SUGGESTED LABORATORY EXERCISES IN PLANT PHYSIOLOGY FOR THE HIGH SCHOOL BIOLOGY CLASS

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PREFACE

This paper is an attempt to correct a deficiency that I have observed in high school biology classes in my area. Today the zoological side of biology seems to predominate in the high school biology classes. It is hoped that the use of some of the experimentation suggested by this paper might motivate the teachers and students to a better presentation and understanding of the botanical section of the biology curriculum. The student may then acquire a balanced understanding and appreciation of the plant and animal kingdoms.

Indebtedness is acknowledged to Dr. Imy V. Holt for his valuable guidance and assistance, as well as for the loan of materials used in this study. I am further indebted to the faculty members of Oklahoma State University for their patience and encouragement in the preparation of this report. I am deeply grateful to the National Science Foundation for the opportunity that it has provided me, and to Lavon Ellis and other members of the summer biology institute for their helpful suggestions. I wish to thank the members of my family for their understanding and cooperation during the writing of this report; particularly my wife, Bernice Payne, for the typing of the final manuscript.

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

The purpose of this report is to present to teachers of high school biology classes activities and laboratory exercises dealing with plant physiology of the higher plants.

Suggested is a collection of experiments which could be utilized either as unit laboratory activities or as individual student projects based upon the more important basic concepts needed for the understanding of plant life and its function.

> The world is confronted by an ever-deepening food crisis. We live in an age of chronic food shortage, a shortage which is intensified as the population of the world increases faster than our current production of food. Considering the globe as a whole, the available food supply in 1949 approximated that of 1939, but in these ten years, the world's population had increased by 15 per cent.[1]

Better training in plant physiology at the high school level would increase the probability that some of these students might develop an early interest that would lead to advanced training in basic or technological research as Plant Physiologists and thus contribute to the solution of the ever-deepening food crisis.

The practicability of each experiment has been considered from several views, including the high school laboratory facilities, cost involved, concepts gained, scientific technique involved, and the consumption of time and motivating effect.

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[1]James Bonner and Arthur W. Galston, Principles of Plant Physiology, (San Francisco, 1959), p. 5.

The experiments presented for consideration by this report will be divided into three categories, according to the school's science facilities: (1) Group C, experiments suitable for use in the school with less than 50 students enrolled in biology; (2) Group B, for the school with several classes in biology, one full-time teacher, and (3) Group A, experiments which might be used in the larger high schools with more than 150 students enrolled in biology classes.

The writer is going on the assumption that the greater the number of sections of a specific course offered, the greater will be the efficiency in equipment utilization, making possible the purchasing and use of the more costly type training aids and laboratory equipment. This assumption is taken solely to assist the user of this report in locating and selecting suitable experiments or activities, according to the facilities available.

Some of the experiments entailing more equipment than is available may be performed by allowing the students to work in teams as high as 3 or 4 students per team, thus allowing each to gain the understanding of the concepts hoped for even though individual equipment is not available. The teacher's own resourcefulness and enthusiasm may permit substitution of equipment and thus allow students to accomplish experimentation far in advance of the equipment eliminating factors apparent during preliminary planning for the laboratory work.

Consideration should be given in project and experiment planning as to sequence of presentation, with the basic physiology offered in earliest part of the course and the more complex concepts later as the course has given wider understanding to the student.

Two factors affecting this normal progression of the course that

must be considered are (1) the seasonal factors as to temperature, humidity, and length of day may necessitate earlier or later presentation of experiments than course context would suggest, and (2) some teachers may prefer to have the student do all the pre-experiment activities, such as planting and controlling the environmental factors during growth in advance of the experiment itself. The individual teacher must weigh the alternative of supplying the student with the plant at the stage of development where experimentation actually starts.

It is intended that the user of this report might use these experiments as an enrichment to his present biology course and in areas of plant study where he does not have sufficient laboratory exercises to put over in an interesting and provocative manner the understanding of such basic concepts as diffusion, osmosis, assimilation, digestion, photosynthesis, respiration, reproductive tissues, leaf, root, stem, and recent developments in tropisms, growth regulators, and isotopes. He might find one of the experiments out of the three suggested in this paper under each concept, of assistance, regardless of the local facilities available to him.

The scope of this paper is being limited by purposely omitting many of the more common laboratory exercises described in the standard high school biology textbook. Also excluded is any reference to field exercises encompassing collecting, preserving, and identification of plants, with only very limited reference to microscopic use or technique.

CHAPTER II

PHYSIOLOGY OF THE CELL

Experiments on Diffusion

Group A - Colorimeter Test of Cell Permeability

Objective: To present a functional concept as to the nature of the cell membrane and its part in diffusion.

Plant Materials	Apparatus	Reagents
10 Beet Slices	1 Beaker (500 ml.) 9 Test Tubes 1 Thermometer 1 Colorimeter	15 ml. water saturated Benzene (1.0 ml. benzene to 1 liter water)

Procedure:[1] Day 0 (40 minutes)

Part I - Effect of Temperature on Permeability

Cut 6 slices of beet root about 1 cm. wide, 4 cm. long and 3 mm. thick. Wash in running tap water for about 5 minutes to remove the pigment from damaged cells. Transfer to distilled water. Heat about 500 ml. of distilled water to 70° C in a beaker, immerse a strip of beet in the water for exactly 1 minute, then transfer to a test tube containing 15 ml. of distilled water at room temperature. Repeat at 65°, 60°, 55°, 50°, and 45° C by allowing the water to cool to these temperatures.

Next, place a slice which has been frozen and an untreated slice

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[1]Leonard Machlis and John G. Torrey, A Laboratory Manual of Plant Physiology, (San Francisco, 1959), p. 55.

in the distilled water at room temperature. One hour after placing each of the 8 slices in the distilled water, carefully remove the slice from the tube, shake the tube, transfer contents to a colorimeter tube, and determine with the colorimeter the amount of red pigment that has diffused out of the slice. Draw conclusions as to temperature effect on diffusion.

Part II - Effect of Organic Solvents on Permeability

Place 1 slice of beet, after washing in tap water as above, in a test tube containing 15 ml. of distilled water saturated with benzene. To a second test tube add 15 ml. of distilled water and insert a slice of washed beet as above. Allow the tubes to stand at room temperature, with occasional stirring, for 1 hour. Then compare the red pigment in the tubes, using the colorimeter. Correct the reading for the benzene by measuring the absorption of the saturated benzene solution. Draw conclusions as to the effect of organic solvent on permeability.

Group B - Determination of Diffusion Pressure[2]

Objective: Diffusion Pressure Deficit represents the force with which water under no pressure tends to enter a cell. The ability to determine this pressure and express it quantitatively is helpful in understanding translocation of substances within a plant.

Plant Materials	Apparatus	Reagents
Potato	Cork-borer (1 cm. diam.) 6 Glass Tumblers Metric Ruler Razor Blade Scales	1.0 M Sucrose (500 ml.)

[2] Ibid., p. 63.

Procedure: Day 0 (2 hours)

Prepare 6 containers (tumblers, beakers, etc.) each containing 100 ml. of one of the following: distilled water, 0.15, 0.25, 0.35, 0.45, and 0.55 M sucrose solutions. Prepare these by making up 500 ml. of sucrose and diluting as required.

The next part of setting up this experiment must be done as quickly as possible. Using a cork-borer of 1 cm. diameter, obtain from one potato 6 cylinders, each 4 cm. long, with all external layers trimmed off. Cut a cylinder into a large number of very thin slices with a razor blade, wash quickly in water, blot dry, weigh, and place in one of the solutions. Do this with each cylinder in order.

Exactly 1 1/2 hours after immersion of a particular cylinder, remove the slices, blot dry, and weigh. Repeat this procedure until all the cylinders have been weighed. Present the data in a table showing original weight, final weight, and change in weight. Make a graph plotting change in weight against sucrose concentration of the solutions. From the graph determine diffusion pressure deficit by interpolation.

Group C - Diffusion Rate[3]

Objective: Distinguish between purely physical phenomena and chemical phenomena of a biological process by use of the temperature coefficient.

Hint: $Q_{10} = \frac{VT}{VT - 10^{\circ} C}$

Plant Materials	Apparatus	Reagents
None	Graph Paper Tape 7 Test Tubes	7 Glass Tubing (agar) Congo Red $CuSO_4$ Eosin B Erythrosin

[3]Ibid., p. 53.

Procedure: Day 0 (20 minutes)

For this experiment pieces of glass tubing filled with 10% agar should be provided. Add to each test tube only enough of the dye solution to reach halfway up the outside of the enclosed agar tube.

