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EXPERIMENTS
IN
PLANT PHYSIOLOGY

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EXPERIMENTS IN PLANT PHYSIOLOGY

A Laboratory Textbook

BY

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To
H. P. L.
and
L. M. S.

PREFACE

“Experiments in Plant Physiology” is a revision, partly rewritten, of the first part of “Methods in Plant Physiology.” The laboratory experiments contained in the earlier volume have been simplified, the more advanced work has been omitted, recently developed experiments on hormone action have been added, and the material has been adapted to use as a laboratory text in elementary and intermediate courses in plant physiology. More experiments have been included than can be covered in the usual course in plant physiology. The authors have intended in this way to adapt the manual to the varying interests and facilities of the classes using it. Experiments requiring more than average skill and attention have been designated by the sign (I). Some of these may be omitted from elementary courses; others should be included as group exercises or class demonstrations.

Particular attention has been given to the development of simple apparatus that can be assembled inexpensively by students or instructor. In spite of simplification of equipment and procedure, however, the exercises included have proved capable of furnishing reliable quantitative data on the problems of plant physiology. Emphasis on quantitative observations and on the interpretation and presentation of data obtained in class will provide training in critical thinking and in the recognition of cause and effect, as well as in the deduction of principles from experimental data.

A limited number of references to original publications have been included in addition to the textbook references at the beginning of each chapter. The citations to original papers will, it is hoped, stimulate a certain amount of general reading among students using the book. The citations are limited to papers bearing directly on the experimental procedures involved and, in most cases, to a few standard publications that will be generally available.

The authors will appreciate criticisms and suggestions from instructors and students who may have opportunity to use the work in classes in experimental plant physiology. By such cooperation any difficulties that may be found can ultimately be removed.

The authors are indebted to all who assisted with the first volume for their contributions to the present, particularly to Mrs. A. L. Dodge for checking the manuscript and citations, to Mrs. Helen Parke Loomis for assistance with both the original and the revised manuscripts and proofs, and to Miss Marie A. Corkle and Mr. Herbert Lenz for the drawings of the illustrations.

WALTER E. LOOMIS,
CHARLES A. SHULL.

AMES, IOWA,
CHICAGO, ILLINOIS,
March, 1939.

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EXPERIMENTS IN PLANT PHYSIOLOGY

CHAPTER I TO THE STUDENT

The Purpose of Laboratory Work in Plant Physiology.—Laboratory work in plant physiology can be made one of the most interesting of tasks or, conversely, it can be made one of the driest and most tedious. At their worst, laboratory exercises are the mechanical repetition of manual tasks; at their best they represent original and creative research which adds new knowledge to that possessed by the student, by his laboratory, or even by the science of plant physiology. Perhaps nothing will aid more in maintaining the latter desirable attitude than the discarding by student and instructor of the idea that experimental results may be right or wrong. The plan of the experiment or the techniques used may be good or poor; but plant responses are data and such data cannot be discarded as wrong. If the conditions of the experiment are not fully known, the data may be valueless, but accurately recorded results are never *wrong* for the conditions under which they are obtained.

The student who feels that his laboratory work is actually experimental; who realizes that, in all probability, exactly the thing he is doing has not been done before; who understands that carefully obtained and honestly recorded data such as he may obtain are the foundation and substance of science, cannot fail to learn lessons in the laboratory that can be learned nowhere else. He learns to observe and to record accurately, to recognize causes and effects, and to be distrustful of incomplete experiments and of unsupported statements. He learns to reserve judgment until all of the facts are known, and he develops skill and confidence in thinking. The textbook and the lecture room are clothed in folds of authority. Thinking based on their data

can never be completely the students' own. In the laboratory we discover that there is no authority in science but the authority of honest and accurate data, and laboratory thinking can be spontaneous and individual.

The Scientific Method.—The foundations of modern science were laid by the Greek philosophers. Although these men made many accurate observations, they depended primarily upon reasoning and logic. If the logic was good, they considered that the scientific conclusion should be correct. Scientists have learned that the logical answer is not always the right one; so the scientific method is based upon observation. Good reasoning and clear thinking are essential in evaluating and correlating data and in planning experiments so that the most profitable observations will be made, but the final test of all scientific work must be not logic or beauty, but accuracy. Logic and beauty are a definite part of the scheme. There are few things more beautiful and nothing more logical than nature. Effect follows cause with unfaltering certainty. The difficulty arises when we attempt to fit human logic, with all of its shortcomings, to the solution of these nicely integrated natural laws. The scientist has learned that he must reverse the procedure of the Greek philosophers and form a logic and a philosophy to fit his most accurate observations. Beautifully constructed concepts of the function of the leaf and of the form of the universe have crumbled and been discarded because they conflicted with increasingly accurate observations.

The scientific method means many things to different people, but to all it is founded upon observation and built with unbiased thought. The facts must be woven into a pattern, must be related to each other, and eventually built into a philosophy. As a student in the scientific method, make your observations painstakingly and relate them, in so far as you are able, to all of your other observations.

Errors in logic are usually obvious and fool no one, but errors in observation are not so easily checked. Even honest errors may delay the progress of science, and dishonest errors in the foundation material of experimental observation cannot be tolerated. You should learn in the plant physiology laboratory to be critically observing, to be thorough, and above all to be completely honest with yourself in all that you do and think.

Planning and Keeping Records of Laboratory Work.—Normal progress in and maximum profit from laboratory or other work can be obtained only by careful planning. Look ahead to the experiments that should be done at certain times of the year or in certain kinds of weather. Start your plant materials so that they will be ready when they are needed. Plan your work in outline for the next period or the next week *before* you leave the laboratory, and replan it in more detail before you come to class so that you will know what equipment to draw, what plants to obtain from the greenhouse, what you are going to do first. Before starting the work of a new chapter, familiarize yourself with the background of the experiments by reading some of the references given at the end of the introduction.

As for records, the most important thing is that they be complete. Always record the date of an experiment and, when they affect your results in any way, keep at least a general record of sunshine, temperature, etc. You will find that such notes may turn your "failures" into your most valuable experiments for they will help you to explain and understand unexpected results. Use a substantial, bound notebook and place all notes and all records *directly* in the book. Keep your book as neat as you can, but place the emphasis upon completeness and accuracy. Your instructor will tell you what final disposition to make of your notes. He may wish them copied, together with the answers to the problems and questions at the end of each experiment, or he may ask you to write the problems into your laboratory notes. At any rate, be careful of your "book of original entry" and be ready to go to court with it at any time.

Remember that you are doing original research; that the experiment always "works," even if it shows nothing more than poor technique; and that no result and no grade are worth the cost of a dishonest record.

Browsing.—The term browsing has been used to designate general, unassigned reading, followed from interest rather than for the solution of some specific problem. When you have a spare hour, page through the physiological periodicals in the library reading room. Textbooks are indispensable and condensed stores of knowledge, but the research periodicals with their reports from the men who are constantly working to widen the field of science are frequently more exciting reading. You may

stumble onto an important contribution to some topic that you have heard vaguely discussed—a paper on the mechanism by which water is lifted to the tops of tall trees, a new technique for studying the unknown processes of photosynthesis, or a report on the use of auxins for changing the growth form of plants. Compare critically the results which the author obtained and the conclusions which he draws from them. Does he consider all the possible explanations of his data or does he appear merely to be trying to bolster some preconceived idea?

Your library will undoubtedly have files and current copies of the *Botanical Gazette*, one of the oldest botanical publications in America; *Plant Physiology*, the official journal of the American Society of Plant Physiologists; *The American Journal of Botany*; the English journal, *Annals of Botany*; and many other American and foreign periodicals which will contain interesting articles.

When you are looking up some of the assigned periodical references, glance at the other papers in the volume, or follow up a topic that interests you by reading the references given at the ends of the papers which you find on the subject. You will be surprised to find how fascinating a sport browsing can be.

CHAPTER II

THE WATER RELATIONS OF PLANTS

INTRODUCTION

Water is the most important plant nutrient and nearly half of the dry weight of plants is composed of the elements hydrogen and oxygen, in the approximate ratio of two hydrogen atoms to one atom of oxygen. Just as carbon is the key substance in organic and biochemistry, so water plays the major role in the physical processes of plants. Water is the solvent in which plant nutrients and plant foods are translocated, and in which they react. Many of these chemical reactions will not proceed in the absence of water. The very life of plant cells depends upon their water content, which keeps the protoplasm hydrated and active and the cells turgid. Many interesting responses, such as the movements of leaves and flowers, are due to changes in turgor which depend in turn upon the quantity of water in the cells. The free carbon dioxide of the air is absorbed in the water on the saturated cell walls of the leaf mesophyll, and the rapid absorption of water by newly divided cells is an essential step in growth.

The Properties of Water.—Water is unique in many of its properties, and life as we know it is closely dependent upon more than one of the unusual and unexpected reactions of this interesting liquid. Water exerts a stabilizing influence upon the temperature of moist bodies and upon climate. Eighty calories of heat are required to change a gram of ice to water without raising its temperature. Similarly, 539 calories are required to change liquid water to water vapor at 100°C. Both of these values are exceptionally high and both reactions, of course, are reversible so that the 80 calories required to melt ice are recovered when the water is refrozen. Cold water gives off heat in freezing, while warm water absorbs heat in evaporation, thus tending to maintain moderate temperatures. In addition,

more heat is required to warm liquid water (specific heat) than nearly any other substance and this heat is again given off when the water is cooled. Water is also relatively transparent to radiation, particularly in the visible spectrum, and it conducts heat better than other transparent materials, to maintain a uniform temperature throughout its mass. All these properties as we have said tend to stabilize the temperature of moist, living organisms, and to make our climate more equable.

A low viscosity, a low vapor tension in comparison with its other properties, and a strong tendency for the molecules to adhere to plant surfaces and cohere with each other are important properties adapting water to the use of plants. The very high dielectric constant of water reduces the attraction between positively and negatively charged particles when they are immersed in water, and makes possible the ionization of electrolytes as well as all the reactions dependent upon dissociation. The dissociation of water into hydrogen and hydroxyl ions is a stabilizing factor in the hydrogen-ion concentration and buffering of plant sap. The ease with which many plant compounds are hydrolyzed or dehydrated with resultant changes in their solubilities and other reactions should be noted also, together with still other properties of water, such as surface tension and polymerization, which play important roles in physiological processes of plant cells.

The experiments given in this chapter illustrate ways in which plants obtain, use, and conserve water and help to show the basic importance of this substance in the physiology of plants. Before starting the experimental work the student should have read some of the following references on the water relations of plants.

References:

- MAXIMOV, N. A. *Plant physiology*. 2d ed. Chapt. VIII; parts of Chaps. IV, VII, and X. New York. 1938.
- MILLER, E. C. *Plant physiology*. 2d ed. Chapt. IV. New York. 1938.
- MAXIMOV, N. A. *The plant in relation to water*. Chaps. I, II, III, and VIII. New York. 1929.
- BAYLISS, W. M. *Principles of general physiology*. 4th ed. Chapt. VIII. New York. 1927.

THE USE OF WATER BY PLANTS

Experiment 1. Water in Plant Tissues. (E)¹

In cooperation with the other members of the class, determine the percentage of moisture in a range of plant tissues. Compare such materials as:

1. Shade and sun leaves.
2. Evergreen and deciduous leaves.
3. Thin and succulent leaves.
4. Herbaceous and woody stems.
5. Fleshy storage tissues (roots, fruits, etc.) and seeds. Or other materials as available.

Make all determinations in duplicate, weighing 25- to 100-gm. samples rapidly to 1 part in 1000² and drying to constant weight at 100°C. Most samples will dry in 24 hr. If they are still losing weight after 48 hr., check the ventilation and heating of the oven. Exchange data and tabulate for reference. Calculate percentages of moisture on the fresh-weight basis.

Questions:

1. Which types of plant tissues are high in moisture content and which are low?
2. What advantages arise from these variations in water content?
3. Why do we calculate the moisture percentage of plants on a fresh-weight basis, but the moisture percentage of soils on dry weight?

Experiment 2. Water and Life. (I)

Spread 50 or more whole bean, sunflower, or similar leaves, with petioles attached, on screen trays in the greenhouse or in a warm room where they will dry uniformly and at a moderately rapid rate. Determine the percentage of moisture in the turgid blade

¹ Experiments marked (E) are elementary, and (I) intermediate in nature.

² Detailed directions for rapid, accurate weighing are given in *Methods in Plant Physiology*. In general, use the cheapest balance which will readily weigh the sample to 1 part in 1000; thus for a 100-gm. sample, use a beam balance accurate to 0.05 gm., and weigh the sample to the nearest 0.1 gm. A 10-gm. sample should be weighed to 0.01 gm. on a prescription balance, and a 1-gm. sample to 0.001 gm. on an analytical balance. Note, however, that if a 1-gm. sample is to be weighed in a 30-gm. crucible, the crucible must be weighed to 0.001 gm. to obtain this accuracy in the sample.

tissue of the leaves as harvested, by separating the blades of 4 to 10 leaves from the midribs, weighing rapidly to 1 part in 1000, and drying to constant weight at 100°C. Make a second moisture determination on the blade tissue when it first shows distinct signs of wilting. At intervals of 30 to 120 min. thereafter, depending upon the rate of drying, carefully remove the blade from one side of four or more leaves without injuring the midrib or remaining tissue, and determine the moisture content of the sample. Quickly cut the petioles of the sampled leaves under water¹ and place the leaves with half of the blade attached in a moist chamber or under moist bell jars, with the petioles dipping into water, and observe for recovery of the wilted blade tissue. Failure of the blade to regain turgidity may be taken as evidence of death. Plot your data and determine from your wilting curve: (1) the moisture content of turgid leaf blades, (2) the moisture content of blade tissue at wilting, and (3) the moisture content at death. The leaves should reach the death point in 3 to 5 hr., and care should be taken that the whole sample dries uniformly.

Questions:

1. Why cannot dried leaves be soaked and brought to life?
2. Why are seeds so much more resistant to drying than leaves?
3. What does the percentage loss of water at wilting tell you of the elasticity of the cell walls of your tissue?

Experiment 3. Water and Growth. (E)

Carefully measure the height of the youngest exposed leaves of two corn plants which are in need of water (just starting to roll), or attach auxanometers² (Fig. 1) to the tips of the leaves

¹ It is well to form the habit of cutting a short section from the base of a plant, while holding it and the knife under water, whenever a cut plant part is to be returned to water after the cut has been exposed to the air for more than a few seconds. During such an exposure, the ends of the water-conducting vessels become filled with air which greatly hinders subsequent water absorption. A sharp knife or razor blade should be used and a slanting cut made to avoid crushing the open ends of the vessels.

² An auxanometer may be made as illustrated with a dowel pin needle and a small pulley to take up the cord as the plant grows. If a 4-in. pulley is used with a needle 20 in. long from the center of the disk, growth will be magnified 10 times. A weight is used to partially balance the needle so that a pull of about 5 gm. will be exerted upward on the plant. If it is

with chewing gum or adhesive tape. Water one plant with a volume of water equal to one-fourth that of the soil in the pot; leave the other plant unwatered. Hold both in a shaded location and measure the height of the central leaves or the drop of the

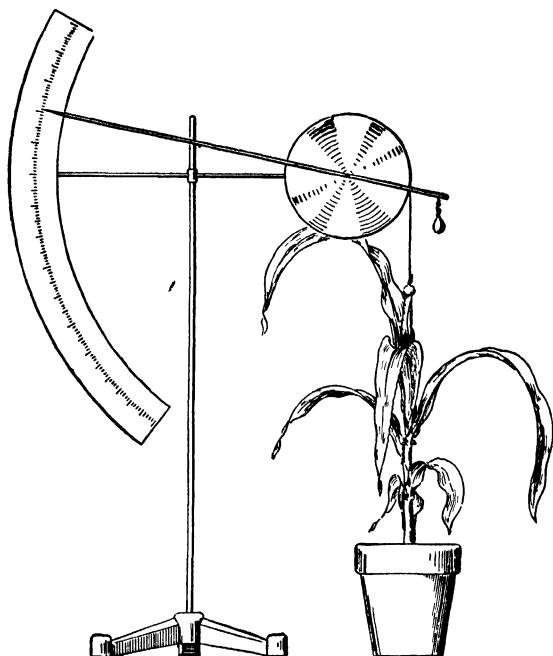


FIG. 1.—Auxanometer attached to measure growth of central leaf of corn. The elongation of the attached leaf is magnified in the drop of the needle on the scale.

auxanometer needles at 15-min. intervals for an hour or more. If plants in dry soil are not available, or as a variation of the experiment, wet one pot with water and one with saturated sodium chloride solution, and observe growth as directed.

Questions:

1. How does water increase the growth of plants?
2. Plants ordinarily grow at night, but may make their most rapid growth on a warm, wet day. How can you explain these reactions from the data of your experiment?

available, a more elaborate recording auxanometer may be used and a continuous record of the growth rate obtained.

Experiment 4. Water and Germination. (E)

Weigh out a sample of corn, bean, or pea seeds; soak them for 1 or 2 hr. in warm water (30 to 40°C.); and hold in wet moss or cotton until germination starts. Determine the percentage of water in the germinating seeds, and by comparison with that present in the air-dry seeds, determine the percentage absorbed in germination.

Weigh 10 air-dry seeds and measure their volume by dropping them into a burette half filled with alcohol and recording the increase in volume. Remove the seeds quickly from the alcohol, wash them, soak in warm water, and germinate. Weigh the germinated seeds and again determine their volume, this time with a burette half full of water. The second measurement should be made before the embryos have grown appreciably. How does the increase in volume compare with the increase in weight?

If a press is available, determine whether the absorbed water is loosely or firmly held within the seeds.

Questions:

1. What processes are concerned in the absorption of water by seeds?
2. How is the physiology of the seed affected by the increased percentage of water?
3. What factors other than water affect germination?

