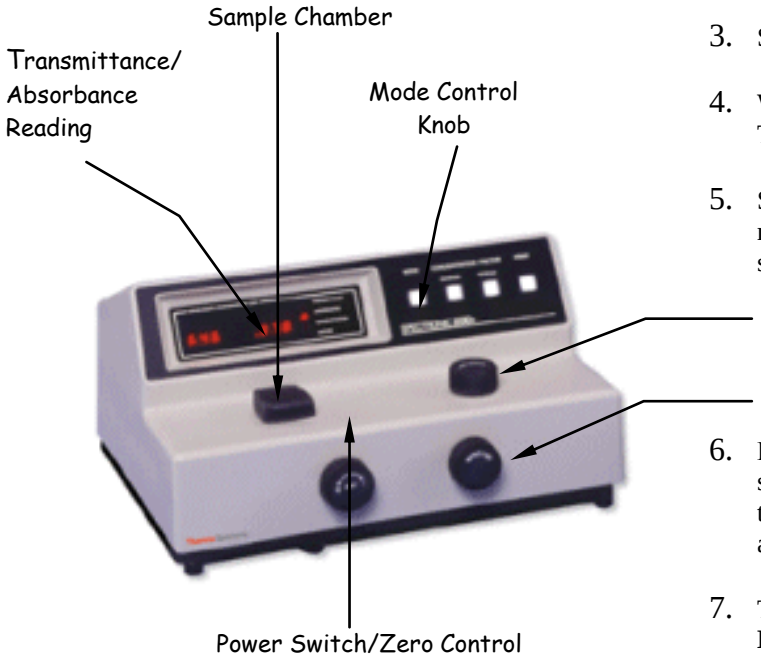


The change in the color of DCIP can be accurately determined by a **spectrophotometer** set at 600 nm, the wavelength of light absorbed by the dye. **If the DCIP changes from blue to colorless, absorbance values will decrease over the course of your experiments. The greater the change (that is, the more the absorbance values decrease), the more photosynthesis is taking place.**

Prior to the experiments, the spectrophotometer must be calibrated.

CALIBRATION OF THE SPECTROPHOTOMETER

1. Turn the spectrophotometer on using the left knob. The spectrophotometer should warm up for 15 minutes before use.



2. Adjust the wavelength control knob (top knob) until the wavelength reads **600 nanometers (nm).**
3. Set the filter level (bottom left) to 600-950 nm.
4. With the sample chamber empty, adjust the Transmittance (left knob) to 0% Transmittance.
5. Set the display mode to Absorbance by pressing the mode control key (left most button on top right of spectrophotometer) until the LED light is next to A.
6. Place your test tube labeled "blank" firmly into the sample chamber, close the chamber door, then adjust the right knob until the spectrophotometer reads zero absorbance.
7. The spectrophotometer is now calibrated and blanked. **Do not turn any knobs after calibration.** The spectrophotometer will now ignore the presence of the chloroplasts and buffer and "see" only the DCIP.