Put an agar tube into a test tube containing 20% $CuSO_4$ solution and another into a test tube of 2% $CuSO_4$ solution. Seal the test tubes with tape and place them in a locker. Determine how far the $CuSO_4$ has diffused in each tube after one week. Measure the tubes at visually estimated points of equal color intensity across the main front of the diffusing material. Ignore any aberrant streaks. Into two other test tubes put 0.1% methylene blue solution, add the agar tubes, and seal with tape. Put one into a basket and incubate at 25° C; do the same for the other at 15° C.

Compare the distances diffused after one week. Calculate the Q_{10} of the process.

Put solutions of 0.001 M eosin B (MW 624), erythrosin (MW 880) and Congo Red (a 0.1% colloidal suspension) into test tubes, add agar tubes, seal with tape, and determine the distances diffused after one week. Make a graph showing the relation between molecular weight and rate of diffusion.

Osmosis Experiments[4]

Group A - Determination of the Osmotic Pressure of Cell Sap

Objective: To demonstrate how the osmotic pressure of the vascular sap of a group of cells may be determined by plasmolysis.

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[4] Ibid., p. 62.

Plant Material	Apparatus	Reagents
Rhoeo Leaves (or substitute)	Dissecting Kit Microscope 5 Microscope Slides and Cover Slips 5 Test Tubes Thermometer (room)	1 M Sucrose solution

Procedure: Day 0 (55 minutes)

Fill small test tubes about 1/3 full with the following series of sucrose solutions: 0.34, 0.30, 0.26, 0.22, and 0.18 M. With a scalpel and a pair of forceps carefully peel off small strips of the lower epidermis of leaves of Rhoeo discolor. Examine a strip of the epidermal tissue to verify that it consists of intact cells containing the red-colored anthocyanin pigment dissolved in the vacuolar sap. Submerge a strip into each tube, at about 4-minute intervals between successive tubes.

Exactly 30 minutes after immersion of each strip, examine the cells under the microscope for plasmolysis, mounting the strip in the same solution in which it has been immersed. Count cells showing any plasmolysis as plasmolyzed and unplasmolyzed in the various solutions, and plot the percentage of cells plasmolyzed against the molar concentration of sucrose. The solution in which half of the cells are plasmolyzed is considered to have the same OP as the cell sap. Interpolate if necessary.

Calculate the OP of the epidermal cells by the following formula:

$$OP = \frac{22.4MT}{273}$$

in which OP = osmotic pressure in atmospheres, M = concentration of external solution at incipient plasmolysis (moles liter), and T = absolute temperature. (0° C = 273° T on the absolute temperature scale).

Group B - Osmotic Effect of Nutrient Salt Concentration on Water Absorption and Plant Growth

Objective: To show that water is frequently in ample supply, but the plant is unable to absorb it because of unfavorable gradient in osmotic conditions.

Plant Materials	Apparatus	Reagents
14 Sunflower Seedlings	7 Corks (2 hole) Cotton 7 Jars Marking Pencil Metric Ruler	0.5 M $CaCl_2$ (200 ml.)

Procedure:[5] Day 0 (40 minutes)

By appropriate dilutions of a 0.5 M $CaCl_2$ stock solution make up 200 ml. each of 0.01, 0.02, 0.03, 0.05, 0.1 and 0.2 M solutions. Place the solutions in half-pint jars, and include one jar with distilled water only. Select 14 uniform, vigorous, 10-day-old sunflower seedlings that have been grown in sand. Place 2 plants in a cork, using cotton for support (keep cotton out of solution), and place the cork in a jar. Measure and record the length of the stem above the cotyledons, using a millimeter ruler. Similarly set up plants in corks for the other jars. Mark the level of the liquid in each jar with a glass-marking pencil.

At the next laboratory period add measured amounts of distilled water up to the mark, and take notes on the appearance of the plant.

After they have been in the solutions one week, remove the plants, and determine the length of the shoots above the cotyledons. Observe the condition of the plants. Determine the amount of water needed to restore each solution to its original level. Make a table showing the results, including the total amount of water used in each jar.

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[5] Ibid., p. 65.

Group C - Osmosis Through Living Membranes

Objective: To demonstrate the physical process of Osmosis through living membranes.

Plant Material	Apparatus	Reagents
2 Carrots (potato may be substituted)	Bottles Clamp and Stand Cork (1 hole) Cork Borer Glass Tubing Tape	Sugar

Procedure:[6] Day 0 (30 minutes)

Slice off the top of a carrot to give a flat surface. With a 15-mm. cork borer cut a cylindrical plug in the center of the cut end of the carrot parallel to the long axis. This plug can be removed readily with a cork screw or may be carefully cut out with a knife. With a knife shape the cut end of the carrot like the neck of a bottle, making the neck about 15 mm. high. This neck need be no more than about 3 mm. thick. Fill the cavity of this carrot about one-half to three-fourths full of granulated sugar. Fit the glass tube into the cork tightly so that there will be no leaks (it must hold liquids). Insert this cork with its tube into the neck of the carrot bottle. Seal it by means of the tape. If the cavity is too full the syrup will rise into the tube. Mark this level. Suspend the carrot in a container of water. Watch for a slow rise of the sugar solution in the glass tube.

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[6]David F. Miller and Glenn W. Blaydes, Methods and Materials for Teaching Biological Sciences (New York and London, 1938), p. 330.

CHAPTER III

METABOLIC PROCESS

Experiments on Assimilation

Group A - Seedling Development in Light and Dark

Objective: To show the difference between light and dark grown plants.

Plant Material	Apparatus	Reagents
60 Peas	2 Beakers 10 ml. Graduated Cylinder 250 ml. Graduated Cylinder Oven Petri Dish Test Tube Torsion Balance	None

Procedure:[1] Day 0 (40 minutes)

Select 60 peas, Alaska variety, of about equal size. Weigh them in three lots of 20 on the torsion balance. Place lot 1 in a weighed test tube, dry in the oven (95°C) for 48 hours, then cool and reweigh. The average weight of the seed coat of each pea is 17 mg.; subtract this weight from the dry weight of the seed to obtain the initial dry weight of the embryo, including cotyledons.

Determine separately the volumes of lots 2 and 3 by placing them

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[1]Leonard Machlis and John G. Torrey, A Laboratory Manual of Plant Physiology (San Francisco, 1959)p. 17.

in a 10 ml. graduated cylinder and covering with a measured volume of water. Place each lot in a Petri dish, half cover with distilled water, label, and allow to germinate in the dark for 48 hours. At the end of the 48 hours, prepare 2 small, paper-lined flats of washed sand, and plant each seed lot about 1/2 inch deep. Put one flat, lot 2, in the greenhouse, and the other, lot 3, in the dark at 25° C. Water each regularly for the next 2 weeks.

This experiment as described shows satisfactory differences between light and dark-grown plants. It may be conducted under more carefully controlled temperature conditions if both flats are grown in the greenhouse with the flat containing lot 3 covered by a specially constructed light-tight box. The box should be approximately 16" high, be painted dull black inside and silver outside, and it should fit snugly around the flat.

On the twenty-first day after soaking the seed, remove the plants carefully from each flat, and rinse off all the sand. Count the total number of plants in each seed lot when they are harvested. This number must be used for all calculations expressed on a per-plant basis. For each lot, measure the shoot length and main root length of 10 plants. Remove the seed coat from any seed where it remains, fold together the plants of each lot, and determine the volume of each lot as before, using a larger graduated cylinder (e.g 250 ml.).

Separate the cotyledons from each of the plants, blot them dry on filter paper, and determine the fresh weight of each lot of cotyledons. Make appropriate corrections for any cotyledons that may have been lost from the plants. Determine similarly the fresh weight of the remaining tissue of each lot of plants. After placing the plants from each lot

in a separate beaker, put the cotyledons associated with each lot in a test tube, and then place each tube in the proper beaker. Dry in the oven (80° C) for 48 hours, and reweigh the cotyledons and dried tissues of each lot of plants.

Determine the percentage of water of seeds and plants as affected by light and dark environment.

$$\frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Group B - Locus of Plant Growth

Objective: To determine the localized regions of cell enlargements as they occur in the primary body of the pea seedling.