Experiment 5. Water as a Nutrient.¹ (I)

The production of water from burning plant material may be demonstrated and roughly estimated by weighing out 1 to 2 gm. of oven-dry absorbent cotton and placing it loosely in a hard-glass combustion tube 15 to 20 mm. in diameter and 25 to 30 cm. long (Fig. 2). Insert a little glass wool at either end of the cotton to hold it in place while burning. Arrange the tube so that a stream of air can be drawn: (1) through a large soda-lime drying tower, (2) through the combustion tube, and (3) through a weighed calcium chloride tube to absorb moisture liberated from the cotton. Start the stream of air and heat the combustion tube carefully in a combustion furnace or with a Bunsen flame, starting, if the flame is used, at the end nearest the calcium chloride

¹ This experiment may be modified by using 5 gm. of cotton and condensing the water in a U-tube packed in ice.

tube and continuing until the cotton is completely burned. Reweigh the chloride tube and determine the percentage of combined water obtained from the cotton. Note that CO_2 production may be measured at the same time by inserting after

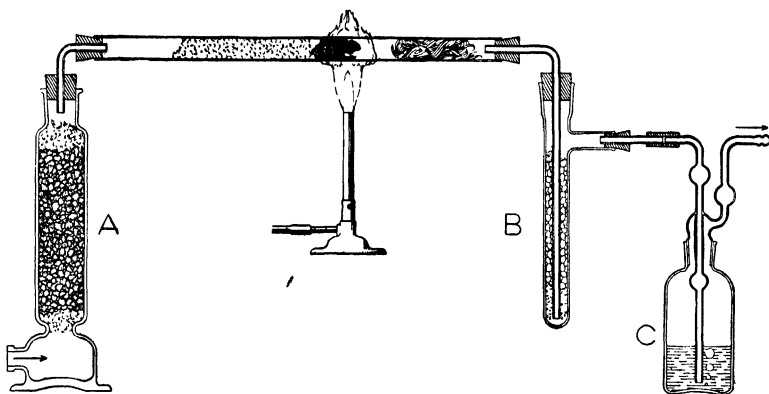


FIG. 2.—Apparatus for demonstrating production of water by burning cotton. The tube *B* is filled with dry calcium chloride and weighed before starting the experiment. Its increase in weight represents water produced. A solution of $\text{Ba}(\text{OH})_2$ may be placed in flask *C* to demonstrate CO_2 production.

the calcium chloride tube an effective absorption tower containing a measured quantity of $\text{Ba}(\text{OH})_2$ (see Experiment 117).

Questions:

1. Assuming that your cotton was pure cellulose, how does your moisture determination check with the theoretical yield?
2. What weight of carbon dioxide should have been liberated? How do you account for the gain in the total weight of the water and CO_2 in comparison with the weight of the cotton burned?

THE ABSORPTION OF WATER

Experiment 6. The Water-absorbing System of the Plant. (E)

Germinate corn, radish, or mustard seeds on moist blotting paper and observe root-hair development. Wash out the roots of several potted plants or observe roots of plants growing in nutrient cultures and if possible trench and peg or wash out the roots¹ of some plant growing in the open or in a large quantity of soil (note Question 3 below).

¹ For details of field studies of roots, see: WEAVER, J. E. Root development of field crops. New York. 1926.

The germinator, developed by M. A. Raines¹ is admirably adapted to studies of seedling root systems. The roots of germinating seedlings are allowed to grow downward between

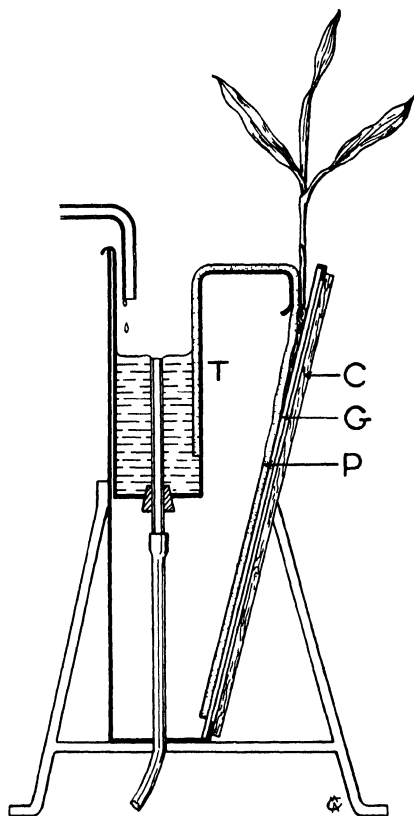


FIG. 3.—Cross section of box for observation of root growth. A pane of double strength glass *G* about 24 by 24 in. is supported at an angle in a metal frame. A dark-colored blotter *P* is laid on top of the glass and bent over into the tank *T*, which runs the length of the apparatus. A fiberboard cover *C* is used to exclude light, and corn or other germinating seeds are placed as shown and their root growth observed through the glass. The water supply to the seedlings may be varied by adjusting the height of the overflow in *T*.

a sloping glass plate and a moist blotting paper (Fig. 3). The paper is kept moist by the capillary movement of water from the trough (*T*) at the top. The glass is covered with a light-tight board which is removable for inspection of the growing roots.

¹ RAINES, M. A. Wick cultures of seedlings with different rates of water flow. *Am. Jour. Botany* **24**: 185-187. 1937.

Questions:

1. Would you expect root hairs to be more useful in absorbing water or in absorbing minerals? Why?
2. How is the root system adapted to its functions of absorption, conduction, and anchorage?
3. How do the weight (determine) and surface area (estimate) of the root system compare with those of the top of the same plant?

Experiment 7. The Absorption of Water by Roots. (E)

a. Thoroughly wet two wilted plants in pots, one with water and one with a molar (5.84 per cent) sodium chloride solution and observe for recovery from wilting.

b. Transfer plants growing in water cultures or small plants whose roots have been *carefully* washed free of soil to beakers containing the following solutions:

Beaker No.....	1	2	3	4	5	6	7
Molality sucrose ¹	0	0.025	0.05	0.10	0.2	0.5	1.0

Place the plants in a warm, light place, preferably in bright sunlight, and observe for wilting after 1 to 2 hr. and after 24 hr. Broken roots will allow the sucrose to enter the plant and will reduce wilting in the more dilute solutions. Fermentation of the sucrose will prevent continuation of the experiment for more than 24 hr.

Questions:

1. What happens when too much soluble fertilizer is applied to plants?
2. What is the water-absorbing power of your plants in terms of mols of sucrose? How is it affected by the rate of transpiration? By time of standing in the sucrose solution?
3. Why are the plants which grow on salt marshes similar in many ways to those which grow in arid regions?

Experiment 8. The Available Moisture of the Soil. (E)

a. Obtain four uniform sunflower, tomato, bean, or similar plants and water two of them with a solution containing 1 gm. of NaCl for each pot and two of them with tap water. Cover all four pots with aluminum shells or wax and oilcloth (see Experiment 20) to prevent water loss from the pot or soil surface, and set on the greenhouse bench. Observe until the plants wilt and

¹ Molar sucrose solution contains 34.2 gm. cane sugar in 100 ml. of solution. The other concentrations are obtained by diluting this solution with water, 1 + 1 (0.5M), 1 + 4 (0.2M), 1 + 9 (0.1M), etc.

do not recover when transferred to a moist chamber. Be sure that the salted pot has reached the permanent wilting point. It may wilt immediately, and then recover and reach permanent wilting very slowly. Separate the soil carefully from the roots and weigh duplicate samples of soil from each pot for determinations of unavailable moisture. Weighings should be accurate to the usual 1 part in 1000 of the weight of the *sample*. Dry these samples in the oven at 100°C. for 24 to 48 hr. and reweigh. Calculate the percentage of moisture on a dry-weight basis. This value is commonly called the "wilting percentage" or the "wilting coefficient." It represents moisture which is not absorbed or is only very slowly absorbed by the roots of the plant.

b. Combine the soil from the two unsalted pots into one sample and place 200 gm. of it into a weighed, friction-top can with perforated bottom. Set the can in water until the soil is thoroughly wetted, cover, and allow to drain for 3 days to a week. The soil is now saturated with the maximum percentage of capillary water. Reweigh the can and wet soil, and calculate the total moisture content, including that originally present as determined in part a. Fifty per cent of this maximum moisture percentage is commonly considered to be optimum although plants may make nearly optimum growth with moisture percentages not much above the wilting point.¹

Questions:

1. What moisture percentages represent the minimum, optimum, and maximum available water for the soil used?
2. What factors affect the percentage of unavailable moisture (wilting percentage)?
3. Why is 50 per cent of saturation taken as optimum rather than saturation?
4. How many pounds of *available* water could be stored in the surface 10,000,000 lb. of an acre of dry soil if it were like the soil used in this experiment?

Experiment 9. Transpiration and Water Loss from Soils. (I)

The important effect of plants upon soil moisture has not been fully realized because some of the older experiments on loss of moisture from soils were set up with the water table within a foot of the evaporating surface whereas, under normal conditions

¹ VEIHMEYER, F. J., and A. H. HENDRICKSON. Soil moisture conditions in relation to plant growth. *Plant Physiol.* 2: 71-82 1927

in the drier sections of the country, the water table may be many feet below the surface, or the moist surface soil may "hang" over a dry subsoil

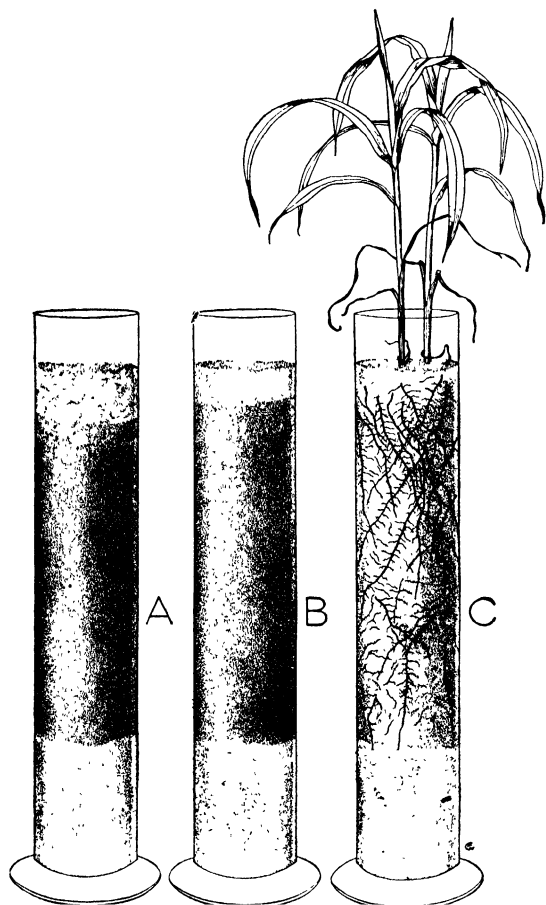


FIG 4 —The movement of water in dry soil Three tubes of soil were partly moistened from the surface. Tube A was "cultivated," tube B allowed to crust, and tube C planted to corn

Pack air-dry loam soil into four tall glass cylinders (1000-ml. graduate cylinders or tall museum tubes may be used) and plant five grains of corn in the top of one tube Measure enough water into each cylinder to cover the soil 1 to 2 cm. for each foot in depth of the soil After the tubes have stood overnight, add

more water as needed to wet the soil to three-fourths of its depth. If all of the soil should be wetted, discard the experiment and repeat with less water. It is essential that dry soil be left in the bottom of the tubes (see Fig. 4).

Weigh the planted tube carefully on a platform balance, and hold it in the greenhouse until the corn is well up; then add water to bring it to its original weight. Keep the remaining tubes in a protected locker and, after 2 or 3 days, determine the percentage of moisture in the soil from the center of one of them. Record this value as the "field percentage"¹ of the soil. If time permits, determine the moisture percentage of the soil at several levels to measure its uniformity.

When the planted tube is ready, determine the weights of the two remaining tubes from the locker. Wrap the three with some insulating material, preferably felt, covered with aluminum painted cardboard or metal; "cultivate" one of the tubes to a depth of 4 to 6 cm.; and hold the planted, the cultivated, and one control tube together in a location which is protected from rain, but conducive to rapid evaporation and transpiration. Record the daily water loss from each of the tubes until the corn plants show evidences of lack of moisture. Plot your data as loss in grams per day. The experiment may be extended by replacing the moisture lost from the tubes, particularly the heavy loss from the planted tube.

Questions:

1. Why does water stop or practically stop sinking in the dry soil?
2. Why is water not drawn back to the surface of the unmulched tube by capillary action? Note that some moisture may be lost by vapor movement.
3. What bearing does this experiment have on problems of cultivation and summer fallowing?

Experiment 10. Root Pressure and the Water Supply in Plants.

(I)

Probably all angiosperm plants show root pressure under certain conditions and the pressures developed may be considerable.² Root pressure is, however, assumed to be of minor impor-

¹ VEIHMEYER, F. J., and A. H. HENDRICKSON, *loc. cit.*

² WHITE, PHILIP R. "Root-pressure"—an unappreciated force in sap movement. *Am. Jour. Botany* 25: 223-227. 1938.

tance in supplying plants with water. Your experiments should show some of the reasons. Obtain three vigorous, uniform *Fuchsia*, *geranium*, *Bryophyllum*, or other plants and treat as follows:

a. Cut one plant to leave a clean stump; cut back the bark and attach a small-bore, bent-arm mercury manometer (Fig. 5). Record the difference in height of the two arms at intervals. Keep the pot well watered but avoid flooding.

After the manometer shows a positive pressure, flood the soil with a saturated sodium chloride solution and observe the effect on root pressure.

b. Prepare the second plant in the same way but save the top. Attach a weighed bottle instead of a manometer and determine the *quantity* of sap excreted by the roots. Recut the base of the top under water and transfer to a weighed flask of water, which is protected against evaporation other than the transpiration of the plant. Expose the top of the plant in the greenhouse or at a south window, determine the grams of water lost in 24 hr., and compare with the excretion from the roots of the same plant.

c. Cut the third plant in the same way, but set the potted stump into a pressure cooker.¹ Connect a piece of glass tubing to the plant stump with heavy-walled rubber tubing. Remove the bark from the region covered by the rubber tubing. Slip a No. 7 rubber stopper over the glass tube. The stopper should fit the tube tightly and should be hollowed out to form a vacuum

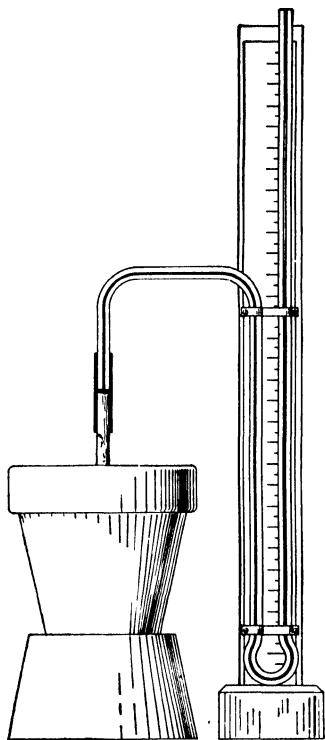


FIG. 5.—Root-pressure manometer. The capillary tube between the cut stump of the plant and the mercury in the manometer is filled with water.

¹ KRAMER, PAUL J. The absorption of water by root systems of plants. *Am. Jour. Botany* 19: 148-164. 1932.

seal against the cover of the cooker. Provide a pressure gauge, tire-valve connection, and open hole in the lid (Fig. 6). Water the pot, place it in the cooker, clamp down the cover, and hold the stopper (*S*) against the lid until enough air can be pumped in through the valve (*V*) with a tire pump to hold the stopper from the inside. A little vaseline may be required to give a tight seal. Increase the air pressure to 10 or 15 lb., attach a weighed flask to collect the plant "sap" forced through the tube, and compare the volume of flow under pressure with

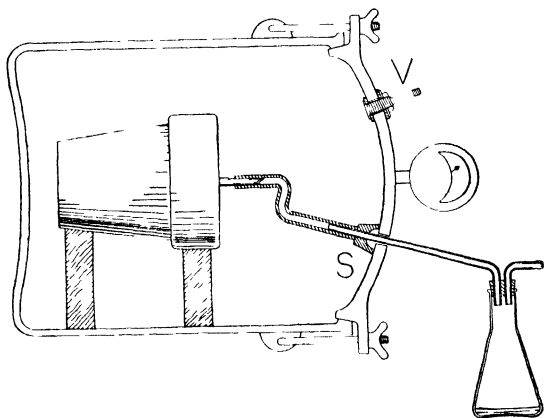


FIG. 6.—Apparatus for collecting sap under pressure. A tire valve *V* is inserted through the lid of a pressure cooker. A pot containing the roots of a plant is connected as shown, with a hollow rubber stopper at *S* to form a seal when air is pumped into the chamber.

the bleeding shown by the plant in part *b* and with the water evaporated from the cut plant top.

Questions:

1. How high would a water column be lifted by the observed root pressure?
2. Why is this force not considered to be the cause of the rise of the transpiration stream?
3. What theories have been advanced to explain root pressure?

Experiment 11. Guttation. (E)

Grow 6 to 10 seedlings of oats, corn, or other cereal in each of two pots and when the plants are 5 to 8 cm. high, moisten the soil in one pot with water and that in the other with 5.84 per cent (molar) sodium chloride solution and hold the cultures in a moist chamber.

Questions:

1. What force is responsible for guttation?
2. Of what advantage are the hydathodes?

THE MOVEMENT OF WATER**Experiment 12. Water Conducting Tissues. (E)**

Study the movement of water in the xylem and phloem of *Fuchsia*, privet, or other plant from which the bark can be conveniently removed. Avoid plants with woody or leathery leaves which will not wilt readily.

a. Cut the stem of one of the plants under liquid, 5 per cent gelatin strongly colored with India ink or Congo red and cooled to 45°C. After leaving in the gelatin for a few minutes, carefully remove a ring of bark to expose an unplugged phloem surface, wash off surplus gelatin, place in a beaker of water in an exposed position, and observe for wilting.

b. Remove the bark from the lower 2 cm. of the second plant and cover the end of the xylem with vaseline before placing it in the gelatin. Afterward remove 1 cm. of the projecting xylem under water and compare with *a* for wilting.

c. At the end of the period or as soon as the two stems show decided differences, cut sections of the xylem from the base of the stems and observe under the microscope. What cells are plugged by the gelatin? What relation does this experiment bear to the theory of water movement by imbibition?

d. Cut the stem of a transpiring *Impatiens* or balsam plant under water, using a sharp razor blade, transfer to a 0.2 per cent safranin-O¹ solution, and allow to stand for a short period. As

¹ Eosin, which is frequently recommended for this experiment, is toxic, spreads rapidly, and washes out readily. Safranin-O is nontoxic and gives a very bright, well-localized, and semipermanent stain. It does not stain the veinlets of the leaf so well as eosin or amaranth and is not so good for measurements of rate of movement. In general, use basic dyes to obtain localized staining and acid dyes for demonstration of movement.