HILL REACTION EXPT 1: EFFECT OF LIGHT INTENSITY ON THE RATE OF PHOTOSYNTHESIS

EFFECT OF LIGHT INTENSITY ON THE RATE OF PHOTOSYNTHESIS

ABSORBANCE READINGS

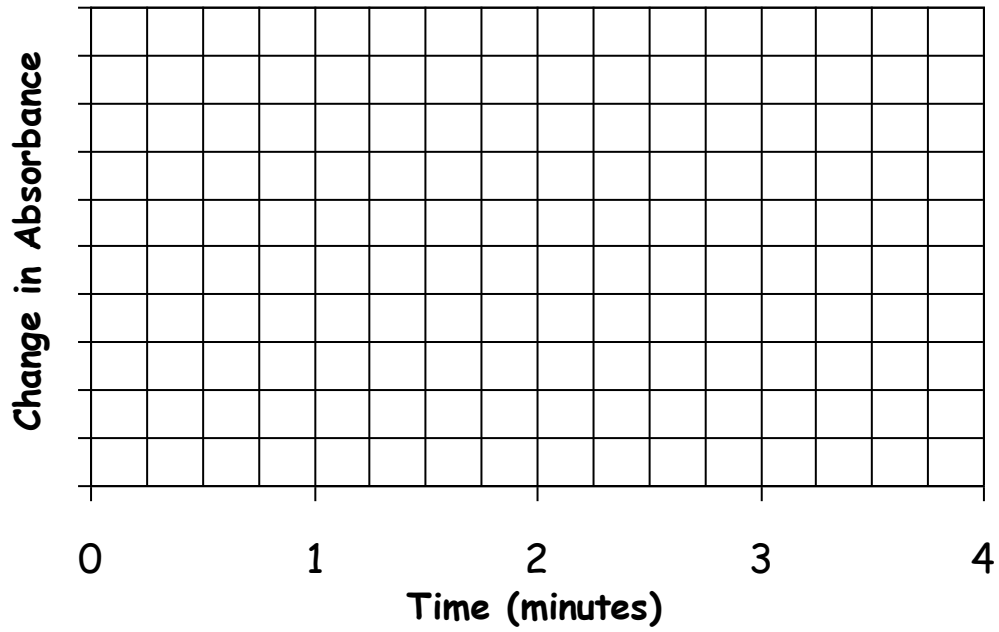
SAMPLE	TIME ZERO	1 MIN	2 MIN	3 MIN	4 MIN
NEAR					
FAR					

CHANGE IN ABSORBANCE

SAMPLE		1 MIN	2 MIN	3 MIN	4 MIN
NEAR					
FAR					

1. Obtain 2 test tubes containing buffer.
2. Using a permanent marker, label each test tube as either "near" or "far".
3. Add 0.5 mL of chloroplast suspension to each tube.
4. Change the pipet tip. Always change the pipet tip to avoid contaminating solutions.
5. Add 0.5 mL of DCIP to each tube (**DCIP is poisonous, handle with care**).
6. Mix the contents of each tube thoroughly, but gently, by covering with parafilm and inverting twice as demonstrated by your instructor.
7. Place the sample labeled "near" into the spectrophotometer.
8. Obtain an initial absorbance reading. Enter the data under "time zero" in top table to the left.
9. Repeat step 7 for the tube labeled "far".
10. Simultaneously place the two samples into the test tube rack illuminated by white light. The sample labeled "near" is placed nearest the light source, while the sample labeled "far" is placed furthest from the light source.
11. After one minute, remove the samples from the light and record their absorbance values. Enter the data under "1 min" in the "ABSORBANCE READINGS" table above (the top table).
12. Place the samples back into the light for another minute, then record absorbance values again.
13. Repeat step 11 twice more for a total of 5 absorbance values.
14. Calculate change in absorbance for each new value by subtracting each new absorbance value from the initial absorbance value at time zero. Enter these values in the "CHANGE IN ABSORBANCE" table above.
15. Plot the **change in absorbance** values on the graph shown below.
    If the DCIP in your test tubes changed from blue to colorless, you should have seen absorbance values going down over the course of the experiment. A greater change in absorbance indicates that more photosynthesis occurred in that tube, causing DCIP to lose its color.