Plant Material	Apparatus	Reagents
6 Pea Seeds (Germinated)	Beaker (600 ml.) Glass Filter Paper Metric Ruler Marking Device Petri Dish	Marking Ink

Procedure:[2] Day 0 (30 minutes) Day 2 (15 minutes)

Root Growth. (Shoot growth could be shown with slight modification). Prepare a moist chamber by lining the sides of a 600 ml. beaker with thoroughly moistened paper toweling. Completely cover a 3 1/4" x 4" glass plate with coarse-grade filter paper soaked with distilled water. Select, from the germinated pea seedlings, one in which the root is fairly straight and at least 2 cm. long. Wipe excess ink from the thread of the marking device. Lay the root on a millimeter ruler, and, starting at the tip, quickly mark 10 thin lines

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[2] Ibid., p. 16.

at 1 mm. intervals with black marking ink. Place the seedling against the moist plate with a rubber band supporting it just under the cotyledons. Repeat the operation until 5 roots are marked. Mark 2 more seedling roots with a single line 10 mm. from the tip as control plants to test the effect, if any, of the marking procedure. Take care to avoid drying the seedlings during the marking. Support each root so that its whole length touches the moistened filter paper. Insert the glass plate supporting the seedlings into the beaker. Cover the beaker with the bottom of a 10 cm. Petri dish, and place the beaker in a locker.

At the end of 48 hours, measure the distance between the marks on each marked root. Average the lengths measured in the first interval behind the apex; do the same for all the other marked intervals. Measure the length of the control roots from the tip to the marked line. Plot the data on a separate sheet of graph paper. Draw conclusions as to the regions of cell growth as noted by your results.

Group C - Light and Dark Effect Upon Growth

Objective: To determine the effect of light on growth.

Plant Materials	Apparatus	Reagents
Seeds (Wheat)	Corks	Water Culture
Seeds (Radish)	Dark Box	
	Glass Jar	
	4 Petri Dishes	
	2 Pots	

Procedure:[3] Day 0 (30 minutes)

Part I - Germinate seeds of wheat, timothy, or other plants in complete

[3] Edmund W. Sinnott and Katherine S. Wilson, Laboratory Manual for General Botany, (New York, Toronto, London, 1956), p. 203.

darkness in Petri dishes lined with moist filter paper. When they have grown to about 2 cm. in height, place them in a dark box with a small opening on one side only, so that the seedlings are exposed to light from one side. Similar but less marked results may be obtained by growing them in front of a window in an ordinary room so that the light comes chiefly from one side. As controls, similar seedlings should be exposed equally on all sides to light. Note the direction of growth of the stem and the leaves.

Grow some seedlings of radish or other suitable plant in water culture in a glass jar so that all parts of the plant are visible, and expose them to one-sided illumination. The base of the stem should be kept firmly vertical with a perforated cork or by other means. Under these conditions compare the growth reactions of stem, root and leaves to light.

Part II - Etiolation. Germinate two pots of seeds in the dark. Upon emergence of the hypocotyl one pot should be placed in good light, the other should be placed in the dark. Light will vary, all other factors remain identical. Observe for color, leaf and growth development, including height and the number of internodes.

Experiments on Photosynthesis

Group A - The Effect of Temperature and Light Intensity on the Rate of Photosynthesis

Objective: The overall rate of photosynthesis may be measured by oxygen evolved, or carbon dioxide absorbed. By the following experiments one could show the effect of light intensity and temperature as a limiting factor in photosynthesis.

Plant Material	Apparatus	Reagents
Elodea (Elodea canadensis)	Audus Microburette Lamp (100 watt) Large Test Tube Rubber Tubing 2 Screw Clamps Stand and Clamp Thermometer (C)	Sodium Bicarbonate Hoagland Solution or Pond Water

Procedure:[4] Day 0 (1 hour, 50 minutes)

In this experiment, measure the amount of O_2 evolved by the water plant Elodea in an Audus Microburette. Cut off under water the base of a healthy sprig of Elodea, and mount with base end up in the collecting tube in a large test tube containing pond water or 1/4 strength Hoagland solution to which has been added 0.5% sodium bicarbonate. Suspend the large test tube containing the collecting tube in the water bath. Gently evacuate the rubber tube attached to the microburette until the entire burette is filled with solution. Close both pinch clamps. Bubbles of O_2 will collect at the top of the collecting tube. Adjust the lower pinch clamp and bring the bubble onto the scale for measurement. After the measurement, by opening the pinch clamp all the way, force the bubble out into the bulb. Make three successive determinations of the gas evolved at temperatures of 30° and 20° with the lamp 48, 24, 12, and 6 inches from the plant. Maintain the temperatures by adding hot or cold water as needed to the water bath.

Before doing the above, make a trial run at 20° with the lamp at 12" to learn how to use the apparatus and to adjust the amount of plant material to give a 2 cm. bubble in not more than 15 minutes, preferably in less time. Begin timing when the bubbles begin to rise to the capillary tube.

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[4] Ibid., p 132

Designate the light intensity at 6" as 100 units. Then remembering that light intensity varies inversely as the square of the distance, make a graph showing the relation between light intensity and the evolution of O_2 per unit time. Calculate the temperature coefficient Q_{10} under conditions of limiting temperature and conditions of limiting light intensity.

Group B - Measurement of the Rate of Photosynthesis

Objective: In order to study the effects of various environmental factors on a process such as photosynthesis, it is necessary to make quantitative measurements of its rate. The rate of photosynthesis can be studied by means of a dye (phenol red) that turns red in an alkaline solution and yellow in an acid solution.

Plant Material	Apparatus	Reagents
Elodea Plants (Elodea canadensis)	Glass Tube 2 Test Tubes	Phenol Red Solution Mercuric Chloride

Procedure:[5] Day 0 (50 minutes)

Place some Elodea plants in a test tube and cover them with tap water. Add five drops of phenol red solution. With a glass tube blow into the water until it just turns yellow; do not blow any longer. Repeat this procedure with another test tube that contains the dye and water but no plant. This tube will serve as a control.

Expose both tubes to diffuse light and determine how long it takes for the dye solution to turn a faint pink. Repeat the experiment using bright light. Repeat in total darkness. What causes the dye to turn yellow after blowing into the solution. Why does it turn pink after

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[5] Erich Steiner, A. S. Sussman, and W. H. Wagner, (Botany Laboratory Manual (New York, 1957), p. 84.

exposure to light? What was the effect of altering the light intensity? The effect of poisons on photosynthesis may be studied by adding a few drops of mercuric chloride solution to the dye solution and repeating the experiments described above.

Group C - Carbon Dioxide and Light Requirements of Photosynthesis

Objective: The unique ability of green plants to change carbon dioxide in carbohydrates in the presence of light makes it important to understand some of the requirements, such as the need of CO_2 or light.

Plant Material	Apparatus	Reagents
Potted Plant	Glass Square Tape Thistle Tube Opaque Paper	KOH (Potassium Hydroxide)

Procedure:[6] Day 0 (15 minutes) Day 1 (30 minutes)

Plants to be used are first placed in the dark long enough to allow the depletion of starch in the leaves.

Part I - A portion of a leaf is deprived of carbon dioxide by filtering the air which reaches it through either KOH (Potassium Hydroxide) or soda lime. This is accomplished by clamping a thistle tube containing the chemical to the lower surface of the leaf, and pressing a piece of glass against the upper surface. Hold in place by tape.

Expose the leaf to sunlight for a day; remove the leaf, extract the pigments in warm alcohol, and treat the leaf with iodine solution. Caution! In heating the alcohol, use a double boiler. Keep the alcohol away from an open flame. How does the area of the leaf which was devoid

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[6] Ibid., p. 77.

of carbon dioxide compare with that to which the gas was accessible? Account for the difference between the two areas.

Part II - Using opaque paper, cut a mask which excludes light from all but a specific part of the leaf. Expose the plant to sunlight for a day, and treat the leaf as in the previous experiment.

Experiments on Respiration

Group A - The Effect of Temperature on the Rate of Aerobic Respiration

Objective: The rate of respiration may be controlled by the concentration of oxygen and the temperature. It is hoped this experiment will show the optimum temperature between a catalyzed reaction and the inactivation of the enzyme.

Plant Material	Apparatus	Reagents
60 g. Pea Seeds (soaked)	5 16 oz. Bottles 50 ml. Burette 4 Cheesecloth Squares 125 ml. Flask Incubator 5 Rubber Stoppers Triple Beam Balance String	0.2N NaOH 30 ml. Barium Chloride Dropping Bottle of Phenolpthalein Solution 0.1N HCL

Procedure:[7] Day 0 (20 minutes) Day 2 (60 minutes)

Place 50 ml. of 0.2N NaOH in each of five 16 oz. bottles, and immediately stopper tightly with rubber stoppers. Weigh out four 10 g. portions of soaked pea seeds, place each in a sack made of a square of cheesecloth tied with a piece of string, and suspend one in each of four of the bottles, with the string held between the stopper and the glass. The seeds and cheesecloth must be secured safely above the alkali.