For more extended studies of leaf venation, stain various leaves with eosin and observe as soon as the dye has penetrated the leaf well; or, heat a leaf of red Prince's Feather (*Amaranthus*) in alcohol and then in I-KI solution and observe under a microscope for striking dichotomous branching; or, place leaves of various plants in an algae tank until the mesophyll has been eaten away by various microorganisms leaving an intact leaf skeleton. Leaf skeletons may be prepared also by boiling leaves in a solution of soda lime.

soon as the dye is clearly visible in the stem, cut thin sections from near the base of the stem and observe the tissues stained by the dye. *Draw.*

Place a second plant in eosin or amaranth and allow to stand for an hour or two with rapid transpiration. Observe the distribution of stain in stem, petioles, and leaves. Observe whole leaves by strong transmitted light and trace out the conducting system. Observe a second plant or the remains of the same one after 24 hr. in the stain.

e. Repeat using a young corn or umbrella plant stem.

f. Repeat adding safranin-O or amaranth (not eosin) to the soil around a growing potted plant. It is important in this test that no broken or injured roots be exposed to the dye and that a nontoxic dye be used. Does the dye penetrate the cortex of the roots? Why does it not reach the tops?

Questions:

1. What do your experiments indicate of the tissue concerned in water movement?

2. What theories of water movement do your data support: Movement in walls or lumina of the vessels; as vapor; or as liquid?

3. Do they offer any evidence concerning the movement of solutes? Explain.

4. How are the various stem tissues adapted to the conduction of water?

Experiment 13. The Length of the Water Conducting Vessels. (E)

Obtain straight and, if possible, unbranched shoots of an assigned wood; remove the bark from the ends of the stick (or from the entire piece), covering all side cuts with hot paraffin; fit the base into a suction flask; attach a small funnel containing mercury to the top, and determine the length of shoot through which mercury can be drawn (downward). If no mercury can be drawn through the piece in 5 min., shorten the stick by 10 per cent¹ and make another test.

Cover a teaspoonful of soda-lime with 100 ml. water, boil rose leaves gently in the mixture for 30 min. or 1 hr., and carefully rub the leaf skeletons free of softened material. The skeletons may be made into permanent mounts or lantern slides if desired.

¹ If two sticks are available, shorten the first 25 per cent at a time to obtain a rapid estimate of vessel length. Cut the second stick to about one and one-half times the estimated length of the vessels and proceed as

When a length is found through which mercury begins to flow, determine the weight of mercury passed in 5 min. Cut the stick in half and determine the mercury drawn through the upper (why?) half in 5 min. What is the ratio of mercury flow in the two lengths?

The following or other woods may be used:

- | | | |
|---------------------|----------|------------|
| 1. Red or black oak | 4. Apple | 7. Rose |
| 2. Maple | 5. Pine | 8. Currant |
| 3. Willow | 6. Elm | |

Questions:

1. Is the large long vessel an efficient or poor water conductor? How would its efficiency vary with conditions?
2. What figure gives maximum length of vessels for your material? Why?
3. How do you account for the fact that a dye can be drawn through a longer section of stem than mercury?

Experiment 14. Movement of Water through Dead Tissue.

(I)

Many theories of the mechanism of water movement in plants are dependent upon the activities of living cells. Test the effect of a dead stem section upon water movement in the umbrella plant with the following modification of Overton's experiment.¹

Obtain several long stemmed umbrella plant shoots (*Cyperus*) and kill sections in the center of two stems by dipping them into a *shallow* pan of boiling water until distinct color changes can be observed in the tissue. Be careful not to break the shoots. Set up these shoots with two checks in weighed bottles of water and determine daily water loss for 3 to 6 days. Transfer the stems to a beaker of water when weighing the bottles; otherwise losses in weight due to drying of the leaves will be recorded as water movement.

To determine the cause of death in the plants with killed stems, set up a second pair of untreated shoots, but place about 30 cm. of freshly boiled stem sections in the water bottle. Compare rate of water loss and leaf health with lots one and two.

directed. Oak sticks of other than the white-oak group, should be 2 m. or more long; most hardwoods, 1 m.; and pines, etc., 20 cm.

¹ OVERTON, J. B. Studies on the relation of living cells to transpiration and sap flow in *Cyperus*. I, II. *Botan. Gaz.* 51: 28-63; 102-120. 1911.

Questions :

1. What force moves water through the stems in this experiment and where is it applied?

2. How could the experiment be repeated with entirely nonliving material?

3. How do you explain the early death of the shoots with dead stem sections?

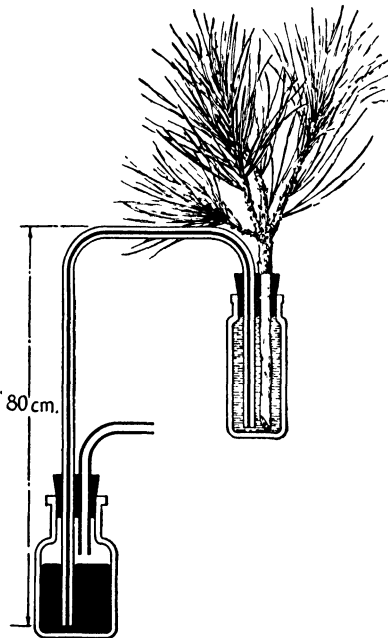


FIG. 7.—The lifting power of evaporation. The upper, water-filled bottle serves as an air trap to prevent air bubbles from plugging the cut end of the transpiring stem.

Experiment 15. The Lifting Power of Evaporation. (I)

a. Cut the stem of a transpiring woody plant under water; remove the bark from the base of the stem and force the xylem carefully through a close-fitting two-hole rubber stopper nearly to the bottom of a small bottle of freshly boiled water. Prepare a capillary glass tube 80 cm. or more in length with a hook bend at the top (Fig. 7). Clean the tube thoroughly with cleaning solution and fill with boiled distilled water; pass the bent end into the bottle containing the plant and dip the lower end into mercury. Exclude all bubbles and seal tightly.

Observe at frequent intervals and determine the height to which the mercury is forced. If possible, determine the manner of failure of the system. Explain.

To show that no vital forces are concerned, the experiment may be repeated with an atmometer bulb replacing the transpiring branch. Fill the bulb with warm water, and, after the mercury has risen to a considerable height, apply gentle suction to the bottle at the base. Explain. Release the vacuum. Does the mercury return to its original height? Note that if too strong a vacuum is applied and water pumped out of the system, the mercury may rise above its former height.

b. Instead of part *a* above, you may set up Askenasy's experiment.¹ Use capillary tubing and clean very thoroughly with warm caustic soda to remove the faintest traces of grease. The connection between the rubber stopper and atmometer also must be made with great care. Use clean redistilled mercury and a new atmometer of the cylindrical type.

Or follow Thut's technique,² which is much like part *a* except that the base of the stem is boiled for an hour to eliminate air bubbles, and all rubber connections are boiled in caustic soda before using. Omit the air trap used in part *a* and attach the branch directly to the top of the carefully cleaned capillary tube. All air must be excluded and a 10- to 20-cm. section of the stem base should be boiled to reduce the possibilities of air working downward through the plant. White fir, box elder, and white and red cedar were used by Thut. With fir 50 per cent of the trials were successful. Care is required when boiling the stem to prevent the leaves being steamed and killed.

Questions:

1. Would you include suction in the forces concerned in the movement of water in plants? How would it act in a plant whose roots are in moist soil instead of water?

2. What are the objections to explaining all water movement in plants upon the basis of cohesion?

Experiment 16. Transpiration and the Movement of Water in the Xylem. (E)

a. Cut two or more straight stems of *Impatiens* or corn under water and allow to stand in water for a few minutes. Transfer to a small quantity of water whose weight is accurately known and hold for 15 min. in locations which you would expect to give widely different rates of transpiration. Transfer the stems quickly to an eosin solution and observe the height to which the color is drawn in 30 or 60 sec. Reweigh the water and container and determine the ratio between transpiration and rate of movement in the xylem.

¹ PALLADIN, V. I. *Plant physiology*. Translated and edited by B. E. Livingston. 3d ed. P. 148. Philadelphia. 1926.

OTIS, C. H. The Askenasy demonstration. *Plant Physiol.* **5**: 419-423. 1930.

² THUT, HIRAM F. Demonstrating the lifting power of transpiration. *Am. Jour. Botany* **19**: 358-364. 1932.

b. Select three thrifty, potted corn plants 1 m. or more in height, and on a bright day treat as follows: leave one dry but not wilted, water one well, and cut the third under water and place in water in a cool shaded place for 15 to 20 min. Cut the first two stalks under water and transfer at once to eosin solution and observe by splitting the stalks quickly the height to which the dye is drawn in 30 sec. Repeat with the cut plant in the cool room and plot the three rates together.¹

Questions:

1. Why do plants growing in moist soil stop growth and sometimes wilt on a hot day?
2. What force carries the dye up into your plants?
3. How can this technique be used as a measure of water deficit in plants?
4. Wilted plants commonly give a low value for dye movement. How do you explain this result, and what does it suggest regarding the mechanism of water movement in corn?

THE CONSERVATION OF WATER

The tissues and morphological adaptations which reduce the rate of water loss from plants are of major importance in plant survival. The problem of water conservation is, of course, complicated by the necessity of permitting ready access of atmospheric carbon dioxide to the photosynthetic tissues.

Experiment 17. Protecting Tissues. (E)

a. Cut a thin transverse section through the skin of an Irish potato and draw a short strip of the outer layers of cells. Mount in concentrated sulfuric acid and observe under the microscope. Sulfuric acid does not readily attack tissues impregnated with oils or waxes.

b. Weigh an uninjured and a peeled potato, exposed on wire trays, at 24-hr. intervals for several days. Handle the peeled potato carefully. After 1 week, section the outer layer of the peeled tuber and examine first in water and then in H_2SO_4 . Plot the percentage water losses and explain your curves.

c. Repeat *b* with an uninjured and a peeled apple. Observe the skin under the microscope and test it in section and on the surface with concentrated H_2SO_4 .

¹LOOMIS, W. E. Daily growth of maize. *Am. Jour. Botany* **21**: 1-6. 1934.

Questions :

1. What types of protecting tissues are developed by plants?
2. Why are potatoes more easily kept in storage than cabbage?

Experiment 18. The Cutinization of Leaves. (E)

a. Obtain leaves of Bryophyllum or rubber plant, tomato or bean, and shoots of Elodea. Weigh the leaves, expose them to laboratory air on screen wire shelves, reweigh at 24-hr. intervals, and plot curves showing percentage rate of water loss. Make two extra weighings in the first 24 hr. to include small differences in initial rates of drying of tomato and Elodea.

b. Kill Bryophyllum, Echeveria, or Sempervivum leaves and shoots of Elodea by steaming for a few minutes and determine their rate of drying as above. Drop concentrated sulfuric acid on a wilted and a freshly steamed Bryophyllum or Echeveria leaf and compare rate of penetration on the upper and lower surfaces of the two leaves.

c. Strip the epidermis carefully from one or two Echeveria or Sempervivum leaves. Place the peeled leaves in a dry place with some normal leaves and observe at intervals for several days.

Questions :

1. An unbroken layer of cutin over the leaves and stems would result in maximum water conservation. Why do we find no plants of this type, even in the desert?

2. Explain why growth and water loss by transpiration may be proportional under favorable conditions. Would you expect the relationship to hold under all conditions? Why?

3. Of what advantage is the stomatal apparatus if it does not "regulate" transpiration?

CHAPTER III

TRANSPIRATION

INTRODUCTION

If a record is kept of the water which is evaporated from a plant during its development, and the weight of the water so lost is divided by the dry weight of the plant produced, a ratio is obtained which is variously termed the "water requirement," the "efficiency of transpiration," or the "transpiration ratio" of the plant under the given conditions. Obviously the ratio obtained will be dependent upon all of the factors affecting the production and accumulation of dry matter by the plant as well as upon the complex external and internal factors which control evaporation from plants. For this reason the older term, water requirement, has been largely displaced by the more accurate, transpiration ratio. The transpiration ratios observed for different plants under varying conditions range from one or two hundred to one or two thousand grams of water transpired for each gram of dry matter accumulated. For mesophytic plants under average conditions a ratio of 300 to 500 may be expected. At these rates the water transpired from an acre of good corn in 3 months would supply the normal needs of a city family of four persons for six years. When plants are grown with deficient moisture supplies, as most of them are for occasional periods, the enormous loss of water in transpiration becomes a most serious problem.

Various "functions" have been assigned to the transpiration process, the most popular of which are the concentration of the dilute soil solution by evaporating out its contained water after it has been "drawn" into the plant, and the cooling of the leaves of plants. Your experiments and a reading of some of the references given will suggest that the importance of both of these effects has been overestimated. It seems probable that plants could be developed which would absorb and translocate soil nutrients and maintain favorable leaf temperatures with

little or no transpiration. Such plants would of necessity, however, be completely covered with an impervious cuticle layer. Such a layer would obviously have a seriously retarding effect upon carbon dioxide absorption from the very low concentrations of this gas present in the air. On the other hand, as soon as the leaf cuticle is perforated by the stomates which make carbon dioxide absorption by the leaves feasible, water is unavoidably lost by evaporation from the moist mesophyll surfaces. The absorption of carbon dioxide is not facilitated and may be hindered by transpiration, but the condition which makes the first process possible makes the second inevitable.¹

The economic importance of transpiration has led to extended studies of this process, especially in the drier sections of the United States, Australia, and Russia. Some of the findings of these researches you can check in the laboratory, particularly the influence of environmental factors upon the transpiration rate and the influence of transpiration upon the physiology and development of the plant.

Before starting the experimental work in this chapter, read one or more of the following general references on transpiration:

References:

- MAXIMOV, N. A. Plant physiology. 2d ed. Chapt. VIII. New York. 1938.
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- BURGERSTEIN, ALFRED. Die Transpiration der Pflanzen. Jena. 1904.
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- DIXON, H. H. Transpiration and ascent of sap in plants. London. 1914.
- LOFTFIELD, J. V. G. Behavior of stomata. Carnegie Inst. Wash. Pub. 314. 1921.

THE MEASUREMENT OF TRANSPIRATION

Experiment 19. Evaporation from Leaves. (E)

Partially fill two small beakers or other straight-sided vessels with water, and cover one to a depth of 5 mm. with paraffin

¹ CURTIS, O. F., WALLACE and CLUM. Leaf temperatures: a critical analysis with additional data. Am. Jour. Botany **25**: 761-771. 1938.

oil. Obtain two long-petioled leaves of rubber plant (*Ficus elastica*), Begonia, or geranium, cover one with a thin but complete coat of vaseline, recut the ends of the petioles under water, and transfer the leaves to two bottles of water which can be tightly closed to prevent any direct evaporation. A 5-mm. layer of purified paraffin oil (Nujol) added after the leaves are in place makes a convenient seal. Weigh all four containers and place them in the greenhouse in partial shade or expose at a north window in the laboratory. After 1 or 2 days, reweigh the dishes and determine the water loss. Calculate the water and leaf surfaces exposed (both sides of the leaves) and express evaporation as grams per square meter per day.

Questions :

1. Why does water evaporate when exposed to the air?
2. How do the oil and vaseline coverings act?
3. Why is the rate of evaporation from the untreated leaf less than from the open dish?

Experiment 20. Evaporation from Growing Plants.¹ (E)

Obtain one or more thrifty potted plants, water to optimum (do not flood), and cover the pot to prevent loss of water by evaporation from the pot or soil. Ganong aluminum shells may be used for this covering if available; the pot in which the plant is growing may be covered with melted paraffin, or transpiration shells may be made inexpensively by plugging the drainage holes of new flower pots tightly with cotton and dipping the entire pot into hot paraffin. The paraffined pots should be large enough to allow the pots of growing plants to be set inside them. Squares of oilcloth are then cut to fit about the plants and tied smoothly over the paraffined shells.

Weigh the potometers as set up to ± 0.5 gm. on a solution balance and hold on a greenhouse bench or in other exposed position protected from rainfall. Place a straight-sided open dish of water with the plants. Reweigh the plants and dish daily or at shorter intervals and replace any water lost. Compare the hourly rate of water loss from plant and from water surface in the day and at night, in exposed and protected locations, or on bright and cloudy days.

¹ This experiment may be combined with Experiment 26, if desired.

After completing the readings, determine the leaf area of the plants¹ and the area of the water surface. From these data both the relative response of the plants and the absolute evaporation rates of plants and water can be calculated.

Questions:

1. How does the rate of evaporation from a plant leaf compare with the evaporation from a free water surface?
2. How do evaporation from plants (transpiration) and evaporation from a free water surface compare in their responses to changing conditions?
3. On the basis of the approximate area covered by the plants used, how many inches of rainfall per month would be lost by transpiration at the observed rates?

Experiment 21. The Transpiration Index. (E)

The potometer method of studying transpiration by weighing potted plants has obvious limitations. It does not permit a study of the transpiration rates of trees, of plants growing under natural soil conditions, or a comparison of the evaporation from different parts of the same plant. The cobalt chloride paper method, while only roughly quantitative, even with careful standardization, has very real advantages in the study of comparative evaporation rates under field conditions.

Prepare cobalt chloride paper by dipping a good grade of filter paper into a 5 per cent CoCl_2 solution, squeezeing the paper moderately dry on a glass or tin plate and drying flat on the plate to insure a uniform color. Cut the dry paper into 2-mm. squares and keep it in a tightly corked bottle over calcium chloride. The blue color of the paper can be restored, as required, by heating for a minute. For field work it is important to have an effective drying bottle to prevent absorption of moisture by the papers before they are used. A 4-oz. screw-top vial half-filled

¹ Leaf area may be determined in any one of several ways. If a planimeter is available the leaves may be placed under glass and their area measured directly, or the leaves may be traced or blueprinted and the area of the tracings determined. If a planimeter is not available, trace or blueprint the leaves, cut out the outlines carefully, and weigh; determine the weight per square centimeter of an accurately measured piece of the paper used, and divide the weight of the paper cutouts by this value to find the area of the leaves. In either case, multiply by two to allow for the two sides of the leaves unless the species used is heavily cutinized and has stomates on one surface only.

with CaCl_2 and fitted with a wire basket to hold the paper squares is convenient, or more elaborate devices may be prepared.