Effect of Light Intensity on the Rate of Photosynthesis



HILL REACTION EXPT 2: EFFECT OF LIGHT WAVELENGTH ON RATE OF PHOTOSYNTHESIS

EFFECT OF LIGHT <u>WAVELENGTH</u> ON THE RATE OF PHOTOSYNTHESIS

ABSORBANCE READINGS

SAMPLE	TIME ZERO	1 MIN	2 MIN	3 MIN	4 MIN
RED					
GREEN					
BLUE					

CHANGE IN ABSORBANCE

SAMPLE		1 MIN	2 MIN	3 MIN	4 MIN
RED					
GREEN					
BLUE					

1. Obtain 3 test tubes containing buffer.
2. Using a permanent marker, label each test tube as either "green", "red", or "blue".
3. Add 0.5 mL of chloroplast suspension to each tube.
4. Add 0.5 mL of DCIP to each tube (**DCIP is poisonous, handle with care**).
5. Mix the contents of each tube thoroughly, but gently, by covering with parafilm and inverting twice.
6. Place the sample labeled "red" into the spectrophotometer.
7. Obtain an initial absorbance reading. Enter the data under "time zero" in the top table to the left.
8. Repeat step 7 for the tubes labeled "green" and "blue.
9. Simultaneously place the red, green and blue samples into racks illuminated by red, green and blue light respectively.
10. After one minute, remove the samples from the light and record absorbance values. Enter the data in the top table to the left.
11. Place the samples back into the light for another minute, then record absorbance values again.
12. Repeat step 11 twice more for a total of 5 absorbance values.
13. Calculate the change in absorbance by subtracting each new absorbance value from the initial absorbance value at time zero. Enter change in absorbance values in the "change in absorbance" table above.
14. Plot the change in absorbance values on the graph below.

Effect of Light <u>Wavelength</u> on the Rate of Photosynthesis

THE ABSORBANCE SPECTRUM OF CHLOROPHYLL AS DETERMINED BY THE SPECTROSCOPE

Sunlight and other forms of white light are composed of different wavelengths ranging from red to blue-violet. In this exercise we will use a spectroscope to separate white light into its component colors. If we place a solution of chlorophyll in the path of the white light entering the spectroscope, we will also be able to see which wavelengths are being absorbed by the various photosynthetic pigments and which wavelengths are not absorbed.

PROCEDURE

1. First look into the spectroscope without the chlorophyll solution to see the complete spectrum of colors comprising white light.

2. Then while still looking into the scope, have your partner or instructor slide the vial of chlorophyll solution in the pathway of the light. Note the dark bands that appear in the spectrum. These are the wavelengths being absorbed by the photosynthetic pigments.

3. Repeat the procedure to make sure you see exactly what colors are being absorbed.

QUESTION

1. Chlorophyll is supposed to absorb in the blue and the red regions of the spectrum. Do your observations with the spectroscope agree with this? Explain.

✍QUESTIONS

1. In what cellular organelle does photosynthesis take place?

2. What is the role of DCIP in the Hill Reaction?

3. In the Hill Reaction experiments, what is the relationship between the **change in absorbance,** as plotted on your charts**,** and the amount of photosynthesis occurring?

4. Chlorophyll is known to absorb from the blue and red regions of the light spectrum, while reflecting or transmitting green light. Did your experiment with the Hill Reaction (the test tubes placed under red, green, and blue light) agree with this? Explain.

5. Based on your observations and the ideas presented here, what colors do you think are "designed into" Gro-Lux™ lamps used in greenhouses. Why aren't greenhouses made of green colored glass?

CLEAN-UP INSTRUCTIONS ARE ON THE NEXT PAGE →

Clean-up:

_____ **Wash and dry mortar and pestle (no soap) as soon as finished using and return to cart at the front of the room.**

_____ **Dispose of D.C.I.P. in waste container on the front table. Dispose of used test tubes in bag on front table marked "used test tubes".**

_____ **Put used cheesecloth in the trash.**

_____ **Pour ice from the Styrofoam container into the sink.**

_____ **Clean the smaller plastic tubes (3 total) with water (no soap). Use wooden sticks by sink to clean pellet in the bottom. Return to Styrofoam container.**

_____ **Do not dispose of remaining NaCl buffer. Leave tube in the Styrofoam container.**

_____ **Reset tray on your table as per instructions next to the tray.**

_____ **Return all other supplies to correct trays on the side counter.**

LABORATORY NOTES