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[7] Leonard Machlis and John G. Torrey, A Laboratory Manual of Plant Physiology (San Francisco, 1959) p. 102.

Place the tightly stoppered control bottle, lacking seed, in a locker. Label the bottles, and keep one at each of the following controlled temperatures: 5° C (refrigerator), 20° C (locker), 25° C (culture room), and 35° C (incubator). At the end of 48 hours, remove the seed from the bottles, and quickly restopper. Then titrate for the amount of CO_2 released in respiration.

Pipette 10 ml. of solution from a bottle into a 125 ml. flask, and add 5 ml. of $BaCl_2$ solution, which will precipitate the CO_2 absorbed by the alkali in the bottle. Add 3 drops of phenolphthalien indicator, and titrate with 0.1 HCl until the color disappears. Do the same thing for a 10 ml. portion of alkali from the control bottle (the one with no seeds). Subtract the first value obtained from the value for the control flask, and multiply by 5. This will give the total amount of acid equivalent to the CO_2 respired. Run duplicate titrations on each flask, using considerable care in reading the measurements. Plot ml. of HCl equivalent to respired CO_2 against temperature.

Group B - The Measurement of Respiratory Rates

Objective: To measure respiration in the laboratory by determining the amount of oxygen absorbed by a plant.

Plant Material	Apparatus	Reagents
40 g. Plant Tissue	Respirometer	Potassium Hydroxide

Procedure:[8] Day 0 (60 minutes)

Respiration is a process fundamental to life. It serves as an indication that an organism is living; moreover the level of respiration

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[8] Ibid., p. 111.

often yields a great deal about what is happening in an organism. Respiratory rates can be studied by measuring the disappearance of food, the consumption of oxygen, the production of carbon dioxide, or the yield of energy.

When it is used properly, a respirometer is exceedingly sensitive to even small changes in gas pressures. Several types of plant materials should be furnished for measurements of respiratory rate. Each laboratory table should be furnished with one or two respirometers in which should be placed 40 grams of tissue. The weighing of this tissue should be performed as accurately as possible.

Procedure for using the respirometer: (1) See that the manometer contains a continuous column of colored fluid. If the column is broken, refill the manometer. DO NOT TIP THE MANOMETER! (2) Carefully remove the stoppers from both bottles and place the weighed plant material in one and a few drops of water in the other. Avoid excessive handling of the bottles since the heat from the hands is enough to cause erratic results. Because of the sensitivity of this instrument heat from other sources such as sunlight or lighted Bunsen burners will also affect the results. (3) Insert the small vial containing 10 per cent potassium hydroxide into the bottle that contains the plant material. Insert a filter paper wick in this vial to increase the absorbing surface. DO NOT ALLOW ANY POTASSIUM HYDROXIDE TO TOUCH THE PLANT MATERIAL. (4) Restopper the bottles tightly and allow the respirometer to come to room temperature in order to dissipate the heat transferred while the bottles were being handled. Usually five to ten minutes will suffice. (5) Apply the screw clamps, first to the bottle containing the plant material

(respiration bottle) and then to the temperature-control bottle. (6) Record the level on the scale where the end of the column of fluid comes to rest. (7) Record the number of millimeters that the level of the fluid travels every ten minutes. The scale is graduated in millimeters so that this distance can be read off directly. Continue these readings for 50 minutes. (8) On the graph, plot the number of millimeters traveled by the manometer fluid against the time when the reading was made; this will show the relationship between respiration and the absorption of oxygen by the plant tissues.

Group C - Release of Carbon Dioxide in Respiration

Objective: To demonstrate the release of carbon dioxide in respiration.

Plant Material	Apparatus	Reagents
Pea Seed	Flasks, 250 ml. Stoppers Tubing	Phenolphthalien Sodium Hydroxide

Procedure:[9] Day 0 (30 minutes) Day 1 (15 minutes)

A prominent feature of the process of respiration is the release of carbon dioxide. This can be demonstrated by connecting a flask containing some germinating pea seeds to another flask containing a solution of phenolphthalien, a PH indicator, which has been turned red by the addition of a small amount of a base, such as sodium hydroxide. If the apparatus is allowed to stand for a number of hours, the carbon dioxide released by the respiring seeds will move through the connecting tube to the second flask, dissolve in the water, which finally decolorizes it. Carbon dioxide and water together form carbonic acid.

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[9] G. Alexander et al., Laboratory Directions for General Biology (New York, 1959), p. 169.

CHAPTER IV

PHYSIOLOGY OF TISSUES

Experiments on Reproductive Tissues

Group A - Potato Bud and Seed Dormancy

Objective: To illustrate the induction and breaking of bud or seed dormancy by the use of plant regulators.

Plant Material	Apparatus	Reagents
3 Dormant Potatoes	Battery Jar	2% Thiourea
20 Seeds (alfalfa, sweet clover, vetch)	5 Petri Dishes	40 mg./liter Coumarin Solution (3 ml.)
150 Lettuce Seeds	Filter Paper	5% Thioures Solution
	3 Paper Sacks	1% Methyl Ester of a-naphthaleneacetic Acid
	5 Strings	

Procedure:[1] Day 0 (35 minutes) Day 14 (15 minutes)

Part I - Select 3 small potato tubers of similar size. Treat potatoes in each of the following ways: Soak in a 2% aqueous solution of thiourea for 1 hour, then air dry. Dip in a 1% aqueous solution of the methyl ester of a-naphthaleneacetic acid (MENA) until thoroughly wet; then air-dry. Soak in distilled water for 1 hour, and then air-dry. When all the potatoes have dried thoroughly, place each one separately in a small brown-paper bag, tie the bag securely, label, and place in the dark.

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[1]Leonard Machlis and John G. Torrey, A Laboratory Manual of Plant Physiology (San Francisco, 1959), pp. 35-38.

Two weeks later, open the bags, and observe the potatoes for sprouting; then retie and continue for another 2 or 3 months, with observations every 2 weeks. Count the number of buds that have sprouted on each potato.

Part II

A. Impervious Seed Coat. Select 20 seeds of Phacelia nemoralis or some other suitable species. Place 10 seeds on a filter paper in one of the Petri dishes. With a razor blade carefully cut through the outer seed coat of each of the other 10 seeds, and remove a small piece of the coat, exposing the inner tissues. Place these seeds on filter paper in a second dish. Add 3 ml. of distilled water to each dish and moisten the filter paper. Label each dish with a number and the date, and place in the dark at 25° C. Compare germination under the two treatments at the end of 48 hours, and record the percentage of germination.

B. Chemical Factors. Place filter paper in the bottom of three Petri dishes, and place 50 dry lettuce seeds (variety Grand Rapids) in each dish. To dish 1 add 3 ml. of distilled water, to dish 2 add 3 ml. of 40 mg. per liter coumarin solution, and to dish 3 add 3 ml. of 0.5% thiourea solution. Cover the dishes, and immediately place in the dark at 25° C to germinate. Observe after 48 hours. Record the percentage of germination in each treatment.

Group B - Parthenocarpy[2]

Objective: To determine whether growth substances will induce the development of seedless fruits.

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[2]Edmund W. Sinnott and Katherine S. Wilson, Laboratory Manual for General Botany, (New York, Toronto, London), p. 187.

Plant Material	Apparatus	Reagents
3 Tomato Plants	Razor Blade	Lanolin Paste Indolebutyric Acid

Procedure: Day 0 (25 minutes)

From tomato plants with flower buds just ready to open, carefully remove the stamens before any pollen is shed. Cut off the style and place on the cut stump a bit of lanolin paste containing 0.2 or 0.3 per cent indolebutyric acid. As control, (1) leave some plants untouched, (2) from others remove the stamens and place lanolin paste without indolebutyric acid on the cut stump, and (3) from others remove the stamens but do not add lanolin.

Be sure to label each flower stating the treatment given. Examine at intervals and compare the development of the ovaries. When growth ceases cut the ovary transversely and determine whether seeds have been formed.

Group C - Apical Dominance

Objective: To determine the influence of a terminal bud on the growth of buds below it.

Plant Material	Apparatus	Reagent
8 Bean Seedlings	8 Pots Razor Blade	Indoleacetic or Indolebutyric Acid Lanolin Paste

Procedure:[3] Day 0 (20 minutes)

Plant bean seeds in small pots. After 2 or 3 weeks, or when the two large primary leaves of the seedling are well developed and the plumular shoot is several centimeters long, cut off the tip of this

[3]Ibid., p. 177.

shoot in several plants and set others aside uncut as controls.