Standardization of the papers is necessary if any attempt at quantitative interpretation of the data is to be made.¹ Standardization consists of finding the time for a square of the paper to

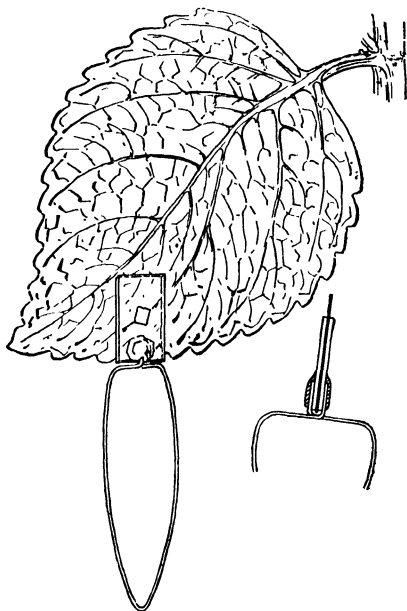


FIG. 8.—Transpiration clips. Spring wire is bent as shown and cemented to small glass slips about $1\frac{1}{2}$ by 2 cm. in size. Two-millimeter squares of cobalt chloride paper are then clipped to the leaves as shown, to measure their relative transpiration rates.

lose its blue color in a saturated atmosphere, when near but not in contact with a free water surface. A rough standardization may be obtained by covering wet blotting paper with a fine wire gauze, placing the cobalt chloride paper on this, covering it with glass, and observing the time for a standard color change. For more accurate standardization methods the apparatus² described by Livingston should be used.

¹ LIVINGSTON, B. E., and EDITH B. SHREVE. Improvements in the method for determining the transpiring power of plant surfaces by hygrometric paper. *Plant World* 19: 287-309. 1916.

² *Loc. cit.* Available from Dr. B. E. Livingston, The Johns Hopkins University, Baltimore, Maryland. Clips with permanent color standards attached may be obtained from the same source.

The determination is made by placing a 2-mm. square of the paper on the leaf, covering it with a glass cover held by a spring clip (Fig. 8), and observing the time for a standard color change in the paper as the result of the absorption of water vapor. Livingston recommends that a stop watch be started when the paper has lost the deep blue of the completely dry preparation and has acquired a clear medium blue color, and that the watch be stopped as soon as the paper has become white. Comparative if less accurate results may be obtained by starting the timing when the paper is attached, provided paper of uniform color is used for all tests. The complete color change is blue to white to pink. The pink color is difficult to estimate and most accurate readings may be made by using the disappearance of the last trace of the blue color as the end point of the change. Many lots of cobalt paper will show a standard color change over a free water surface in approximately 30 sec.

The time, usually in seconds, for a standard color change, divided by the time for the same color change on the leaf, gives the transpiration index. It is convenient to multiply the index by 100 to obtain the evaporation from the leaf as a percentage of the evaporation from a free water surface.

$$I = \frac{S \times 100}{E}$$

where E is the experimental and S is the standard time in seconds. Three precautions are required: the color readings should be uniform; the paper should be uniformly but not absolutely dry since the completely dry paper resists wetting, and the slips should be adjusted to reduce to a minimum the effects of atmospheric humidity.

Study the transpiration index of some of the following or make similar comparative measurements.

1. Young and old leaves on the same plant.
2. Leaves on watered and on dry plants.
3. Leaves in the shade and in the sun.
4. Succulent and thin leaves.

Questions:

1. List some specific experiments in which the cobalt chloride paper method of estimating transpiration would be valuable.
2. What errors besides those of manipulation are involved?

Experiment 22. The Transpiration Ratio.¹ (I)

When time permits it is interesting to grow oat, corn, bean, tomato, or other quick-growing plants in sealed containers, and to keep a record of the water required to maintain the pots at uniform moisture. After the plants have made a good growth, they are dried in an oven. Their dry weight divided by the water used in their growth gives their transpiration ratio. Glazed gallon or larger jars, with the soil covered with a mixture of vaseline² and paraffin, are convenient containers for experiments of this type. Water may be applied through a funnel or a glass tube into a small inverted flower pot set into the soil and waxed over. Autoirrigator pots are ideal for this experiment when they are available.

Compare the transpiration ratios of:

1. Plants grown in fertile and nonfertile soil
2. Plants grown at high and low humidity but with the same light and CO₂ supply.
3. Plants grown with varying shades.
4. Different varieties or species of plants.

Questions:

1. Is the transpiration ratio constant for a given plant variety?
2. What conditions would you use to obtain a minimum ratio?
3. Why is so much water used by plants?

EXTERNAL FACTORS AFFECTING TRANSPIRATION

The evaporation of a liquid involves the absorption of the energy required for the liquid-vapor phase change and the dispersion of the vapor formed. The energy used in the vaporization in the leaf may be absorbed slowly from the surrounding air or more rapidly from sunlight. Direct sunlight, because it supplies the energy for vaporization of water and tends to

¹ May be combined with Experiment 155. In this case, cover the pots with a 3- to 5-cm. mulch of dry sand instead of the wax. See also Experiment 42.

² Twenty per cent vaseline in warm weather and 30 to 40 per cent in cold. If the wax cracks away from the jar, reseal it with vaseline or a hot iron. The wax is applied after the plants are up. Level and firm the soil in the pot; wrap the plant stems with a little cotton, and apply the melted wax at a temperature of 50 to 55°C., tipping the pot to insure an even covering. Wax-covered pots must be protected from full summer sunlight at all times.

raise the leaf and air temperatures, is a major factor in the determination of transpiration rates. The dispersion of water vapor in transpiration depends upon diffusion through the stomates, and the transpiration rate, other things being equal, is thus dependent upon the diffusion gradient of water vapor from the intercellular spaces of the leaf to the air. This gradient is calculated as vapor pressure expressed in millimeters of mercury, and is dependent upon the temperature and the relative humidity of the air and of the intercellular spaces of the leaf. For example, the pressure of water vapor in a leaf at 20°C. would be expected to be near 17.54 mm. Hg, or 100 per cent humidity. If the pressure outside were zero, thus at 0 per cent humidity, and the temperature was again 20°C., the gradient would be equal to a pressure difference of 17.54 mm. Hg over whatever distance was involved. If the relative humidity was 60 per cent, the pressure outside would be 10.52 mm. Hg, the difference or gradient only 7.02 mm. Hg, and the transpiration rate would be expected to fall to 40 per cent of its value at 0 per cent humidity. Similarly, at higher or lower leaf or air temperatures the difference in vapor pressures determines the transpiration gradient for any leaf and the relative transpiration rate, unless a water deficit in the leaf, stomatal closure, or other factors interfere.

Experiment 23. The Water Absorbing Power of the Air and the Transpiration Rate. (E)

Use a leafy branch with a woody stem which will resist crushing of the vessels; white pine or white fir is excellent; recut under water to eliminate air plugging, and mount in a small stoppered flask of water or attach to a 1-ml. pipette graduated in 0.01 ml. (Fig 9). Determine the transpiration rate of the shoot, in the first case by weighing actual loss to 0.01 gm. and in the latter by measuring water absorption. The weighing method is less convenient, but it is not affected by lags between transpiration and absorption which sometimes cause difficulty.

Compare the transpiration of your potometer at such variations of temperature and humidity as are available in the laboratory, in a moist chamber or room, and under a shade in the greenhouse. Determine relative humidity and temperature, calculate diffusion gradients in millimeters of mercury for each location, assuming air temperature and 100 per cent humidity within the leaves,

and plot transpiration rates against water-vapor gradients. Use the same or a similar setup for Experiments 24 and 25.

An interesting comparison may be obtained by setting up an atmometer with the plant potometer and determining the relative response of plant and atmometer.

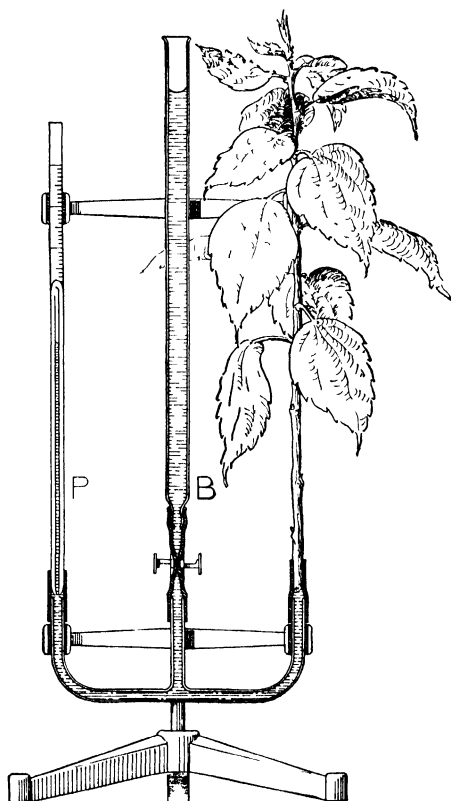


FIG. 9.—Potometer for measuring transpiration. The absorption of water by a transpiring shoot is measured in the graduated pipette *P*, which is refilled as needed by opening the pinch clamp of the burette *B*.

Questions:

1. Do your data indicate a direct proportionality between gradient and transpiration?
2. Under what conditions would you expect deviations from such a proportionality?
3. If the transpiration rate of a leaf at 30°C. and 55 per cent relative humidity is 100, what will be its transpiration rate if the temperature is

reduced (as at night) to 20°C. without changing the water content of the air¹ or the opening of the stomates?

Experiment 24. Air Movement and the Transpiration Rate. (E)

With the same apparatus which you used in Experiment 23, determine the effect of a gentle fanning on the transpiration rate in one or more locations. Determine the transpiration and, from an atmometer, the evaporation rates with and without fanning, first in the shade and then in sunlight.

Questions:

1. Does wind affect the relative humidity of the air? How do you account for its effect in increasing transpiration? How may wind reduce transpiration?

2. How do you explain differences in the responses of plant and atmometer?

Experiment 25. Radiation and the Transpiration Rate. (E)

With a plant and atmometer setup similar to that employed in Experiments 23 and 24 except that two atmometers, one black and one white, should be used, determine the effect of radiation on transpiration and evaporation rates. Set up the mounted plant and the two atmometers under a movable shade in a sunny greenhouse. After determining evaporation rates, remove the shade and make new readings. A 1000-watt Mazda bulb, backed by a good reflector and held 60 to 100 cm. from the plant and atmometers, may be used in lieu of sunlight but is not so effective as bright sun.

Questions:

1. Why is sunlight an important factor in transpiration? List its direct and indirect effects.

2. Why will plants in the light transpire in saturated air? Would you expect the transpiration rate under these conditions to be greater in green or in etiolated leaves?

Experiment 26. The Water Supply and the Transpiration Rate. (I)

Select two potted plants² which have been allowed to dry out but have not wilted and enclose with shells and oilcloth as

¹ While the water content of the air in grams per cubic meter will not change with temperature unless the dew point is reached, the pressure of the vapor will change according to the gas laws.

² Soft-leaved geranium plants are desirable.

in Experiment 20. Water one pot well but avoid overwatering; leave the other pot in its dry but not wilted condition; weigh the two plant setups carefully and place them on a greenhouse bench together with a straight-sided water dish of known weight. Weigh and record water loss daily and refill all containers to their original weight. If the "dry" pot loses weight as rapidly as the watered one, do not replace the water lost from it in the first 2 or 3 days.

Continue the experiment for 2 or 3 days after a clear-cut difference in transpiration rates has been obtained. At the end of this period, use the plants for Experiment 29*b* and *c*, and then remove the leaves and determine the fresh weight of the leaves and of the roots. Determine the area (both surfaces) of the leaves by means of the planimeter or otherwise (see footnote, page 29). The leaves may be outlined or pressed if it is desired to measure them at a later period. Calculate the transpiration for the last two or three days of the experiment on the basis of grams per gram of roots, grams per gram of leaves, and grams per square meter or decimeter of leaf surface. Calculate the ratio between the wet and dry plants on the three bases.

Calculate the evaporation from the dish during the same days on the basis of grams per unit of water surface. What is the ratio between the evaporation from the free water surface in the dish and from an equal area on each of the plants?

Questions:

1. Which method of calculating transpiration do you consider most accurate? Most rapid? Could the rapid method be used in your experiment without appreciable error in the comparison of wet and dry plants?
2. Under what conditions might an error be introduced into the green weight of leaf comparison? Would dry weight of leaves be a better basis?
3. Considering its exposure and the very large interior surface, is the leaf structure efficient in reducing evaporation?

INTERNAL FACTORS AFFECTING TRANSPIRATION

Experiment 27. The Leaf as a Transpiring Organ. (E)

Obtain leaves of several thin-leaved plants and examine them for venation. Can you make a pinhole through the leaf without striking one of the tiny veinlets?¹ Cut sections of the leaves

¹ Exhaust leaves under water and observe by strong transmitted light or use one of the techniques given in the footnote on page 19.

and measure their thickness with an eyepiece micrometer. What size would your leaves be if they were enlarged until they were an inch thick?

Strip off the lower epidermis of some thin shade-grown young leaves and look at the exposed leaf tissue with a high-power dissecting binocular using reflected light, or mount in toluene or kerosene and observe at 50 to 100 magnifications.

Mount pieces of the lower epidermis of leaves in water containing a trace of ether and observe stomates. How many stomates do you find in an area the size of a pinhole?

Questions :

1. List the leaf factors which tend to increase transpiration. How is excessive water loss prevented?
2. Why are the transpiration-reducing features of desert plants, such as small thick leaves with few stomates, not selected for dry-land crop plants?

Experiment 28. The Relation of Stomates to Transpiration. (E)

Determine the transpiration indices of the upper and lower surfaces of transpiring leaves with cobalt chloride paper. Run the lower and upper surface determinations simultaneously on such leaves as catalpa, Norway maple, lilac, or Fuchsia, which show marked differences in the number of stomates on the upper and lower surfaces. Compare also geraniums or other plants on which the stomatal ratio is 5 or 10 to 1.

Strip off the upper and lower epidermis of the leaves used and determine, as the average of several counts, the number of stomates per field under high power (400 $\times$). Measure the diameter of the microscope field with a stage micrometer and calculate the number of stomates per square centimeter of leaf area. If you do not have a stage micrometer, estimate the field diameter of a microscope with a 4-mm. objective and 10 $\times$ ocular, at 0.34 mm.

The celloidin method¹ of counting stomates may be conveniently used in this experiment, particularly for observing the upper surfaces of the leaves. The celloidin mixture, described under Experiment 46, may be brushed or smeared thinly on the leaf surface to be examined. As soon as the celloidin film is

¹LONG, FRANCES L., and FREDERIC E. CLEMENTS. The method of collodion films for stomata. *Am. Jour. Botany* **21**: 7-17. 1934.

dry enough to peel, remove it carefully, mount dry, and count the stomate prints in a microscope field with high-power magnification. For accurate counts, follow the precautions against shrinkage of the film given by Long and Clements. These precautions may be omitted when determining the ratio of the stomates on the two surfaces of the leaf.

Multiply the time for the cobalt paper to change by the average number of stomates per microscope field to obtain the theoretical time to change with a uniform number of stomates.

Questions:

1. What deductions can you make from your data regarding the relative importance of cuticular and stomatal transpiration?

2. Would you expect a leaf with twice as many stomates as another to transpire twice as much water? Explain.

Experiment 29. The Measurement of Stomatal Opening. (I)

The measurement of stomatal opening in transpiration and photosynthesis studies is a difficult problem. Three methods are in use.

a. *Direct observation* of the stomates on the leaves as used by Lloyd.¹ Pass direct sunlight or strong electric light through a round 1-l. flask of 2 per cent copper sulfate solution to remove some of the heat waves. Focus this cooled light on the leaf with a mirror and condenser, and observe the stomates directly with a 4-mm. long-focus objective. There will ordinarily be little difficulty in observing the stomates, but the accurate measurement of their openings requires practice and a familiarity with the material, and even with the copper sulfate filter there is serious danger that the leaves may be overheated.

b. *The porometer* permits a convenient measurement of relative porosity of the leaf, but gives no index of actual stomatal opening since the rate of passage of air through a leaf depends upon the compactness of the mesophyll and the number of stomates on the upper surface as well as upon stomatal aperture. The cup of the apparatus illustrated in Fig. 10 is carefully cemented to the surface of the leaf to be tested with warm gelatin, rubber cement, chewing gum, or other material, air is removed at *E* to raise water

¹ LLOYD, F. E. Leaf water and stomatal movement in *Gossypium* and a method of direct visual observation of stomata *in situ*. Bull. Torrey Botan. Club **40**: 1-26. 1913.

in the tube *T* to a predetermined level, and the time for the water column to drop a standard distance with the pinch clamp at *E* closed is determined with a stop watch. The resistance of the leaf to air movement is the factor measured, and with comparable

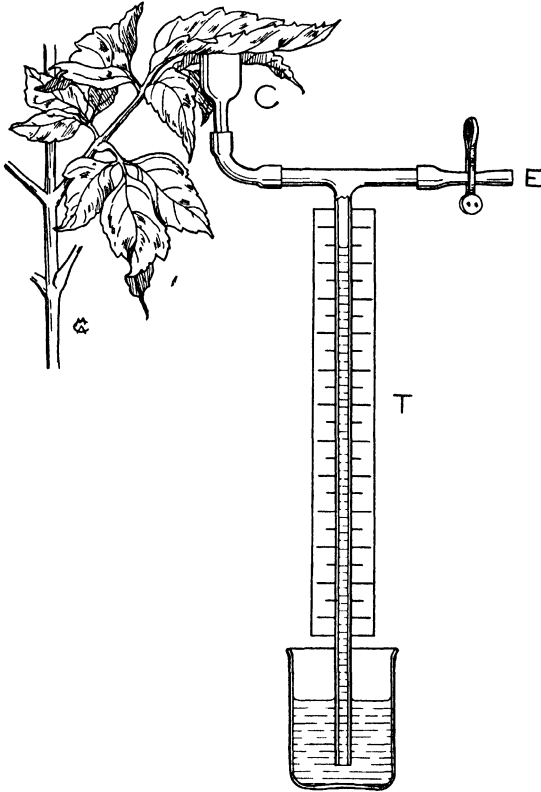


FIG. 10 —Porometer. The cup *C* is cemented to the surface of the leaf to be studied. Air is removed at *E*, the clamp closed, and the time for the water column in *T* to drop a standard distance is measured.

plant material, particularly with the same leaf under varying conditions, this resistance is proportional to stomatal opening.