With other plants decapitated in the same way, place a bit of lanolin paste to which about 0.2 or 0.3 per cent of indoleacetic or indolebutyric acid has been added on the decapitated stump.

On still other decapitated plants, as controls, place a bit of lanolin paste which does not contain growth substance on the cut surface.

Recapitulation--four groups including the controls:

1. Decapitated
2. Uncut (control)
3. Decapitated + hormone in lanolin
4. Decapitated + lanolin (control)

At weekly intervals note what differences as to bud development there are among these four groups of seedlings.

Experiments on the Leaf

Group A - Effects of Environmental Factors on the Rate of Transpiration

Objective: Use a potometer to demonstrate transpiration and how it is affected by environmental factors.

Plant Material	Apparatus	Reagents
Geranium (Leafy Branch)	Fan Hot Plate Plastic Hood 2 Photofloods Potometer	None

Procedure:[4] Day 0 (50 minutes)

A Geranium shoot or that of another appropriate plant should be cut off at ground level and the cut end immediately put into the water.

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[4]Erich Steiner, A. S. Sussman, and W. H. Wagner, Botany Laboratory Manual, (New York, 1957), p. 88.

The shoot is fitted into the potometer. All air bubbles must be removed from the plant chamber when the potometer is set up. The measurement of transpiration is carried out as follows:

When the instrument is not in use the stopcock below the water reservoir should be open, but a pinch clamp should be placed on the rubber tube connecting the capillary tubing to the main body, or plant chamber, of the potometer. The capillary is filled before the clamp is applied. When ready to measure the transpiration of the plant, mark the end of the column of water in the capillary, close the stopcock, and open the pinch clamp. As water is transpired from the plant, the end of the column of water in the capillary will move toward the chamber of the potometer.

Lay a centimeter ruler along the capillary, and measure the number of centimeters traversed by the end of the water column during one minute (or a longer period, depending upon the speed with which water is lost). Repeat the experiment, using one or more of the following conditions: (a) Cover the plant with a plastic hood and tie it at the base of the plant; (b) expose the plant to a gentle air current by means of an electric fan; (c) use a strong air current; and (d) repeat, using a hot plate between the fan and the plant, or heat lamps.

Group B - Sugar-Starch Interconversions in Leaves

Objective: To illustrate the temporary storage of photosynthetic starch and to show that all leaves do not use starch as a form of storage of carbohydrates.

Plant Materials	Apparatus	Reagents
1 Geranium Leaf	Razor Blade	95% Ethyl Alcohol
1 Iris Leaf	50 ml. Beakers	I_2KI
9 Corn Leaves (etiolated)		25 ml. 0.5 Sucrose
		Glucose
		Fructose
		H_2O

Procedure:[5] Day 0 (50 minutes) Day 2 (15 minutes)

Part I - Starch Storage in Leaves. Obtain, from plants that have been in bright light for several hours, one leaf of variegated Geranium and one of Iris. Immerse the leaves in 95% ethyl alcohol on the steam bath for 20 minutes. Then wash them in hot water, and immerse them in I_2KI solution with water, and spread them out. A dark purple coloration indicates the presence of starch in the leaf.

Part II - Interconversion of Sugars. Put into 50 ml. beakers or half-pint jars 25 ml. each of the following: 0.5M solutions of sucrose, glucose, and fructose, and distilled water. Pick 9 etiolated corn leaves, and place the basal ends of 2 of them in each of the solutions. Mark (by holes or small cuts) each pair in such a way that they can be identified. Keeping the basal ends in the solutions, cut off about 1 cm. of the base of each. Place the beakers on the shelf in the locker. Test the odd leaf for starch as in Part I above. After 48 hours, test the leaves in the solutions for starch.

Group C - Relative Rates of Water-Vapor Loss from Leaves of Various Plants

Objective: To measure the relative rates of water vapor loss of various plants due to transpiration.

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[5]Leonard Machlis and John G. Torrey, A Laboratory Manual of Plant Physiology (San Francisco, 1959) p. 91.

Plant Material	Apparatus	Reagents
Assorted Plants	Millimeter Ruled Paper Paper Clip	Cobalt Chloride Disks Collodion Solution

Procedure: Day 0 (40 minutes) Day 1 (40 minutes)

Obtain a bottle of cobalt chloride disks mounted on folded celluloid. The disks are blue when dry and turn pink as they become moist. The bottle, which also contains anhydrous calcium chloride, should be kept tightly closed and opened as short a time as possible when strips are taken out. Take a strip out quickly, and attach it to a leaf with paper clips so that the blue disks are in close contact with the two leaf surfaces. Measure the time it takes for each disk to turn pink, one disk measuring the time for the upper leaf surface and the other for the lower. Repeat this measurement for a number of different plants. If the time required exceeds 30 minutes, consider water-vapor loss to be negligible.

After each of the above measurements, remove the leaf from the plant. Paint both surfaces with collodion solution. Allow the solution to dry, then peel the collodion off and mount it on a slide. If the leaf is especially hairy, the collodion peel may first remove only the hair. Then repeat to remove the epidermis. Count the number of stomata visible in the low-power field. Determine the approximate leaf area by placing the leaf on millimeter-ruled paper and counting the number of squares covered.

Experiments on the Root

Group A - Root Initiation

Objective: Study the stimulatory effect of auxin treatment on root formation.

Plant Material	Apparatus	Reagent
30 Bean Seeds	Beaker	Hoagland Solution,
	Heavy Aluminum Foil	1/4 strength
	4 Jars, Half-pint	Indoleacetic Acid
	Seed Flat, Sand	(1.0 mg.)
	Tags	Micronutrients

Procedure:[6] Day 0 (30 minutes) Day 7 (30 minutes)

Soak 30 seeds of common garden bean, Rhaseolus vulgaris, for about 1 hour in tap water in a beaker. Plant the seeds well apart and about 1/2" deep in a small, paper-lined flat of sand. Water the sand thoroughly; then germinate in the dark at 25° C for 5 days, until the hypocotyls begin to show. Transfer the flat to the greenhouse, and grow for an additional 7 days, until the plants have formed a pair of simple leaves.

Completely cover the outside of 4 half-pint screw-cap jars with heavy aluminum foil. Tie a tag around each jar neck with string. Fill each jar with 200 ml. of one of the following solutions and label:

1. Distilled water.
2. Quarter-strength Hoagland solution with added micronutrients.
3. As in No. 2, plus 0.1 mg. indoleacetic acid per liter.
4. As in No. 2, plus 1.0 mg. indoleacetic acid per liter.

Cover each jar with the five-holed tin lid, and screw it in place.

With a sharp razor blade, excise each bean plant at the level of the earth, remove both cotyledons, cut the hypocotyl 5 cm. long, as measured from the point of cotyledon attachment to the cut base. Immediately put the hypocotyl through one of the holes in the lid and into the solution, with the pair of leaves projecting above the lid and the cotyledonary stumps below the lid. Repeat the procedure, 1 plant at a

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[6]Ibid., p. 28.

time, working rapidly to avoid drying of the plant, until 5 plants have been placed in each jar. Select plants as similar as possible. Place the jars on the laboratory bench.

One week later, measurements may be made. Unscrew the cap, remove the lid, and cut each hypocotyl just above the cotyledonary stumps to facilitate handling. Make the following measurements on each hypocotyl: (1) Number of rows of lateral roots, (2) number of lateral root primordia (shorter than 1 mm.) in each row, (3) number of lateral roots (longer than 1 mm.) in each row, (4) length of lateral roots in mm.

Determine the averages for each treatment.

Group B - Measurement of Root Pressure

Objective: Determine root pressure with a Water Monometer.

Plant Materials	Apparatus	Reagents
Potted Plant (Geranium)	Knife Label, Gummed Ring Stand Tubing, Capillary Tubing, Rubber	Methylene blue

Procedure:[7] Day 0 (2 hours)

Part I - For this experiment obtain a well-established, preferably woody-stemmed, potted plant. Any of the following plants are particularly good for this experiment: Fuchsia, Coleus, Pelargoniom, Geranium, tomato, Begonia, sunflower, Salvia. Thoroughly moisten the soil in the pot. Using a sharp knife or pruning shears, cut off the stem of the plant 1-2" above the soil. Slip a piece of tight-fitting rubber tubing over the stump, leaving an inch of tubing above the surface. Using

[7] Ibid., p. 66.

soft wire, secure the tube to the stump. Fill the tube with 0.1% methylene blue solution, and allow the plant to stand for 15 minutes.

Insert into the rubber tubing one end of a 3-foot length of capillary tubing of 1-2 mm. bore, previously fitted with a cork for supporting it, so that the dye solution is forced up the capillary. Support the capillary in a vertical position by securing the cork in a clamp attached to a stand. Wire the rubber tubing to the capillary. Be sure that there are no air bubbles in the column of solution. Mark the level of the liquid in the capillary immediately with a gummed label. Every 15 minutes for the next 2 hours measure and record the level of liquid, using the initial mark as the zero point. A drop in the level should be indicated with a minus sign.

Record changes in the height of the column of liquid during the time of observation.

Part II - To convert your measurements of column height into atmospheres of pressure, the following figures, which will be approximately correct for normal laboratory conditions, may be used as a basis for calculation:

Water Column (specific gravity of water = 1.0)

1,029.5 cm. of water = 1 atm. pressure

Mercury Column (specific gravity of mercury = 13,546)

76.0 cm. of mercury = 1 atm. pressure

Before dismantling the experiment, and while the level of the liquid in the capillary is still high, pour 50 ml. of saturated NaCl solution on the soil. Observe the level of the liquid in the capillary.

Group C - Effect of Vitamin B_1 on Plant Development

Objective: Observe the stimulating effect of thiamine chloride.

Plant Material	Apparatus	Reagent
20 Tomato Seedlings	4 Pots	10 Vitamin B_1 Tablets

Procedure:[8] Day 0 (30 minutes)

Secure from the field or garden a number of seedlings six to eight inches in height--almost any plant will do, for example: tomato, Marigold, cabbage, or Zinnia. Treat one lot by allowing them to stand with roots immersed for about twenty minutes in a B_1 solution (made up by dissolving 10 tablets in 1 quart of water). The other lot should be kept in plain water. Transplant both lots back to a similar situation to that in which they grew originally. Continue to water the treated group with the B_1 solution about once a week. Always water the other at the same time with plain water. Try to keep these plants until they reach maturity. The B_1 plants should grow more sturdily and have more and larger fruits.

Experiments on the Stem

Group A - Stem Structure and Function

Objective: To gain in insight into the functions of an herbaceous dicot stem, through the understanding of its structure.

Plant Material	Apparatus	Reagents
Sunflower Plant	Cover Glass Microscopes Razor Blade Slides	Hydrochloric Acid Phloroglucinol Acid

[8]Turtox Service Leaflet 54, General Biology Supply House (Chicago, Illinois)

Procedure:[9] Day 0 (60 minutes)

Make an external examination of a young sunflower plant, then make two thin cross-sections of the stems with a wet razor blade, one section near the tip of the stem and the other near the base. Place the sections in a drop of phloroglucinol solution for one minute and then mount in a drop of concentrated hydrochloric acid under a cover glass. This treatment will partially stain the fresh-cut section. Study under low-power of the microscope and note the grouping effect of different types of cells. Pith (thin-walled cells near the center); xylem cells (red-stained cells in a ring just outside the pith); phloem (greenish in color, just outside the xylem); Pericyclic cap (red-stained); cortex and epidermis are greenish, at the periphery. Red color is the test for lignin. Various observations could be made as to the region of the stem which contains chloroplasts, conducting or vascular tissues, and other structures important in the functioning of the plant. By use of modifications of this procedure and employment of different stains, a wide variety of cell structures and functions could be identified.

Group B - Plant Movement

Objective: The following experiment shows the effect of Auxin upon the movement of plants.

Plant Material	Apparatus	Reagent
2 Tomato Plants	2 Pots	Indoleacetic Acid Lanolin

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[9] G. Alexander et al., Laboratory Directions for General Biology (New York, 1959), p. 134.

Procedure:[10] Day 0 (20 minutes)

One way in which plants respond to external stimulus is through movement of either the entire plant or of some of its parts. Those movements which result from differential growth of plant organs are called tropisms. Tropisms are described according to the nature of the stimulus. The growth of a stem towards light is called phototropism. Tropism such as this may be explained partially by differences in auxin concentration or activity in the various parts of the plant organ concerned.

Smear a small amount of lanolin containing 0.5% indoleacetic acid upon one side of a stem of Lypersicon (tomato) of Kalanchoe. The lanolin should be placed 1" or 1 1/2" from the tip. To another plant apply plain lanolin in the same area of the stem. Observe both plants after 48 hours.

Group C - The Rise of Fluid in a Stem

Objective: To study the rise of fluid in the stem.

Plant Material	Apparatus	Reagent
Sunflower Plant, (1 month old)	Dissecting Kit Ice Cubes Scissors Tripod	Acid Fuchsin Dye

Procedure:[11] Day 0 (50 minutes)

Cut roots of the sunflower plant to two centimeters in length by

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[10]Erich Steiner, A. S. Sussman, and W. H. Wagner, Botany Laboratory Manual (New York, 1957), p. 129.

[11]G. Alexander et al., Laboratory Directions for General Biology (New York, 1959), p. 142

cutting with scissors while under water. Place in a .25 per cent solution of acid, fuchsin dye solution. Note time--place in front of strong light and observe through the stem tissue. Time required for the dye to rise to a given distance, such as 40 centimeters. When the dye has reached the highest point in the plant, cut cross sections from different areas such as the leaf, petiole, stem and root, and determine where the dye has risen. By use of the microscope or tripod lens, observe dye penetration through the various parts. Various areas of larger plants may be studied in a similar fashion, such as flower parts, leaf venation, and general plant translocation.

Similar experimentation with ice cubes and varying temperatures will bring about interesting results.

CHAPTER V

MISCELLANEOUS PHYSIOLOGY

Experiments on Growth Regulators

Although we are far from a complete understanding of the means by which growth in the various parts of the plant is regulated, some important discoveries have been made which show that plant hormones aid in achieving control of growth. The most important of the plant hormones that have been discovered is indole-acetic acid. Another is gibberellin, which has recently been found to have a striking influence on seed germination and stem growth. These compounds, called auxins, belong to a general class of substances known as growth regulators. There are auxins which are not true plant hormones in the sense that they do not occur naturally in plants but are made synthetically. Such an auxin is the weed killer, 2,4-dichlorophenoxy acetic acid, which has never been found in plants, and is, therefore, not considered to be a hormone.

Research has shown that auxins may perform a variety of functions in the plant. They control cell enlargement and may be able to stimulate or inhibit growth, depending upon the concentration present.

Group A - Root-Forming Experiments

Objective: To demonstrate the effect of hormones and gibberelic acid on the roots and leaves of various plants.

Plant Material	Apparatus	Reagents
2 Geranium Cuttings	Box, Propogating Jar, Battery Pins 2 Pots Sand, Moist	Gibberellic Acid Turtox Hormone Powder

Procedure:[1] Day 0 (35 minutes)

Part I - Powder. Since the actual hormone used to induce the formation of roots must be administered to the cutting in trace percentages, it is possible to dilute it either in water or in a dry inert powder or dust. The latter is very handy to use since the basal portion of the cutting is dipped into the dust prior to planting.

Select eight cuttings (the number can be varied to suit the convenience of the experimenter) which are as nearly identical as possible. Any length from two to six inches should be decided upon and the cutting should be selected from among these: Begonia, Carnation, Geranium, grape, ivy, Poinsettia, and rose. Dip four of the cuttings into Turtox Hormone Powder to a depth of an inch, shake off the excess powder and plant in one box. Label the boxes carefully, keep a record of everything which is done subsequently and let the care of the two groups be identical.

At the end of about three or four weeks, the treated plants should have rooted. Is any difference in growth perceptible? Remove one treated plant carefully; wash off the roots. If present, compare with those of an untreated specimen. If roots have not grown on either, wait another week and compare results. Make herbarium specimens of these plants and arrange them as permanent exhibits.

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[1]Turtox Service Leaflet No. 54, General Biological Supply House (Chicago, Ill.)

Part II - Leaf cuttings are of unusual interest and are not so hard to induce as might be thought. Plants which have heavy thick fleshy leaves lend themselves to this type experimentation. Examples are: Sansevieria, Gloxinia, Bryophyllum, Sedum, Peperomia, Saint Paulia, and Geranium. Select the smaller, mature leaves, remove them from plant and keep them on moist sand for a few days to dry partially--but not shriveled. Cut several veins in the leaves, moisten and apply a hormone powder to the under surface. Pin the leaf, with treated surface downward, to the surface of the sand in a propogating box. Place a battery jar over the leaf (or leaves) until roots are about 1/2" long. The leaves may now be removed and potted and will give rise to new plants.