Use the porometer on the attached leaves of the wet and dry plants of Experiment 26 and compare the results obtained with direct measurement of stomatal opening in alcohol.

c. Direct measurement of stomatal opening is possible, provided the epidermis is stripped off the plants and the stomates are fixed in absolute alcohol so rapidly that the guard cells do not

dry or become plasmolyzed. Even with the most rapid handling, however, turgor changes may be involved in the stripping of the epidermis which will affect the stomatal opening. The fixed epidermis is mounted in absolute alcohol and the average opening of a number of stomates measured with a standardized ocular micrometer.¹ The area of an elliptical opening is obtained by multiplying the product of the two axes by 0.7854. The perimeter, which is a better index of diffusion capacity, may be obtained with the approximation equation:

$$P = \frac{\pi}{2} \left(a + b + 2\sqrt{\frac{a^2 + b^2}{2}} \right)$$

in which a and b represent *one-half* the length and width of the ellipse. The results obtained with this equation are slightly low, but are more nearly correct than those obtained with the usual $\pi(a + b)$, particularly for narrow ellipses like partially closed stomates.

Determine the average stomatal opening and the number of stomates per square centimeter on leaves of the dry and watered plants from Experiment 26. (Save the leaves.) Calculate the percentage of leaf area through which diffusion was taking place. Compare the loss from the wet and dry plants and the free water surface for the last 24-hr. period of Experiment 26 and determine the ratio between evaporation from a free water surface and evaporation from the open area of the stomates.

Questions:

1. What agreement do you find between porometer readings and open area?
2. Why is the perimeter a better basis for predicting diffusion through stomates than the area or even the average diameter?
3. How much difference would you expect in the diffusion through a fully open and a half-open stomate of the same size, and under the same conditions?

¹ The standardization of an ocular micrometer is accomplished by placing the micrometer in the eyepiece of a microscope, on the flat side of the ring built into the focal plane of the eyepiece for this purpose. Focus the microscope on a stage micrometer marked in tenths and hundredths of a millimeter, and determine the apparent length of the entire ocular scale in millimeters. If the ocular is graduated with 50 lines and its total apparent length is 0.179 mm., dividing 0.179 by 50 gives 0.00358 mm. or 3.58 μ as the length of each graduation. This standardization cannot be used for any other optical combination than the one upon which it was obtained.

Experiment 30. Stomatal Movement. (I)

The earlier idea that the opening and closing of the stomates were important factors in controlling the transpiration rate has proved to be unfounded, but the topic is still a fascinating one of importance in studies both of transpiration and photosynthesis.

Remove the epidermis from some easily stripped leaf, mount in water containing a little ether, and observe stomatal opening. The ether apparently increases the permeability of the guard cells to water and speeds up their movement. What causes the stomates to open?

Study changes in stomatal opening, using a porometer, or epidermal strips in absolute alcohol: (a) when the plant is transferred from darkness to light or vice versa, (b) when it is allowed to wilt, (c) when it is held at high temperatures, 35 to 38°C., with liberal light and water. The effects of light quality, light intensity, temperature, and CO₂ concentration on stomatal movement may be studied as time permits.¹

Questions:

1. Why can the stomates act as automatic safety devices to prevent excessive water loss from the plant?
2. How can stomates open so quickly when lighted (3 to 5 min.)?
3. What advantages arise from having the stomatal opening regulated by the CO₂ concentration of the guard cells?

THE EFFECTS OF TRANSPIRATION**Experiment 31. The Cooling Effect of Transpiration. (I)**

Transpiration is frequently assigned a major role in cooling plant leaves. The large quantities of water transpired and the high latent heat of water require a great deal of heat energy, but a moment's reflection will show that plants are likely to encounter the highest temperatures during the driest weather when their stomates may be closed by wilting.² Shull³ has calculated that a

¹ For suggestions for further work see: SAYRE, J. D. Opening of stomata in different ranges of wave lengths of light. *Plant Physiol.* **4**: 323-328. 1929. Also SCARTH, GEORGE W. Mechanism of the action of light and other factors on stomatal movement. *Plant Physiol.* **7**: 481-504. 1932.

² CURTIS, O. F. Leaf temperatures and the cooling of leaves by radiation. *Plant Physiol.* **11**: 343-364. 1936.

³ SHULL, C. A. The mass factor in the energy relations of leaves. *Plant Physiol.* **5**: 279-282. 1930.

green elm leaf in moderate sunlight will heat at the rate of 40°C . a minute if not cooled by transpiration, convection, or reradiation. A dead elm leaf, because of its lower specific heat, should heat even more rapidly and would quickly burst into flame if its cooling depended upon transpiration.

Expose transpiring leaves, dried leaves or pieces of green paper, and leaves covered with a very thin film of vaseline, to bright sunlight and see whether you can detect any striking temperature differences due to transpiration. Normal and vaselined leaves may be wrapped around the bulbs of sensitive thermometers to give rough readings of leaf temperatures. Compare these with the air temperature in the shade near the leaves. The vaseline should be applied thinly and uniformly to both surfaces of the leaf and then wiped off with a soft dry cloth to fill the stomates without greatly changing the leaf surface. If fine thermocouples are available, clamp these on the surfaces of the leaves after the manner of Miller and Saunders,¹ or, better, insert the thermocouples into the leaf tissue.² Measure air temperature by shading the thermocouple with a piece of insulating board. Do not measure air temperature in the shade of a leaf only, for leaves are fairly transparent to infrared radiation.

Compare also the temperatures of thin leaves and the thick leaves of succulents such as *Bryophyllum* and *Echeveria* or *Sempervivum*.

Questions:

1. Why are wilted, non-transpiring or slowly transpiring, leaves not quickly killed by overheating?

2. Why can leaf temperatures change several degrees a minute with gusty wind or with alternate shade and sun?

Experiment 32. Transpiration and Mineral Absorption. (I)

A second function assigned to transpiration is the "drawing" into the plant of the dilute soil solution with its dissolved minerals.

¹ MILLER, E. C., and A. R. SAUNDERS. Some observations on the temperature of the leaves of crop plants. *Jour. Agr. Research* **26**: 15-43. 1923.

² CLUM, H. H. The effect of transpiration and environmental factors on leaf temperatures. I and II. *Am. Jour. Botany* **13**: 194-216; 217-230. 1926.

This viewpoint fails to take into account the fact that solutes diffuse into the roots of plants independently and that a solute may be absorbed relatively much more rapidly¹ or much more slowly² than water.

Grow four or more cultures of sunflower, pea, wheat, or other plants on modified Knop solution (see Experiment 42) until 3 or 4 weeks old and large enough to transpire rapidly. Change the solutions at weekly or semiweekly intervals toward the latter part of the growth period.

Make up enough modified Knop solution for half of the cultures, with enough extra for tests of total mineral content, using only half of the recommended quantities of salts. That is, use 25 ml. of each of the six stock solutions of Experiment 42 in each liter of diluted solution. Make up a second lot of double-strength solution using 100 ml. of each of the stock solutions in a liter. To prevent the formation of precipitates when mixing the solutions, pour them into the 400 ml. of distilled water required for each liter with rapid stirring, and add the KH_2PO_4 and ferric tartrate solutions last. You should have 100 to 200 ml. of each solution left for mineral tests. Carefully clean two or four weighing bottles, dry at $100^\circ\text{C}.$, cool in a desiccator, and weigh on a quantitative balance to ± 0.0001 gm. Add 10 to 25 ml. of the culture solutions to each of two bottles or, better, make duplicate determinations on each solution. Evaporate the solutions in an oven at $100^\circ\text{C}.$, pipetting in more solution to a total of 40 to 100 ml. Evaporate to dryness, cool, and reweigh the bottles to determine the total salt content of your solutions.

Fill two sets of culture jars (Fig. 16), one with the dilute and one with the concentrated solution, transfer paired lots of plants to the jars, weigh carefully (± 0.5 gm.), and expose all cultures in the greenhouse to conditions favorable for transpiration until one-fourth to one-third of the water has been evaporated. Reweigh the cultures to determine total transpiration, shake the

¹ PARKER, F. W. Soil phosphorus studies. III. Plant growth and the absorption of different phosphate concentrations. *Soil Science* **24**: 129-146. 1927.

² MUENSCHER, W. C. The effect of transpiration on the absorption of salts by plants. *Am. Jour. Botany* **9**: 311-329. 1922.

LOOMIS, W. E. Schloesing's experiments on the relation of transpiration to the translocation of minerals. *Plant Physiol.* **4**: 158-160. 1929.

solutions to suspend any precipitate which may have formed, pipette into weighing bottles, and evaporate down some of the residual solutions to determine their total mineral content. If mineral absorption is proportional to transpiration, there will be no changes in the mineral concentrations of the cultures.

Questions :

1. Explain on a basis of diffusion gradients the apparently selective absorption of water or minerals. (Compare Experiment 46.)

2. Why do plants on infertile soils grow better in wet weather when the transpiration rate is low and the soil solution dilute than in drier weather when conditions for mineral absorption might seem to be more favorable?

Experiment 33. Transpiration and Growth. (E)

Obtain two vigorous potted corn plants about 30 cm. high, water both plants, and cover the pots (see Experiment 20); weigh, and attach auxanometers to the central leaves as illustrated in Fig. 1, page 9. Place one plant in a shaded moist chamber and the other in strong sunlight and determine their transpiration and growth rates over a period of 2 to 6 hr.

Questions :

1. Why do desert and salt marsh plants tend to be short?

2. Why can plants be "hardened" by increasing their transpiration rate? Hardening is correlated with sugar accumulations in the plant.¹

Experiment 34. Transpiration and Differentiation. (I)

Obtain samples of plants grown under high and low transpiration conditions, *e.g.*, shade and sun leaves from the same plant, and study their relative differentiation by determining: (1) percentage of dry matter in the growing plant, (2) leaf thickness, stem strength, or other measure of differentiation, (3) percentage of crude fiber in the dry tissue. Use the autoclaving method, outlined in Experiment 155, and calculate results to original green weight.

Questions :

1. Why is the best tobacco for cigar wrappers grown under shade?

2. How would the quality of lettuce be affected by hot dry weather?

3. Would you expect a high daytime transpiration rate to give marked differentiation if night conditions were favorable for rapid growth?

¹ DEXTER, S. T. Effect of several environmental factors on the hardening of plants. *Plant Physiol.* 8: 123-139. 1933.

CHAPTER IV

PLANT NUTRIENTS

INTRODUCTION

The utilization of nutrients, and particularly soil nutrients, by plants is perhaps the division of plant physiology which has received the most attention from practical plant growers. Relatively little can be done about the temperature, CO₂ concentration, or the mean daily sunlight. It is expensive to attempt to increase or decrease the water supply of a given area, but wide changes in the soil nutrient supply are relatively inexpensive and practicable. Even though all of the nitrogen, phosphorus, and potassium used by our crops had to be supplied in fertilizers, they would represent on an average only 2 or 3 per cent of the dry weight of the harvest.

Plant nutrient research has been largely concerned with three main problems: (1) efficiency in the use of the fertilizer or macro-nutrients such as nitrogen and calcium, (2) identification of the micro-nutrients such as manganese and zinc, and (3) the determination of the functions of the various elements. In spite of the large amount of published research on plant nutrition, none of these problems has been satisfactorily solved, and our lists of essential elements and our knowledge of the functions of the various nutrients are in a state of flux. The following classification of nutrients and their functions is therefore subject to many additions and revisions.

Framework Elements.—Carbon, hydrogen, and oxygen are the most important plant nutrients from the standpoint of bulk, and constitute 90 or more per cent of the dry weight of common plant materials. These elements make up the cellulose and the lignin, the protective waxes, the reserve foods, and most of the protoplasm of plants. Fortunately for the farmer, they are normally furnished free by the air and the rainfall, and are not considered in the usual program of plant nutrition.

Protoplasm Elements.—In addition to carbon, hydrogen, and oxygen, the proteins and other compounds which make up protoplasm require nitrogen, phosphorus, and sulfur. Notice that these are all absorbed as anions and that nitrogen and phosphorus are our most important fertilizer elements.

The functions of four elements, carbon, hydrogen, oxygen, and nitrogen are well understood. Most of the phosphorus and some of the sulfur can be accounted for although we do not fully understand their action. Beyond these six elements we face conjectures and hypotheses. Calcium is known to occur in certain cell-wall constituents and magnesium in chlorophyll although both elements appear to have other uses. Two tentative groupings may be made on the basis of probable functions.

"Balancing" Elements.—The basic elements, absorbed as cations, are in some way concerned with maintenance of ionic balance in the plant. Calcium, magnesium, and potassium come in this group and apparently sodium should be included for marine but not for land plants. If chlorine or other members of the halogen group are shown to be essential, it seems probable that they will be found to function as anions in the maintenance of this balance. There is considerable evidence that potassium and possibly magnesium may function in the following group also, acting, perhaps, as inorganic catalysts rather than as enzyme formers.

"Catalytic" Elements.—The elements used by plants in small quantities have been assigned catalytic or enzyme-forming roles, rather largely on the assumption that the minute requirements would be insufficient for any other purpose. Sommer and Lipman,¹ for example, found that zinc was essential for tomatoes but that a total of 0.000125 gm. of zinc sulfate per plant was adequate for normal development. Because of the small requirements, these elements have been termed "infinitesimal" or perhaps better, "micro-nutrients." Iron, in this group, has been recognized as an essential element since the beginning of water-culture work. Increasing evidence indicates that manganese, boron, and zinc are essential elements and there is less conclusive evidence for copper. Many other elements have been suggested

¹ SOMMER, A. L., and C. B. LIPMAN. Evidence of the indispensable nature of zinc and boron for higher green plants. *Plant Physiol.* 1: 231-249. 1926.

and future work will probably establish some of these, largely minerals, as essential.

References:

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HUGHES, H. D., and E. R. HENSON. *Crop production*. Chaps. VII and VIII. New York. 1930. Emphasizes the cropping viewpoint.

RUSSELL, E. JOHN. *Soil conditions and plant growth*. 7th ed. London and New York. 1937. Includes some theoretical as well as practical material.

BRENCHLEY, WINIFRED E. *Inorganic plant poisons and stimulants*. Cambridge. 2d ed. 1927. Reviews the earlier work on micro-nutrients.

ELEMENTS PRESENT IN PLANTS

The liberation of water from burning plant tissue was demonstrated in Experiment 5, and the production of charcoal (carbon) from plants is an everyday phenomenon. It should not be forgotten that these elements, oxygen, hydrogen, and carbon, are the most important plant nutrients from the percentage standpoint although they will not be given further consideration in this chapter.

Experiment 35. Plant Ash. (E)

Obtain a sample of living material, that is, bark or wood from a green tree, green leaves, or air-dry grain, and determine its moisture content by oven drying. Grind the dry sample and hold in a desiccator for ash determinations.

Heat three clean porcelain crucibles at low red heat for $\frac{1}{2}$ hr. in a blue flame. Cool until the yellow color fades and then cool for an hour or more in a desiccator and weigh with quantitative precautions to ± 0.1 mg. The crucibles must not be touched except with clean tongs or exposed more than momentarily to moist or dusty air. Weigh three samples of the oven-dry material into the crucibles, using from 0.5 to 10.0 gm. and weighing

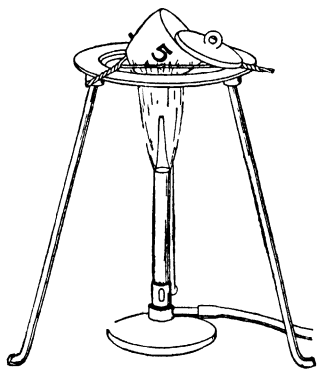


FIG. 11.—Crucible and lid arranged for ashing over Bunsen burner.

again to ± 0.1 mg. Heat the crucibles in a Bunsen flame with crucibles and covers arranged as shown in Fig. 11. The lid deflects warm air onto the sample and hastens oxidation. When flaming stops, set upright, cover, and heat at low red heat until free of black or gray color, heat in an electric furnace at low red heat for 3 hr. If the carbon color persists, cool, moisten the ash with a drop or two of concentrated nitric acid, and reheat. Cool for a minute in the air and then in the desiccator and reweigh.

Calculate the percentage of total ash in the oven-dry material and in the living tissue. Tabulate the data of the class. The following or similar materials may be used:

1. Wheat or corn grain.
2. Wood, twigs, shavings, or bark.
3. Wheat straw.
4. Potato tubers or beet roots.
5. Leaves of alfalfa or clover.
6. Leaves, stems, or roots of any desired plant.

Be sure that root samples are thoroughly washed to remove sand and soil.

Questions:

1. What correlations can you find between tissue function and ash analysis?
2. The ash of wheat grain is about one-half phosphoric acid. Does this mean that wheat is high in phosphorus and that the crop has a high phosphorus requirement? Check percentages of ash and acre yields.

Experiment 36. The Elements Present in Plant Ash. (E)

To demonstrate the presence of various elements in plant ash, perform the following qualitative tests on an ash sample, preferably the ash from young but fully grown leaves.

Dissolve about 0.2 gm. of ash in 10 ml. of warm $1 + 4\text{HCl}$ ¹ and make to a volume of 100 ml. with distilled water.

Sulfur.—Add a few drops of BaCl_2 solution (2 to 5 per cent) to a 10-ml. portion of the filtered ash solution. A white, very fine crystalline precipitate is BaSO_4 .

Calcium.—Take a second portion of 20 ml. and make slightly alkaline with $1 + 1\text{NH}_4\text{OH}$. Filter and add a few drops of a

¹ One part concentrated (37 per cent) HCl and four parts distilled water by volume.

saturated ammonium oxalate solution. A white precipitate is calcium oxalate. Save the solution.

Magnesium.—Add an excess of ammonium oxalate to the calcium test solution to precipitate all calcium. Filter and evaporate the filtrate to a volume of 10 ml. Add 1.0 ml. saturated Na_2HPO_4 solution, cool, and allow to stand. Crystals are NH_4MgPO_4 . Crystallization may be hastened by rubbing the inside of the vessel with a glass rod.

Iron.—To a few drops of the ash solution on a white plate, add a drop of potassium thiocyanate solution. A red color indicates $[\text{Fe}(\text{CNS})_6]^-$.

Sodium.—Test a loopful of the solution, concentrated if necessary, in a clear flame on a sodium-free wire. A yellow flame color and a bright yellow line in the yellow region (589 $\text{m}\mu$) of the spectrum indicate sodium.