When initial experiments on the rooting of soft-stemmed cuttings have been mastered, try the evergreens and hardwoods. They are not overly difficult but one should not begin by using them. The time required for rooting is considerably longer.

Group B - The Effect of Auxins Upon Plant Growth[2]

Objective: This experiment will show the effect of auxin upon the elongation and inhibition of plant tissues and will also demonstrate techniques designed to measure auxin concentrations.

Plant Material	Apparatus	Reagents
Radish Seedling	Atomizer	Indoleacetic Acid
4 Tomato Seedlings	4 Capsule Dishes	2,4-D Maleic Hydrazide
	Hand Lens	
	Metric Ruler	
	2 Pots	
	Razor Blade	

[2]Leonard Machlis and John G. Torrey, A Laboratory Manual of Plant Physiology (San Francisco, 1959), p. 33.

Procedure: Day 0 (30 minutes)

Part I - Use five-day old seedlings of radish, sunflower, or wheat which were grown in the dark. Cut only one section, 5 mm. in length, from the apical end of the hypocotyl. The section should be cut 2 to 3 mm. below the cotyledons. Using the millimeter scale of a ruler, measure the exact length of the section under a hand lens. Carefully float these sections on water solutions of auxin in capsule dishes. Both indole-acetic acid and 2,4-D should be used in varying concentrations of 1/100, 1/1000, 1/10,000, and 1/100,000. Allow the sections to remain in the solutions for forty-eight hours, then measure their length again. Note which concentration of each compound was most effective in stimulating the growth of sections. Repeat this experiment using sections of roots of sunflower, wheat, or cucumber which should be obtained from seedlings germinated in the dark on moist filter paper.

Part II - Transplant two 4" high tomato seedlings to each of two 4" pots, water them and allow them to grow for an additional week in the greenhouse. Label each pot. On the seventh day, using an atomizer, spray one entire plant with a sufficient volume of 0.4% aqueous solution of maleic hydrazide to thoroughly wet all surfaces of the plant. Continue the untreated plant as the control.

Observe the two plants over a period of 4 weeks. At the end of the fourth week, make the following observations: (1) total height of each plant in centimeters; (2) development of axillary buds and form of leaves; and (3) color and appearance of the plant.

From these observations it should be apparent that maleic hydrazide inhibits, rather than stimulates, plant growth.

Group C - Auxin and Leaf Abscission

Objective: To illustrate the relationship between auxin supply and leaf abscission.

Plant Material	Apparatus	Reagents
Coleus Plants	Reinforcement Rings	0.1% Indoleacetic Acid Lanolin Paste

Procedure:[3] Day 0 (30 minutes)

With young Coleus plants in pots, starting with the most macroscopically visible leaf below the apex, cut the petiole of the leaf at the base of each leaf blade of three successive pairs of leaves. To the cut surface of three debladed petioles apply plain lanolin paste; to the other three apply small dabs of 0.1% indoleacetic acid in lanolin paste (1,000ppm.). Measure the length in mm. of each petiole immediately after deblading and at weekly intervals until the end of the experiment, noting the time at which the petioles drop off. Determine the average time in days for petiole drop for each treatment.

Experiments on Isotopes[4]

The following experiments dealing with Radioactive materials suggest the use of Phosphorus-32 in amounts not exceeding 10 microcuries. This material is available from suppliers and has been officially recognized as presenting no health hazard to the student or the teacher in carrying out laboratory experiments with Radioactive P^{32} in amounts not exceeding 10 microcuries.

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[3]Ibid., p. 30

[4]Samuel Schenberg, Laboratory Experiments with Radioisotopes for High School Science Demonstrations, USAEC, Supt. of Documents, (Washington, D. C., July, 1958), pp 27, 29, 35.

Group A - Translocation of Radioactive Phosphorus in the Geranium Plant via the Stem

Objective: To show that a stem conducts liquids in both directions.

Plant Material	Apparatus	Reagents
2 Tall Potted Geranium Plants	2 Band-Aids Geiger Counter Lead Foil Razor Blade	1 Microcury of P^{32} as Na_3PO_4

Procedure: Day 0 (50 minutes)

Part I - With a razor blade scrape bare a small area of the stem 1/2" in length by 1/4" wide approximately halfway up the stem. Test all parts of the plant for radioactivity. Impregnate one Band-Aid with 0.5 microcuries of P^{32}. Put in close contact with scraped area of the plant stem. Remove one end of the Band-Aid with scissors and wrap the Band-Aid firmly around the stem. Place a lead shield around the Band-Aid. With a Geiger Counter test for radioactivity above and below the scraped area every 20 minutes. Continue for several hours. Marked radicacitvity should be observed below the scraped area within an hour, indicating only downward translocation of P^{32} via the phloem. There should be no increase in activity above the scraped area.

Part II - On the side of the stem of the second plant, make an incision into the xylem tissue and lay bare an area of the same size as in Part I of this experiment. Apply the Band-Aid impregnated with 0.5 microcuries of P^{32} as before. Place a lead shield around the Band-Aid. Measure the radioactivity above and below the incision every 20 minutes for several hours.

Results: After 1 to 2 hours, a rapid progressive increase in radioactivity will be noted in the stem above the cut, and finally in the

leaves. (Some radioactivity will be noted below the cut). Rapid increase in radioactivity above the cut indicates upward translocation of P^{32} via the xylem tissue. Some downward translocation is indicated by radioactivity below the cut.

Group B - Translocation of Radioactive Phosphorus in Tomato Plants via the Roots

Objective: To show that roots will absorb a mineral such as phosphorus and that this compound will be distributed to the stem and leaves.

Plant Material	Apparatus	Reagents
Pot-grown Tomato Plants, 6" High	Bottles, 200 ml. Geiger Counter Lead Foil	10 Microcuries of Radioactive Na_3PO_4, containing P^{32}

Procedure: Day 0 (60 minutes)

Remove the soil from the roots of the tomato plants. Wash the roots carefully in tap water. Test all parts of the plants for radioactivity. Add 10 microcuries of radioactive phosphorus to the tap water in the bottle which will hold the plant. Determine the counts per minute of radioactivity in the above solution. Place the tomato plant in this solution. Shield the bottle containing the plant with lead foil to ensure correct readings from the plant structures. Measure the activity in the leaves at intervals of 10 minutes, beginning with the lower leaves. Set up a control plant without radioactive phosphorus.

There should be a gradual increase in activity within 1 hour, beginning with the lower leaves, indicating the translocation of radioactive phosphorus. Celery and Coleus plants may also be used with favorable results.

Group C - Preparation of Autoradiographs

Objective: To make autoradiographs of roots, stem, and leaf of bean plant.

Plant Material	Apparatus	Reagents
Bean Seedlings, 6"	2 Boards Black Paper 2 Bottles Cellophane 2 Corks No-screen X-ray Film Lead Foil	Developer Fixer 10 Microcuries of P^{32} as Na_3PO_4

Procedure: Day 0 (30 minutes)

Remove 2 bean plants from the seed flat when about 6" in height. Wash roots under tap water until all soil has been removed. Add 10 microcuries of radioactive phosphorus to the tap water in the bottle which is to hold plant A. Prepare a second plant in a similar manner but do not add radioactive phosphorus. Label it as a control. Place the bean plants in the solutions. Shield both bottles with lead foil. Remove the plant from this nutrient in 24 hours. (Time element can be varied to show rate of phosphorus uptake). Place the plants inside cellophane envelopes and place on covered film (covered with standard black light-proof paper). Flatten and place the plant against the film. Place the film and plant between 2 boards. Keep in this position to expose the film for 2 to 4 days, depending upon the intensity of radiation. Develop the film, print with high-gloss paper. Concentrations of Radioactive material will be visible in the veins, tips and lobes of the leaves. These pictures, accumulated over a period of time, should prove useful to the teacher as an additional teaching aid.

Experiments on Tropisms

Group A - Rooting Effects of Plant Hormones

Objective: To show the effect of varying concentrations on the bean plant.

Plant Material	Apparatus	Reagents
50 Bean Seedlings	4 Jars Flat of Vermiculite	Ethyl Alcohol Indoleacetic Acid

Procedure:[5] Day 0 (50 minutes)

The rooting effects are particularly marked and may be demonstrated in the following way. A 0.01% solution of indoleacetic acid is prepared by dissolving 100 milligrams of crystals in a few milliliters of ethyl alcohol and stirring one liter of water rapidly into the alcohol solution. Serial dilutions giving concentrations of 0.001%, 0.0001%, and 0.00001% are prepared from the first solution. Select 50 uniform seedlings from the seeds which have been germinated in flats of sand in the dark, and cut to a hypocotyl length of twelve to twenty centimeters. These are then divided into five groups; four groups are treated by immersion of the cut ends in one of the hormone solutions, while the fifth group is placed in pure water as a control. The treatment should be continued for 24 hours in the dark, and then the cuttings may be placed in a flat of vermiculite and sand in the dark for four more days.