Potassium gives two spectroscopic lines in the dark red (770 and 767 $\text{m}\mu$). Compare with lithium, which gives a bright red line at 671 $\text{m}\mu$.

Chlorine and Phosphorus.—Dissolve a second sample of ± 0.1 gm. of ash in $1 + 9\text{HNO}_3$ and divide into two unequal portions. To the smaller add a few drops of silver nitrate solution and observe for a heavy white precipitate of AgCl as an indication of the presence of chlorine.

To the larger portion add molybdate solution (see footnote, page 51), heat on a steam bath for a few minutes, and cool. A yellow precipitate is ammonium-phospho-molybdate.

Questions:

1. What elements, other than those observed, are commonly present in plants?
2. To what extent can the essential nature of an element be determined by ash analysis?
3. Why is an ash analysis of a crop plant of so little value in planning a fertilization program?

Experiment 37. The Types of Plant Ash. (I)

The ash of growing or storage organs is relatively high in phosphorus and potassium and the ash of older tissues and stems is relatively high in calcium and silicon. Obtain samples of the indicated materials; determine percentage of moisture, grind, ash fairly large samples, and test as follows:

Calcium Type.—Ash 2 to 10 gm. of the chips of some wood. If green twigs are used, remove the bark before drying and grinding. Cool the ash, weigh, dissolve in hot $1 + 4\text{HCl}$ and dilute to 50 ml. Make the extract slightly alkaline with $1 + 1\text{NH}_4\text{OH}$ and filter if a precipitate forms. Heat the filtrate and add saturated ammonium oxalate solution a drop at a time until no more precipitate is formed. Allow the calcium oxalate precipitate to stand for several hours to crystallize. Filter, wash the precipitate, dissolve in hot $1 + 1\text{H}_2\text{SO}_4$, and titrate with standard KMnO_4 solution. One milliliter of $0.1N$ potassium permanganate solution is equivalent to 2.0 mg. of calcium in calcium oxalate.

Calculate calcium as a percentage of the ash *and* as a percentage of the original tissue sampled. The calcium is present in the ash as the oxide and allowance may be made for the weight of the oxygen when calculating to percentages of ash. When determining the percentage of calcium in the original tissue, the calcium should be calculated as the element.

Silica Type.—Ash 2 to 10 gm. of wheat straw or *Equisetum* stems, cool, and weigh. Digest the ash in 10 ml. of hot $1 + 4\text{HCl}$, then evaporate to dryness, and heat for 2 hr. at 110 to 120°C . on a sand bath to dehydrate the silica and render it insoluble. Digest again with 50 ml. hot $1 + 9\text{HCl}$, filter through an ashless filter paper, wash with hot water, dry, ash the paper and residue in a tared crucible, and weigh the silica, with quantitative precautions in cooling and handling, to ± 0.0001 gm. Calculate silica (SiO_2) as a percentage of the ash and of the original sample.

Potassium Type.—Dry 50 to 100 gm. of washed slices of pared sugar beets or Irish potatoes and determine their moisture content. Grind the dry material and ash a 2- to 10-gm. sample, determining the weight of the ash. Loosen the ash from the dish with a drop or two of HCl , warm with a little water, breaking up the precipitate to hasten solution, and transfer ash and solution to a 100-ml. volumetric flask. Add an excess of NH_4OH and ammonium oxalate to the flask to precipitate iron, aluminum, phosphates, and calcium. Cool the flask, make to volume, mix thoroughly, and filter through a dry paper into a dry flask.

Evaporate a 75- or 80-ml. aliquot of the solution to dryness in a porcelain dish, add 1 ml. of $1 + 1\text{H}_2\text{SO}_4$, and ash to whiteness at full red heat. Dissolve the residue in 10 ml. of water; add a few drops of HCl and an excess of 2 per cent platinum

chloride solution. Evaporate to a thick paste and take up in 80 per cent alcohol (K_2PtCl_6 is slightly soluble in water but less so in alcohol), transfer to a weighed Gooch filter, wash thoroughly with 80 per cent alcohol, dry, and weigh as K_2PtCl_6 . Multiply by the factor 0.1938 to obtain K_2O or by 0.1608 to obtain K. Calculate percentage of K_2O in the ash and percentage of potassium in the dry matter and in the *green sample*.

Phosphorus Type.—Ash 4 to 20 gm. oven-dry wheat or ground corn and determine the percentage of ash. Dissolve the ash in $1 + 9HNO_3$, heat, and add an excess of molybdate solution.¹ Digest at 60 to 70°C. for an hour, add a few drops of molybdate solution to be sure no more precipitate forms, and filter onto a weighed Gooch crucible. Wash with cold water, dry at 100°C., and weigh with quantitative precautions as ammonium-phospho-molybdate, $(NH_4)_3PO_4(MoO_3)_{12}$. Multiply by the factor 0.01653 to obtain phosphorus or 0.03783 to obtain P_2O_5 . Calculate the percentage of P_2O_5 in the ash and of phosphorus in the dry sample.

Questions:

1. Do beets and potatoes have a high potassium content or a normal potassium content and low total ash?
2. Which will remove the more potassium from an acre, a 12-ton crop of sugar beets or a 2.5-ton crop of red clover hay?
3. What is the basis of the idea that phosphorus makes grain? Would you expect to be able to grow cabbage or alfalfa without phosphorus?

Experiment 38. The Nitrogen of Plant Tissue. (I)

Obtain fresh samples of stems, leaves, and seeds, or dried samples whose original green weight is known so that the percentage of nitrogen in the living tissue can be calculated. If fresh material is used, determine its moisture content by drying to constant weight at 100°C. Grind the material and weigh out duplicate samples for nitrogen determinations, using 0.250 gm. dry leaf or seed material and 1.000 gm. woody stem tissue.

Kjeldahl Digestion.—Transfer the weighed sample quantitatively to an 800-ml. Kjeldahl flask, and add 30 ml. concentrated H_2SO_4 in which has been dissolved 1.0 gm. salicylic acid. Allow

¹ Dissolve 25 gm. molybdic acid (MoO_3) in a mixture of 36 ml. NH_4OH and 68 ml. water. Stir this solution slowly into a mixture of 122 ml. HNO_3 and 287 ml. water, allow to stand in a warm place for several days, decant the clear liquid, and hold in a glass-stoppered bottle.

the acids to react with the cold sample for 30 min. with occasional rotation to bind nitrate nitrogen in organic combination. Then add 5 gm. sodium thiosulfate, rotate, and allow to stand for 5 min. Add 8 or 10 gm. Na_2SO_4 and a small crystal of CuSO_4 , and digest in a Kjeldahl digestion rack for 20 min. after a clear blue-green color is obtained. Use c.p. sucrose samples for blanks, and digest in the same manner.

Cool the flasks until sulfate crystals just begin to form in the liquid or until they can be touched with the bare hand, then add 200 ml. distilled water, dissolve all crystals, and allow to cool. Stopper the flasks if they are left overnight at this point.

Distillation of Ammonia.—Pipette 20 ml. 0.1N HCl into 500-ml. Erlenmeyer flasks, add a drop or two of methyl-red solution, and connect to the Kjeldahl still with the delivery tubes *just* dipping into the acid. Add 90 ml. of 40 per cent NaOH by weight *carefully* down the side of the flask. Do not wet the top of the neck or mix the solutions in the flask. Quickly add 2 to 5 gm. mossy zinc, and connect tightly to the still. If the *zinc* is omitted the sample may be lost.

With the flask tightly connected, rotate to *thoroughly* mix the acid and alkali, then (*never before* mixing) light the burner, and distill 200 ml. of liquid into the standard acid in approximately 1 hr. A dark blue color on mixing, turning black on heating, indicates a proper excess of NaOH solution. Watch the standard acid, and, if it turns from pink to yellow, indicating complete neutralization, add 10 ml. more standard acid at once and include in the calculation.

Calculation.—Titrate the residual acid in the receiving flasks with 0.05N NaOH. Subtract the experimental titrations from the blank (sugar) titrations. The difference D_t represents standard acid neutralized by the ammonia from the sample. The nitrogen in milligrams per sample is obtained with the equation:

$$N = D_t \times N_b \times 14$$

where D_t is the difference in milliliters between the blank and the sample, N_b is the normality of the base, and 14 is the molecular weight of nitrogen. From the weight of the sample used and its percentage of dry matter, calculate the nitrogen as a percentage of the green and dry weights of the tissue. For more

detailed description of the Kjeldahl method, see Methods in Plant Physiology, by Loomis and Shull.

Members of the class may use different materials and exchange data, or they may all use different parts of the same plant.

Questions:

1. What types of plant tissues are high in nitrogen? Why?
2. What is the objection to multiplying total nitrogen by a factor to obtain "crude protein"?
3. What happens to the nitrogen when corn stalks or other plant materials are burned? Does this constitute an important argument against burning?

Experiment 39. Microchemical Tests for Mineral Nutrients in Plants. (E-I)

Most of our knowledge of the form and location of minerals in plants must be obtained from microchemical data so that these experiments cover an important technique.

a. Iron.—Extract the chlorophyll from leaves with warm alcohol and test sections of the material with potassium ferrocyanide. Use only a *clean* razor blade in sectioning and handle sections with glass needles. Place sections on a slide in a 2 per cent solution of potassium ferrocyanide. After about 15 min. add a drop of 2 per cent HCl (free from iron). As soon as the acid has penetrated the tissue, wash with distilled water. A dark blue color indicates the presence of iron. If the tissue is acid, the blue color will appear before the addition of HCl. Where is the color localized? Repeat with a yellow leaf or a yellow portion of a variegated leaf.

To etiolated leaves and to the joints of cornstalks grown in "acid" soil, add first a few drops of a 10 per cent solution of potassium thiocyanate and then a few drops of 1 + 2HCl solution. A red color indicates iron. This is the test used by Hoffer to indicate potash starvation.

Questions:

1. Does the location of iron deposits suggest use or accumulation?
2. What data can you get from comparisons of young and old leaves that bear on this problem?

b. Nitrates.—Nitrates give a deep blue color with diphenylamine (0.1 gm. diphenylamine in 10 ml. of 75% H_2SO_4). The colored compound is soluble in water and fades on standing to a

yellowish brown, so that very rapid handling is required for satisfactory localization of the test.

Place carefully made sections on a dry slide, mount under low power, adjust the light carefully, and observe the localization of colored areas as the reagent is added from one side of the section. Compare weak and succulent, normal, and stunted plants of various kinds. Compare plants of the same sort grown in shade and sunshine.

Water tomato plants which show little or no nitrate with 0.5 per cent NaNO_3 and determine the time for nitrates to appear in

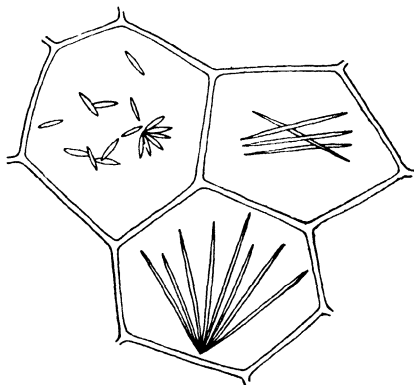


FIG. 12.—Calcium sulfate crystals.

the leaves under conditions favoring high and low transpiration rates.

Questions:

1. Do your results indicate that nitrates are normally present in quantity in plants making a good growth?
2. How do you explain the fact that Thomas has been unable to identify nitrates in the leaves, twigs, or fruit of apple?
3. Would you expect to find nitrates in maturing corn grain, or potato tubers? Why?

c. Calcium.—Test the leaves and stems of leguminous and non-leguminous plants and determine their relative calcium content. Compare young and old leaves.

1. *Calcium Sulfate Formation.*—Put sections of tissue on a slide and cover. Then place a drop of water at the side of the cover glass, add a drop of concentrated H_2SO_4 , and allow them to run under the cover glass together. Large needle crystals of calcium

sulfate (Fig. 12) will appear if calcium is present. Some of these go over into plate crystals. Twinning of the plates is frequent. If crystals do not form readily by this method, heat the tissue on the slide in 5 per cent H_2SO_4 and add a few drops of alcohol to the warm solution.

2. *Calcium Oxalate Formation*.—Put sections of tissue directly into hot 3 per cent ammonium oxalate on a slide, heat gently for a minute over a beaker of boiling water, and wash with water. Tetragonal crystals of calcium oxalate (Fig. 13) appear in the presence of calcium (one molecule water of crystallization). Or, put pieces of tissue into 3 per cent ammonium oxalate or oxalic acid. After 15 min. wash with water, harden in alcohol, section, and observe. Small tetragonal crystals of calcium oxalate (three molecules H_2O) appear. The crystals can be seen more clearly if the tissue is cleared in chloral hydrate.

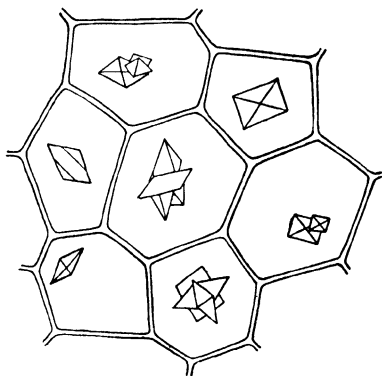


FIG. 13.—Calcium oxalate crystals.

Calcium oxalate crystals may accumulate in considerable quantities, particularly in succulent and evergreen (Angiosperm) leaves.

Questions:

1. Do you consider the calcium shown by microscopic tests to be primarily essential or excess calcium?

2. What type of evidence supports the contention that calcium is required to neutralize toxic accumulations of oxalic acid? Is it possible that the organic acids neutralize the calcium?

d. *Phosphates*.—Phosphates are demonstrated with difficulty in most plant material and tests for the localization of organic phosphorus are generally unsatisfactory. If phosphorus tests on the tissue sections are negative, ash a small quantity of the tissue and test the ash microchemically for phosphorus. Calcium phosphate accumulations may be identified by heating sections gently in a 1 per cent solution of cobalt nitrate. A blue pre-

precipitate indicates calcium phosphate, but the reaction should be checked by one of the phosphorus tests.

1. *Ammonium-magnesium-phosphate Crystal Formation.*—Treat sections with magnesium mixture.¹ If inorganic phosphorus is present, ammonium-magnesium-phosphate crystals are formed (Fig. 14). These crystals are insoluble in ammonia, but soluble in acids, even in acetic acid.

2. *Ammonium-phospho-molybdate Crystal Formation.*—Put sections on a slide in a solution of ammonium molybdate in HNO_3

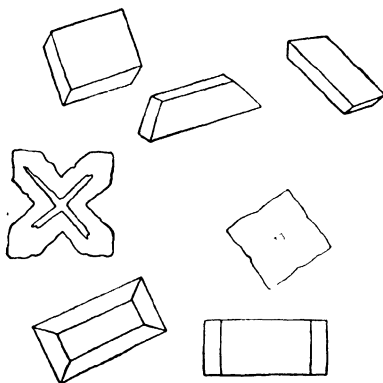


FIG. 14.—Ammonium-magnesium-phosphate crystals.

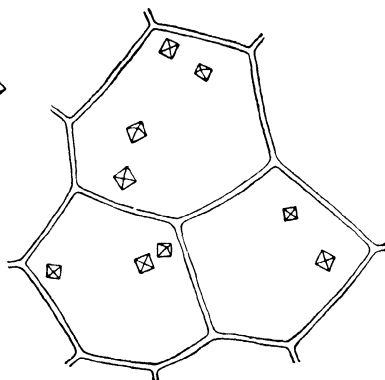


FIG. 15.—Potassium chlorplatinate crystals.

(1.0 gm. ammonium molybdate in 12 ml. HNO_3). If phosphorus is present, there appear at once small yellow drops with a black border, then spherocrystals, and later cubes and octahedrons. The crystals are soluble in excess of phosphoric acid, in ammonia and other alkalies, and in dilute mineral acids. Wash in dilute HCl and add a drop of one per cent phenylhydrazine hydrochloride; a blue-green color appears.

Question:

1. How do you account for the fact that phosphorus is demonstrated with such difficulty?

e. Potassium.—Place sections of the material directly in an aqueous solution (1 + 10) of platinum chloride. Yellow octahedral crystals of potassium chlorplatinate are formed if potassium is present (Fig. 15). The tissue should be neutral or only

¹ To prepare magnesium mixture, combine 25 ml. saturated water solution of MgSO_4 , 2 ml. saturated solution of NH_4Cl , and 15 ml. water.

slightly acid as acid increases the solubility of the crystals in water. Alcohol may be used as a precipitant. Compare tissues of plants making rapid and slow growths, and, if possible, test plants known to be deficient in potassium, making sections through the meristematic regions for comparison with other regions.

Questions :

1. Do your results indicate that potassium ion is relatively abundant?
2. What conclusions regarding the action of potassium can you draw from a study of the tissue in which the mineral is accumulated?

ESSENTIAL ELEMENTS

The determination of the macro-nutrients required by plants is relatively simple and either washed quartz sand or water cultures can be used. The determination of the necessary micro-nutrients is frequently a very difficult matter, requiring the most exacting attention to purity of water and nutrient salts, and the composition of the container. Most plants, for example, grow normally on the boron dissolved from the walls of pyrex culture vessels. Sand cultures are not ordinarily feasible for these experiments because of the impurities carried by the sand.

Water cultures have been considered to be an abnormal habitat for land plants, and the failure of many plant species to grow in these cultures has been assigned to an unfavorable root environment. Most of the trouble appears now to have been due to the omission of essential micro-nutrients or to a failure to buffer the solutions adequately against rapid changes in acidity and ion balance.

Experiment 40. The Growth of Plants in Nutrient Solutions.

(I)

a. Starting Plants.—Soak several times as many seeds as you wish to grow plants for 2 hr. in a 0.1 per cent Merko or Semesan solution. Spread the soaked seeds on a paraffined wire screen which just touches a weak nutrient solution or distilled water in a pan below the screen, and leave them uncovered in a moist chamber in diffuse light until the radicles have reached the water surface. Move the plants into full greenhouse light as soon as possible. Light is particularly important for cereal seedlings where a very short mesocotyl is necessary if the basal node of the

stem is to be placed below the cork of the culture jar where the nodal roots can develop normally. A 1 + 9 dilution of a full nutrient solution is a desirable starting medium, but distilled water should be used when the necessity of the various nutrients is to be tested. Large-seeded plants, particularly, may be started in clean moist sand in the bottom of a glass moist chamber held in strong light. Flood the sand with water as soon as the seeds germinate. Transfer plants to the permanent cultures as soon as they can be handled conveniently.

b. Knop Solution.—One of the simplest and most used solutions is the four-salt Knop solution. Make up five stock solutions as follows:

1. 5 gm. KCl in 1 l.
2. 5 gm. KH_2PO_4 in 1 l.
3. 5 gm. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 1 l.
4. 20 gm. $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ in 1 l.
5. 0.2 gm. FeCl_3 in 1 l.

Fifty milliliters of each of the stock solutions mixed together and made to 1 l. gives a Knop solution.

c. A Buffered Solution.—Knop solution is convenient to use, particularly when substituting the various nutrients, but is poorly buffered and must be renewed frequently. In the following solution¹ ammonium nitrate, ferric sulfate, and colloidal tricalcium phosphate are used to maintain a fairly uniform pH in a solution which need not be changed oftener than once a week, but should, for best results, be agitated daily or oftener to bring the colloid into better contact with the solution.

1. 4 gm. KNO_3 in 1 l.
2. 6 gm. NH_4NO_3 in 1 l.
3. 10 gm. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 1 l.
4. 4 gm. $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$ in 1 l.
5. $\left\{ \begin{array}{l} 10 \text{ gm. colloidal } \text{Ca}_3(\text{PO}_4)_2 \\ 5 \text{ gm. KCl} \end{array} \right\}$ in 1 l.

Mix 50 ml. of each of the first four solutions with 100 ml. of the phosphate suspension (No. 5) and make to 1 l.

The colloidal phosphate mixture is prepared as follows: Dissolve 13.69 gm. potassium phosphate (K_3PO_4) in 1 l. of water and

¹ ZINZADZÉ, C. Nutrition artificielle des plantes cultivées. I. Mélanges nutritifs à pH stable. *Ann. Agron.* 2: 809-853. 1932. See also *ibid.*, 3: 53-72. 1933.

10.73 gm. c.p. anhydrous CaCl_2 in a second liter. Pour the two solutions together with vigorous stirring to produce a finely divided colloidal suspension of $\text{Ca}_3(\text{PO}_4)_2$. If the supernatant liquid is not acid to phenolphthalein, add a few more drops of CaCl_2 solution to bring to neutrality. Reduce the KCl content of the mixture by allowing it to settle for a day or two in a graduated cylinder and decanting the clear KCl solution to leave a volume of 694 ml. Refill to 1 l., and use 100 ml. to contain 1.0 gm. $\text{Ca}_3(\text{PO}_4)_2$ and 0.5 gm. KCl. The suspension should be shaken thoroughly and poured rapidly to insure uniformity when measuring out the required 100 ml.

Zinzadze's solution is excellent for general work and is particularly good for plants such as corn (maize) which tend to become chlorotic in water cultures.

d. Shive Solution.—A three-salt solution, No. R5C2, developed by Shive,¹ has been extensively used. The Shive solution is essentially a concentrated modification of the Knop solution with only three salts used to carry the six macro-nutrients.

1. 49.01 gm. KH_2PO_4 in 1 l.
2. 24.56 gm. $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ in 1 l.
3. 73.95 gm. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 1 l.

Fifty milliliters of each of these stock solutions plus 4 or 5 mg. ferric phosphate and water to make 1 l. form a full nutrient solution.

e. Micro-nutrients.—With the exception of iron, the micro-nutrients are supplied in the seed and as impurities in ordinary c.p. salts in sufficient quantities to give a good growth with many plants. When repurified salts are used or certain plant species such as tomato and sunflower are grown, it becomes necessary to add micro-nutrients to obtain normal plant development. Since most of the elements in this group, including iron, are toxic when present in excess of requirements, they must be added carefully. Ten milliliters of the following stock solution in each liter of nutrient solution should be sufficient. Notice that the salts are chosen to contain no other nutrient ions such as NO_3^- or SO_4^{--} . The absence of these nutrients is convenient when testing for

¹ SHIVE, J. W. A study of physiological balance in nutrient media. *Physiol. Researches* 1: 327-397. 1915.

SHIVE, J. W. A three-salt nutrient solution for plants. *Am. Jour. Botany* 2: 157-160. 1915.

essential elements (Experiment 42). The solution is made as follows:

1. 0.10 gm. MnCl_2 , equivalent to 1 p.p.m. in the diluted nutrient solution.

2. 0.05 gm. ZnCl_2 , equivalent to 0.5 p.p.m.

3. 0.10 gm. H_3BO_3 , equivalent to 1 p.p.m.

4. 0.005 gm. CuCl_2 , equivalent to 0.05 p.p.m. Do not use more.

5. Water to make 1 l.

f. The Growth of Plants in Water Culture.—Grow an assigned plant species in water cultures. Start the plants as directed above and transplant into notched corks in 1- or 2-qt. Mason jars (Fig. 16). The jars may be wrapped in brown paper or set into brown paper sacks to reduce the growth of algae or, better, they may be given one coat of black and a second coat of white paint or two coats of aluminum paint, which both reduce light and reflect heat so that the solutions do not become too warm when held in direct sunlight. Use four or five small plants or two or three larger plants in each culture and grow duplicate or triplicate cultures in each solution.

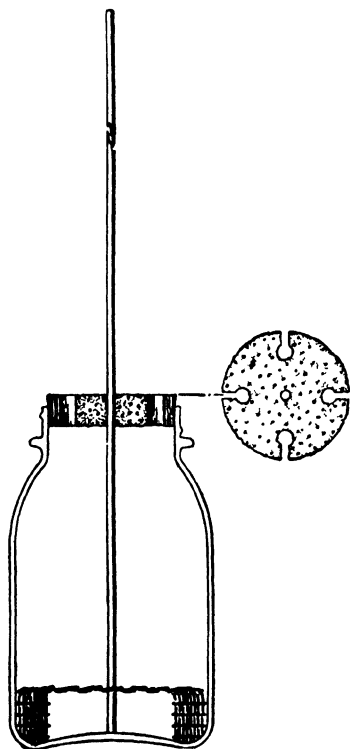


FIG. 16.—Flask for growing plants in water culture. The bent iron screen in the bottom of the jar and the heavy wire support should be dipped in hot paraffin. The cork is cut to permit the rapid insertion of seedling plants, which are held in place with cotton.

Compare the growth of your plants in Shive or double-strength Knop solution alone and in the same solution plus 5 or 10 gm. of powdered animal charcoal, with their growth in the $\text{Ca}_3(\text{PO}_4)_2$ buffered solution. Add the micro-nutrient solution to any cultures not known to develop without it. Add distilled water as needed to replace transpiration loss, and add 1.0 to 5.0 ml. of freshly made 0.05 per cent ferric tartrate solution to each culture

daily. Careful attention to the iron supply makes the difference between success and failure in water-culture work. Do not wait until the plants are already chlorotic before adding iron, and do not use iron solutions decomposed by standing. Change the solutions first after 3 weeks, then after 2 weeks, and thereafter at 1-week intervals, for a total of 6 or more weeks.

Measure the length and obtain the green and dry weights of roots and tops, and take notes on color, freedom from disease, and other characteristics of the plants at the end of the experiment.

Questions:

1. Would you expect a simple or a more complex nutrient solution to be most generally satisfactory? Why?
2. Why do charcoal, silica gel, and colloidal calcium phosphate act as buffers?
3. Why is organic matter recommended for alkali and other toxic soil conditions?

Experiment 41. The Adaptation of Roots to Growth in Nutrient Solution. (I)

Start several seedlings of corn in moist sand and a second lot in water according to the outline above. A week to 10 days after the seeds have germinated, wash some of the plants from the sand and set up in a buffered nutrient solution in comparison with the plants started in water. Observe the behavior of the transplanted and of new roots. Section roots grown in sand using pith and a sharp razor blade to make thin sections, and compare their cortical structure with that of similar roots grown in solution. If time permits, determine whether the corn roots from the sand will survive in continuously aerated culture, and test the ability of water-culture roots to survive even when manure extract (25 to 50 ml.) is added daily to maintain a continuous fermentation in the solution. For the last test the plants may be grown in 1-l. Erlenmeyer flasks plugged with cotton and grafting wax to reduce aeration to a minimum. The experiments may be repeated with rice, comparing it with corn, particularly in its resistance to transfer from sand to water.

Questions:

1. How are the roots of corn adapted to growth in water or flooded soil? Study a root starting above and growing into water to see if the adaptation is local.

2. Why can corn be grown in water cultures when it is killed by standing water in the field?

Experiment 42. The Essential Elements. (E-I)

Working with another group or with the entire class, determine the necessity of the different nutrient elements for the growth of some plant. Wheat, barley, oats, buckwheat, and sunflower are well adapted to water-culture work. Corn, soybeans, and tomatoes may also be used. Corn and soybeans have large seed reserves which delay the appearance of phosphorus-, sulfur-, and magnesium-deficiency symptoms. The small seeds of



FIG. 17.—The effect of boron and iron on the growth of tomatoes in water culture. Left to right: minus iron and boron; minus iron; minus boron; complete nutrient.

tomatoes and their high boron requirement (Fig. 17) make them a desirable water-culture plant although the seedlings are somewhat difficult to start. Sunflower also has a high boron requirement and is a very satisfactory plant for general use. Buckwheat shows the effects of omitted macro-nutrients quickly.

Set up the following nutrient solutions in 1-qt. Mason jars, painted or wrapped in brown paper, and covered with corks which have been dipped in melted paraffin and then, after cooling, into a suspension of 5 gm. of an organic mercury dust such as Merko in 100 ml. ethyl alcohol. This dip will leave the corks coated with a covering of the organic mercury, which will retard seedling diseases of various sorts. The jars from which "other elements" are omitted should be lined with paraffin to prevent

solution of zinc and other elements in the glass. Clean and dry these jars carefully and add 30 to 50 gm. hard paraffin. Seal the jars tightly and roll them in boiling water until the inside of the jar is uniformly covered with a closely adhering coat of paraffin. Use four uniform vigorous wheat or other small seedlings, or two or three larger plants in each culture, and set up the experiment in a cool greenhouse in such a manner that it is protected from cockroaches and mice

Jar	Solution
1a and b	Distilled water
2a and b	Full nutrient solution
3a and b	Minus nitrogen
4a and b	Minus phosphorus
5a and b	Minus potassium
6a and b	Minus calcium
7a and b	Minus magnesium
8a and b	Minus sulfur
9a and b	Minus iron
10a and b	Minus other elements

For this experiment use a modified Knop solution made up in six parts which are combined in equal proportions and diluted with water to form a full nutrient solution. All the nutrients should be dissolved in carefully distilled water and only distilled water should be added to replace transpiration losses. Gentle heating will be required to dissolve the ferric tartrate, but boiling, which will hasten hydrolysis of the salt, should be avoided.

Stock solution	Salt	Grams per liter
1	KCl	6 00
2	MgSO ₄ 7H ₂ O	9 00
3	Ca(NO ₃) ₂ 4H ₂ O	10 00
4	KH ₂ PO ₄	3 00
5	Ferric tartrate	0 50
6	"Other elements" ¹	

¹ Use 10 ml. of the concentrated solution of Experiment 40 and 40 ml. of water

Fifty milliliters of each of these solutions plus 700 ml. distilled water makes 1 l. of full nutrient solution.

Substitution solutions should be made up for each element to be omitted, to supply the other element of the salt and maintain the concentration of the culture.

Element substituted	Salt	Grams per liter
1. Nitrogen.....	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ for $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	7 28 ¹
2. Phosphorus.....	KCl for KH_2PO_4	1.64
3. Potassium.....	NaCl for KCl, and $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ for KH_2PO_4	4.71 3 04
4. Calcium.....	NaNO_3 for $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	7 19
5. Magnesium.....	$\text{Na}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ for $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	9 79
6. Sulfur.....	$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ for $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	7 42
7. Iron.....	Distilled water	
8. Other elements	Distilled water	

¹ Because of its low solubility, it is necessary to make up a solution of 1.82 gm. $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ per liter and to use 200 ml. of this solution to replace 50 ml. $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, at the same time omitting 150 ml. water.

The substituted salts are added in concentrations equimolar with those displaced except that NaNO_3 is used in equivalent concentrations.

Keep a record of the transpiration of each culture by watering at 2-day intervals with distilled water from a graduated cylinder and recording the water required to fill each jar. One- to five-ml. portions of a fresh 0.05 per cent iron tartrate solution should be added daily to all but cultures 1 and 9 to maintain a normal green color. Include the volume of the iron solution in the transpiration record.

All solutions should be renewed after 15 to 20 days, and then at 7- to 10-day intervals until the experiment is taken down after 4 to 8 weeks.

Record the lengths and the green and dry weights of roots and tops for each of the treatments, and take careful notes on the growth and appearance of the plants, recording any apparent symptoms of nutrient deficiency. From the combined dry weights of roots and tops and the total transpiration per culture, calculate the transpiration ratio for each treatment.

Questions:

1. How do you explain the fact that some of the elements appear to be "more essential" than others?
2. How should this experiment be modified to test the necessity of zinc for plant growth?
3. What relationship do you find between mineral nutrition and transpiration ratio?

Experiment 43. Antagonism. (E-I)

Solutions of single salts are commonly toxic to plants, but the toxicity may be reduced by using suitable combinations of

salts. Test the action of calcium in antagonizing sodium by growing duplicate lots of wheat or other seedlings in each of the following solutions. All jars should be carefully lined with paraffin and only redistilled, conductivity water handled in paraffin lined bottles used in making up the solutions and replacing transpiration loss. Protect all paraffin lined vessels from heat or *direct sunlight*.

Number	Solution
1	. Redistilled water. ¹
2	50 ml. 0.76M NaCl (mol. wt. 58.5) and 900 ml. redistilled water.
3.	50 ml. 0.19M CaCl ₂ (mol. wt. 111.0) and 900 ml. redistilled water.
4	. 50 ml. 0.76 M NaCl + 50 ml. 0.19M CaCl ₂ + 850 ml. water.

Harvest the plants when some of them begin to show signs of serious distress, recording the following data: (1) condition of roots and tops, particularly any evidences of toxicity; (2) length of tops and roots; (3) green and dry weights with roots and tops of plants recorded separately.

Questions:

1. Calculate the molar concentrations of the two salts used at final dilution.
2. What theories have been advanced in explanation of antagonism?²

Experiment 44. Buffering. (I)

Buffering capacity has been stressed as a characteristic of a good culture medium. By buffering we mean a resistance to change, and more specifically to change in active acidity or pH. Organic acids, acid salts, and colloidal substances are the most important biological buffers

Titrate 10 ml. of 0.1N HCl with 0.1N NaOH using methyl red as an indicator. Observe the end point or color change of the indicator carefully. Titrate 10 ml. of 0.1N citric, acetic, or other organic acid in the same way, observing the color change of the methyl red indicator. Where is buffering apparent in the organic acid titration?

The buffering action of a good garden loam or compost soil can be demonstrated by shaking 20 gm. moist soil with 50 ml. of freshly boiled distilled water, centrifuging a portion of the

¹ See Loomis and Shull, *Methods in Plant Physiology*, for directions for preparing conductivity water.

² SKEEN, JOHN R. Experiments with *Trianea* on antagonism and absorption. *Plant Physiol.* 5: 105-118. 1930.

solution and determining its pH. Mix 10 ml. of 0.1*N* HCl with 40 ml. boiled distilled water and determine the pH of the solution before and after shaking with 20 gm. of the soil. What is the effect of the soil? Repeat using 10 ml. 0.1*N* NaOH with 40 ml. H₂O and 20 gm. soil. The initial pH of the alkali should be about 12, which is beyond the range of the quinhydrone electrode, so that a glass or hydrogen electrode or colorimetric comparison must be used.

Determine the pH of Knop solution, and compare with the pH of a mixture of 40 ml. Knop solution and 10 ml. *N*/10 HCl. Is the solution well buffered? Repeat with Zinzadze's buffered solution.

Questions :

1. What indicator and procedure would you use for determining total acidity of a plant sap?
2. Why are sandy soils more sensitive to improper fertilization than loam soils?
3. How can a water-culture medium be buffered?

CHAPTER V

THE ROLE OF DIFFUSION IN PLANTS

INTRODUCTION

The part played by diffusion in the absorption of water, soil nutrients, and carbon dioxide, in the reaction of these materials within the plant, and in their movement throughout the plant, is so important that an understanding of this physical phenomenon is essential to all students of plant physiology. In general terms, diffusion is the tendency of any substance to distribute itself uniformly throughout the available space. Available space and rates of diffusion will obviously be very different for carbon dissolved in a steel bar, sugar dissolved in a cup of tea, or carbon dioxide dissolved in the atmosphere, but the same principles of diffusion may be applied to all.

a. Materials diffuse from regions of higher activity of the diffusing substance to regions of lower activity. The activity of a substance will depend upon the number of particles in unit volume, *i.e.*, upon its concentration, but it will depend also upon the temperature, the pressure, and upon any intermolecular magnetic or adsorption forces which interfere with the free movement of the diffusing particles. Dye will diffuse from a solution into a cloth, or phosphorus from a dilute soil solution into a root, because the activity or effective concentration of the diffusing substance is greatest in the solution, although the total or apparent concentration may be greater in the cloth or root.

b. The rate of diffusion is proportional to the activity gradient of the diffusing substance. Different materials in varying environments have their own characteristic diffusion speeds, but for any given condition and substance the rate is directly proportional to the activity difference, and inversely proportional to the distance which separates the two levels. In other words, materials diffuse down hill, from the high to the low molecular or ionic activity, at a rate which is proportional to the steepness of the hill.

c. Materials diffuse independently. Sugar dissolved in water diffuses independently of the water and at the same time the water diffuses independently of the sugar. Even the ions of a salt may diffuse separately, provided the electrical charges of both are balanced by some other movement.

In the experiments which follow, you will study three phases of diffusion particularly applicable to plants: (1) the diffusion of substances (solutes) dissolved in water, (2) the diffusion of water itself, and the measurement of water concentrations by the freezing-point method, and (3) the effects of membranes on diffusion. The term osmosis as applied to diffusion through membranes is not used. Although both relative and absolute diffusion rates may be changed by the properties of a given membrane, if the material diffuses at all the laws of diffusion still hold and most students find it simpler to retain the general term, and to use the general concepts of diffusion.

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GORTNER, R. A. Outlines of biochemistry. Chapt. XI. 2d ed. New York. 1938. A discussion of the special case of equilibrium across a membrane known as Donnan equilibrium.