Remove the cuttings from the rooting medium and count the number of roots on each cutting for each treatment. Determine the average

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[5] G. Alexander et al., Laboratory Directions for General Biology (New York, 1959), p. 147.

number of roots on each cutting for each treatment. Determine the average number of roots per treatment and make a graph of the results.

Group B - Geotropism Effect on the Stem

Objective: To determine the effect of gravity on growth.

Plant Material	Apparatus	Reagents
Corn Seed	Glass-sided Box Paper, Blotting Sawdust Turntable	None

Procedure:[6] Day 0 (30 minutes)

Part I - In a glass-sided box plant corn grains which have been soaked in water for 24 hours and are beginning to germinate. Orient them with the embryo facing the glass and so that some are right side up, others upside down, and still others sidewise. They may be held firmly in position with a sheet of white blotting paper behind which is packed moist sphagnum moss or sawdust. Study the growth responses for a week or more and record the results.

Part II - Select some straight-stemmed potted plants, of almost any fast-growing herbaceous species such as tomato or sunflower which are from 10 to 30 cm. long. Place one pot on its side so that the plant is in a horizontal position but is rotated slowly. All sides of the plant are thus exposed equally to the stimulus of gravity. Place it in the dark. Compare the growth of these two plants over the next several days.

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[6]Edmund W. Sinnott and Katherine S. Wilson, Laboratory Manual for General Botany (New York, Toronto, London, 1956) p. 205.

Group C - Plant Tropism

Objective: To observe plant responses to gravity.

Plant Material	Apparatus	Reagents
Corn Seed, (Germinated)	Filter Paper Marking Pencil Paper Toweling Petri Dish Razor Blade	None

Procedure:[7] Day 0 (40 minutes) Day 2 (40 minutes)

Obtain corn seeds that have been soaked overnight in distilled water. Select 6 seeds, place them in a Petri dish with embryos down, and arrange them across the center of the dish with all the pointed ends oriented in one direction. Using moist filter paper slightly larger than the dish, and then soaked paper toweling, cover the seeds and press them firmly into place so that they will not change position. Prepare a second Petri dish in exactly the same way. Place the Petri dishes on edge in a holder, with the points of the seeds down. Mark the upper edge of each dish with a wax pencil. The holder should be transferred to the dark at 25°.

Forty-eight hours later observe the seeds. Carefully excise with a razor blade the terminal 2 mm. of all the roots in one dish. Arrange all the roots so that they are parallel, replace the moist filter paper, and turn the dish 90° so that the roots are now horizontal. Arrange the roots in the other dish in the same manner, but leave them intact. Rotate 90° to make the roots horizontal. Return the dishes to the dark at 25° C. At the end of 2 hours, observe the two sets of roots.

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[7]Leonard Machlis and John G. Torrey, A Laboratory Manual of Plant Physiology (San Francisco, 1959), p. 23.

Experiments on Chromatography

Group A - Paper Chromatography of Amino Acids

Objective: To introduce a method used to identify Amino Acids.

Plant Material	Apparatus	Reagents
None	Filter Paper	Asparagine
	Glass Rods	Butanol
	Hood	Glacial Acetic
	Oven	Acid
	Jar, Quart	Leucine
	Paper Toweling	Ninhydrin
	Stapler	Proline

Procedure:[8] Day 0 (50 minutes) Day 1 (50 minutes)

Obtain a piece of filter paper 6 1/4" x 8 1/2". At all times handle the paper by the edges only. Draw a pencil line across a short side of the paper 3/4" from the edge, and let this side be the bottom. Beginning 1 1/4" from the left-hand edge, make 5 marks 1" apart, numbered from 1 to 5 along the pencil line. With the glass rods provided for the solutions, apply a drop of a solution containing asparagine, leucine, and proline to mark 1, a drop of a solution of asparagine to mark 2, of leucine to mark 3, of proline to mark 4, and of an unknown to mark 5. Allow the drops to dry. Now staple the long edges of the paper together, forming a cylinder. Make sure that there is a space between the edges of the paper--i.e., that the edges do not touch at any point.

In the hood, put 40 ml. of a solution of butanol, glacial acetic acid, and water (4:1:1) into the bottom of a quart mason jar, and stand the cylinder of paper in the jar. Handle the solution with care;

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[8]Ibid., p. 121.

it will burn the skin and clothing. Screw on the metal lid. Place the jar in a locker for two hours. At the end of two hours, move the jar to the hood, remove the paper from the jar, cut the staples, and hang the paper in the hood to dry until the next laboratory period. Use clips to hang the paper from the glass rods.

At the next laboratory period, spray (in the hood) the chromatogram uniformly with the ninhydrin reagent. While it is still damp, lay the chromatogram in the oven at 80° C on pieces of clean paper toweling. Color development will take place in 5-10 minutes. Mark the limits of the amino acid and amide spots with a pencil, and record the color of each spot. The distance from the point of application of the solution to the spot is characteristic of each particular substance. By comparing the location of the three spots obtained in column 1 with the single spots in columns 2, 3, and 4, and by comparing the colors, name the three substances in column 1. Similarly, identify the unknown.

Group B - Leaf Pigment Separation by Chromatography

Objective: To separate the leaf pigments of spinach by the chromatographic technique, using filter paper as an adsorbent.

Plant Materials	Apparatus	Reagents
Spinach	Filter Paper	Acetone Benzene Petroleum Ether

Procedure:[9] Day 0 (40 minutes)

Cut a strip of filter paper so that it is long enough to protrude

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[9]Erich Steiner, A. S. Sussman, and W. H. Wagner, Botany Laboratory Manual (New York, 1957), p. 81.

one inch above the rim when it is resting on the bottom of a test tube. Point the lower end of the filter paper strip. Carefully pour into the test tube about 1 ml. of a mixture of equal parts of benzene and petroleum ether. Make concentrated pigment extract of leaves by chopping them in a small amount of acetone. With a medicine dropper apply a drop of the extract to the filter paper strip 2 to 3 cm. from the pointed end. Place the filter paper strip, pointed end down, into the test tube containing the solvent. Be careful not to splash the solvent over the paper.

Cork the test tube and observe the migration of the pigments up the filter paper strip. From the information given below map the chromatogram which you have developed by identifying each of the bands with a known pigment. (a) Carotene and xanthophyll are respectively chrome yellow and greenish yellow. (b) Chlorophyll a is blue-green while chlorophyll b is yellow-green. (c) Anthocyanin pigments are water-soluble and do not migrate in an organic solvent.

Alternative method: Squash a small piece of leaf on the filter paper strip about 2 to 3 cm. from the pointed end. Press hard enough for the pigments to be absorbed by the paper, but be careful not to tear the paper.

Group C - Simplified Paper Chromatography[10]

Objective: To illustrate the principles of chromatographic analysis.

Plant Material	Apparatus	Reagents
None	Filter Paper Strips Graduate Cylinder	Shaeffer's Permanent Jet-Black Ink

[10]A. P. Burrus, Demonstrations in Science, (Texaco Research Center, Beacon, New York), p. 2.

Procedure: Day 0 (30 minutes)

Cut a strip of filter paper one inch longer than the graduate cylinder. Put a small amount of water in the cylinder. Place a dot of ink on the strip near one end. Lower the "ink dot" end into the cylinder so that the end of the filter paper is immersed in the water and so that the "dot of ink" is above the surface. Hold the strip in place by a stopper. Set aside and allow the chromatogram to develop.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The present trend of emphasizing the zoological side of high school biology and the practice of lightly slipping through the botanical side with only elementary type experimentation or bookish lectures, could be reduced and plant physiology could be made an interesting and important area of student activity and learning.

The collection of experiments in plant physiology offered in this report is organized in such a way that the important basic concepts could be illustrated by experimentation, regardless of the size of school. Experiments selected by the teacher could help him to (1) broaden and supplement his present laboratory experiments, (2) improve his technique or procedure in present laboratory experiments, (3) modify some of these suggested experiments to suit his facilities, and (4) inspire him to further study and investigation in the botanical phase of biology.

The writer recognizes the limitations in scope of this report and this collection of experiments serves only as an introduction to the field of plant physiology at the high school level. It is hoped that biology teachers will continue to compile suitable experiments along this area of study and will make available to the high school students an evaluation of their findings.

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