THE DIFFUSION OF SOLUTES

Experiment 45. Diffusion in a Gel. (E)

The diffusion of a dissolved material can be observed most accurately if it is allowed to move through a gel where it will not be subject to convection currents or accidental stirring.

Completely dissolve 3.2 gm. agar in 200 ml. boiling water and when partially cooled add 20 drops of a methyl red solution and a few drops of 0.1N NaOH if necessary to give a neutral (yellow) color. Fill eight test tubes three-fourths full with the agar mixture and allow to harden. Cover the agar with 4-ml. portions of the solutions as indicated, stopper the tubes tightly, hold in a cool place (20°C.), and record the diffusion of the various solutes

in millimeters per day. The top of the gel should be marked before the solutions are added to facilitate measurements, and where two color lines are apparent diffusion records should be taken on both.

Tubes	Solution
1 <i>a</i> and <i>b</i>	4 ml. 0.2 per cent methylene blue
2 <i>a</i> and <i>b</i>	4 ml. 0.4 per cent methylene blue
3 <i>a</i> and <i>b</i>	4 ml. 0.1 <i>N</i> HCl
4 <i>a</i> and <i>b</i>	2 ml. 0.2 <i>N</i> HCl and 2 ml. 0.4 per cent methylene blue

Make up the methylene blue solution to 0.4 per cent and use with equal parts of water for tubes 1*a* and 1*b*. Note that tubes 4*a* and 4*b* contain both 0.2 per cent methylene blue and 0.1*N* HCl, and compare the movement of these materials diffusing together, with their respective concentrations acting alone. If the diffusing acid does not give a clearly visible red color as it changes the methyl red indicator, use more indicator in a fresh lot of agar.

Questions :

1. What evidence do you have of varving particle size in the materials used?
2. What is the relation between concentration and diffusion of methylene blue?
3. How could you demonstrate that materials diffuse independently?

Experiment 46. Precipitation and Diffusion. (E)

Carefully prepare two moderately permeable celloidin tubes¹ approximately 2.5 by 10 cm. in size, fill one tube three-fourths

¹ Dry celloidin in air and dissolve 10 gm. of the material in 200 ml. of equal parts of absolute alcohol and anhydrous ether to make a solution the consistency of maple sirup. One or more days with occasional stirring will be required to dissolve the celloidin. Pour 5 to 10 ml. of the solution into a clean dry tube of about 25 mm. diameter and preferably not over 12 cm. deep; tilt the tube and spin carefully to obtain an even coating of the celloidin. Return excess solution to the stock bottle. If the solution does not spread readily, thin with ether and alcohol. Spread the material thinly enough on the tube walls so that it sets quickly and uniformly with a little blowing to remove ether fumes. The tube must be turned constantly during this setting process to insure a uniform wall thickness. Remove most of the ether with a current of air blown carefully into the bottom of the tube, then fill with water and allow to stand for a few minutes. If too much ether is left in the celloidin, it will turn white and hard in the water; if too much alcohol is evaporated out, the membrane will be very slowly permeable.

full with water and the other with a 3 or 4 per cent starch paste and immerse them in 50-ml. portions of 0.01 per cent iodine solution in potassium iodide (Fig. 18). Tall bottles or vials which can be stoppered tightly with paraffined corks to prevent evaporation of the iodine should be used. Observe the tubes at frequent intervals for 48 hr. or longer. Test the external solution around the starch-filled tube for iodine, using starch paste. Observe the color of the solution within the water-filled tube and compare with the external color. If the celloidin tube is dried too long

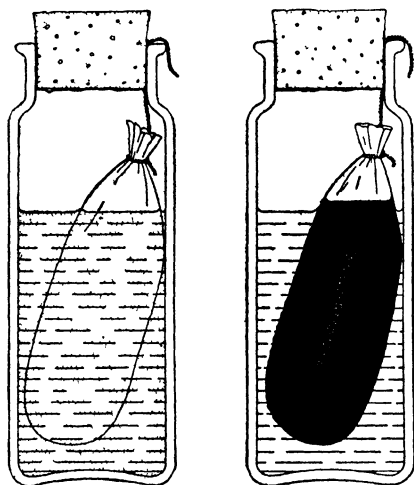


FIG. 18.—Precipitation and diffusion. The celloidin tube in the bottle at the left was filled with water and that in the bottle at the right with starch paste. Both bottles contained dilute I-KI solution.

in making, you may find that you do not obtain a good equilibrium across the membrane because of its low permeability.

Questions :

1. Explain your results in terms of diffusion equilibria.
2. What analogies can you draw between this experiment and the absorption of nitrate and chlorin ions by plants?

Experiment 47. Solubility and Diffusion. (E)

Pour the following liquids very carefully into a test tube to the depth and in the order given: (1) chloroform—30 mm., (2)

Loosen the membrane from the tube with a blunt instrument and hold under water until used.

water—5 mm., (3) ether—30 mm. Three layers should be formed, and the ether must be added carefully to avoid disturbing the water. Stopper the tubes tightly with a cork which has been dipped in hot paraffin; mark the position of the three layers and measure their thickness. Set aside and record the thickness of the different layers at intervals. Look up the solubility of ether and chloroform in water and in each other.

Questions:

1. Explain the factors and processes involved.
2. What relation does this experiment have to diffusion in plants?

Experiment 48. The Cause of Diffusion.¹ (E)

Observe Brownian movement by mixing a little India ink into a drop of water and mounting under high power or oil-immersion lens with reduced light, or better, with dark-field illumination. If possible, trace the movement of some of the diffusing particles. Remember that you are observing a substance (carbon) in the colloidal state, a form in which the particle movement is so slow that it has been called nondiffusing.

Questions:

1. What keeps the carbon particles in constant motion?
2. Why would a motion of this type cause diffusion from a region of higher to a region of lower activity?

THE DIFFUSION OF WATER

The viewpoint of the chemist that water is something in which to dissolve his chemicals is inadequate in physiology where water may be the most important ingredient in the solution. The concept of water as a solute as well as a solvent, with its own molecular activity and its own diffusion gradients is essential to an understanding of the diffusion phenomena of living cells.

Experiment 49. The Dilution of Water. (E)

Fill a moderately to slowly permeable diffusion tube (see Experiment 46 for directions for making) with molar (34.2 per cent) sucrose solution colored with safranin-O and tie very care-

¹ A good demonstration of diffusion for an elementary class can be obtained with a simple ultramicroscope filled with cigarette or other smoke; Central Scientific Company's Brownian movement apparatus is convenient and inexpensive.

fully to a one-hole rubber stopper fitted with a capillary tube 60 cm. or more in length (Fig. 19). Immerse the membrane in water and measure inward diffusion of water by the rate of rise of the solution in the capillary tube, which should be 2 cm. or more a minute. Transfer the tube from the water to a 3*M* (17.5 per cent) sodium chloride solution and again measure direction and rate of diffusion of water. Why does water diffuse from pure water into a water-sugar solution? Which dilutes water more rapidly, sucrose or salt? Why? To show that sucrose does dilute water, place 10 ml. water in a 25-ml. graduated cylinder, add 3.4 gm. of sucrose, dissolve, and observe the volume of the solution.¹



FIG. 19.—
Celloidin
membrane
and capillary
tube for dem-
onstrating
the diffusion
of water.

Immerse the tube in 50 per cent by volume ethyl alcohol and record the *initial* rate and direction of water movement. Alcohol penetrates celloidin rapidly and, if a readily permeable membrane is used, the initial fall of water in the capillary tube may be quickly reversed by the inward diffusion of alcohol. Keep the tube in alcohol for a short time only, and if a rapid outward diffusion of water is not obtained use a harder membrane. Determine the density (specific gravity) of your sugar and alcohol solutions and define the diffusion of water between them in terms of density. Do you get the definition usually given? Why?

Observe any diffusion of the safranin-O dye. Also observe the osmometer while it is immersed in water, by holding it against the light. Sugar diffusing outward has a high specific gravity and settles to the bottom of the vessel. Its presence may be detected by a change in the refractive index of the water which makes the sugar solution visible.

In what direction is the sucrose diffusing when water is diffusing inward through the membrane?

¹ The apparent dilution of water, as measured by change in volume, has little quantitative relation to the molecular activity of water within the solution. The demonstration will, however, help to fix the idea that *water* may be *diluted* by a dissolved solid.

Questions :

1. Under what conditions is the definition, "Osmosis is the diffusion of water from a less dense to a more dense solution," correct?
2. Formulate a definition which will hold for all conditions and substances.
3. Place the solutions which you have used in the order of their water concentration or activity.

Experiment 50. Vapor Tension and Diffusion. (E-I)

Fill three containers with the following solutions, weigh carefully, place them under a small sealed bell jar with minimum free area, and hold for several weeks at constant temperature.

Number	Solution	Fill container
1	4M NaCl (mol. wt. 58.45)	$\frac{1}{2}$ full
2	0.5M NaCl	$\frac{3}{4}$ full
3	Distilled H ₂ O	$\frac{3}{4}$ full

Make final weighings and determine the increase or decrease in the weight of the solution contained in each vessel.

Questions :

1. Explain any changes in the weights of the vessels.
2. Why does a nonvolatile solute lower the vapor tension of water?

Experiment 51. Pfeffer's Membrane. (E)

Fill a test tube with 0.5M CuSO₄·5H₂O solution (mol. wt. 249.71). With a pipette, very carefully introduce a few drops of 0.5M potassium ferrocyanide (mol. wt. 422.33) beneath the surface of the copper solution. A globule consisting of the ferrocyanide solution surrounded by a gelatinous membrane of copper ferrocyanide (Pfeffer's membrane) should form. Hold the tube against the light and observe the changes occurring at the surface of the membrane. Explain! Allow to stand for several hours and observe.

Questions :

1. Explain the behavior of the globule.
2. Why does water diffuse between solutions of equimolar concentrations? (How do the two salts ionize?)

Experiment 52. The Diffusion of Water and the Gas Laws. (I)

While water tends to diffuse from its own high to all lower concentrations, it is possible to increase the tendency of water

molecules to diffuse by applying mechanical pressure. Such pressure increases the activity or diffusion tension of the water molecules without appreciably changing their concentration. If pure water is placed outside a differentially permeable membrane and, for example, a sucrose solution within, the tendency of water to diffuse inward on a concentration gradient can be balanced by applying pressure to the solution until the lower concentration of water inside the membrane has the same *activity* as the higher concentration outside. This balancing pressure is termed the equilibrium osmotic pressure for the solution used. Obviously the equilibrium pressure is a function of the reduced activity of water in the uncompressed solution. The more the activity of water is reduced by added sucrose, or other solute, the higher will be the pressure required to restore diffusion equilibrium.

When the copper ferrocyanide compound formed in Experiment 51 is precipitated within the walls of a porous clay vessel, it forms the membrane which has been most used in quantitative experiments on the diffusion of water and the potential ability of such diffusion to develop an osmotic pressure. Two laboratory groups working together may prepare such a membrane as follows:¹

Obtain a new, unshellacked, cell-type Livingston atmometer; dry in the oven, cover with warm 0.5*M* CuSO₄ solution in a beaker, place the beaker in a vacuum desiccator, and alternately evacuate and release to fill the pores of the cell with the solution. Hold in the CuSO₄ solution until used.

Prepare a mercury manometer of the reservoir type (Fig. 20) using 6-mm. glass tubing and fitting the stoppers carefully to withstand pressures of two atmospheres. Remove the atmometer cell from the copper sulfate, quickly wipe dry on the inside with a clean cloth, and fill with the designated ferrocyanide mixture. A membrane will be formed just at the inner surface of the cell and the presence of the two solutions will make it self-repairing. Wrap a cloth or rubber dam about the top of the cell to prevent spilling ferrocyanide on the outside and insert the stopper and capillary connecting tube with a minimum of air. Dip the cell into the CuSO₄ solution occasionally to keep it wet.

¹ LOOMIS, W. E. A quantitative demonstration of osmotic equilibrium. *Plant Physiol.* 6: 365-367. 1931.

Cut four to six pieces of medium-size (No. 16) copper wire 10 or 15 cm. long, bend them down over the stopper, and tie the ends under the shoulder of the cell. Tighten the wires with

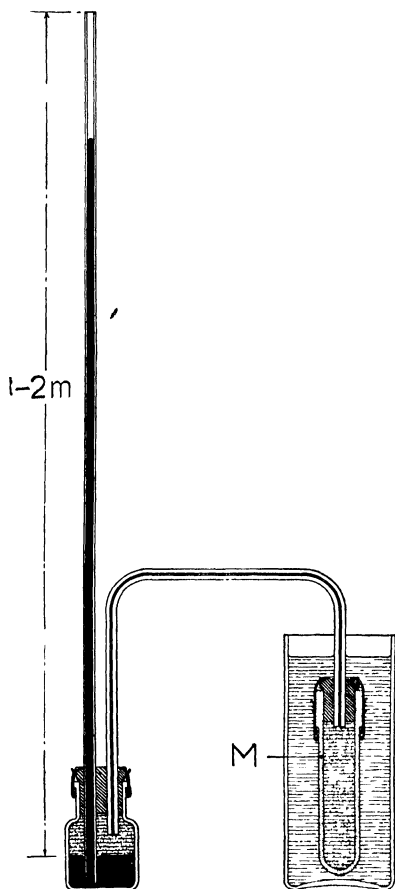


FIG. 20. Osmometer for the quantitative measurement of diffusion tension. (See text for description.)

pliers and bend the ends up to hold in place. The greatest care in wiring is necessary to prevent leaks under high pressure.

Place a small quantity of mercury in the bottom of the manometer reservoir, fill the reservoir with water and wire in the stopper as above. Cover the cell with 0.5M CuSO_4 solution and add mercury to the manometer until equilibrium is estab-

lished. Avoid any considerable rise or fall of the manometer which involves a change in concentration of the solution being measured. Leaks developing around the stoppers may be checked by tightening the wiring. If the ferrocyanide solution is forced through the cell and forms a membrane on the outside, start over with a new atmometer cup.

The following solutions may be used by the class. Solutions stronger than $0.05M$ require considerable skill in wiring to prevent leaks. The solute to be tested should be weighed to 0.001 gm. and dissolved with the ferrocyanide so that the solution made to volume (500 ml.) is standard for both solute and ferrocyanide.

1. $0.1M$ sucrose (342.17) and $0.25M$ potassium ferrocyanide.
2. $0.05M$ sucrose and $0.25M$ ferrocyanide.
3. $0.03M$ sucrose and $0.25M$ ferrocyanide.
4. $0.25M$ ferrocyanide alone as a check on the equilibrium between the two salt solutions.

Questions:

1. Calculate the percentage of the total sucrose diffused outward in 2 weeks. (How determined?)
2. Calculate the osmotic value of the solution used from van't Hoff's equation:

$$P = \frac{nRT}{V}$$

where P is the potential pressure in atmospheres, n is the mols of solute, R is the gas constant, 0.082 , T is the absolute temperature, and V is the volume in liters (in which n is contained). How do the calculated agree with the observed pressures?

Experiment 53. Measuring the Concentration of Water. (I)

At the freezing point liquid water is in equilibrium with ice and the vapor tensions of the two substances are equal. If the water is diluted with alcohol or sugar, its vapor tension is lowered and the freezing point is shifted to a new equilibrium at a lower temperature where the vapor tension of pure ice is again at equilibrium with the lowered vapor tension of the water in the solution. The freezing point of water solutions is thus a direct and easily utilized measure of the effective concentration or activity of water, and it is the method most frequently utilized for determining the water concentration and thus the osmotic value or potential osmotic pressure of biological fluids.

Accurate measurements of the freezing point of water solutions may be made with the Beckmann thermometer, which is a large thermometer graduated usually in $0.01^{\circ}\text{C}.$ over a range of about $5^{\circ}\text{C}.$ and so made that its readings can be adjusted by varying the mercury content of the thermometer. Adjust a thermometer so that the freezing point of water comes near the top of the scale and standardize the instrument by making three determinations of the freezing point of distilled water which agree within $0.005^{\circ}\text{C}.$ Handle the standardized thermometer very carefully; avoid jarring, and, when it is not in use, rinse, dry, and stand vertically in a dry test tube padded with cotton. Keeping the thermometer cool, by immersing the containing tube in cracked ice, will reduce the chances of disturbing the standardization and will permit more rapid freezing-point determinations.

The sample to be frozen is placed in a test tube which is enough larger than the thermometer bulb to permit the sample to be stirred with a looped wire while it is being frozen. The sample tube is held in a larger tube (Fig. 21), which serves as an air jacket to insure slow and uniform cooling of the sample. The air jacket is immersed in a freezing mixture of salt and chipped ice or snow which should have a temperature of about $-10^{\circ}\text{C}.$ If a Beckmann thermometer is not available, approximate freezing-point determinations can be made with a good $\frac{1}{10}$ -degree thermometer and the same type of freezing bath and air jacket.

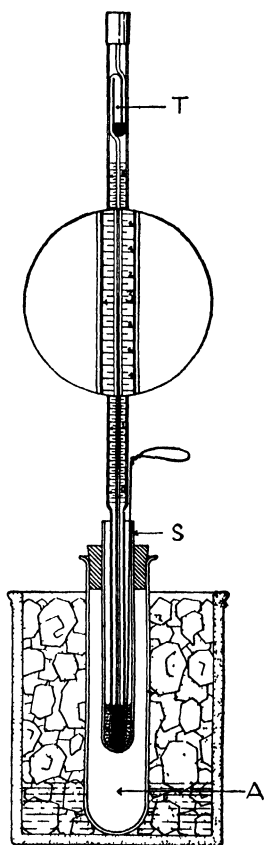


FIG. 21.—Simple freezing-point apparatus. *T*, mercury trap of the thermometer; *S*, sample tube; *A*, air jacket, to insure uniform cooling of the sample.

Determinations may be made more rapidly if the samples are precooled by setting the tubes into cracked ice and water (no salt). Transfer the cooled tube to the air jacket in the bath;

insert the thermometer, *cleaned, dried, and precooled*, and stir steadily until the determination is complete. The temperature should fall at a uniform rate until it is below the freezing point and then rise quickly to this value as ice formation begins. Watch the thermometer carefully, and record the lowest temperature reached and the highest temperature obtained, with vigorous stirring, after freezing starts. The difference between these is the amount of undercooling u in the equation below. The difference between the high reading and the freezing point of pure water is the apparent freezing-point depression or d . Since some of the water is frozen out in warming the undercooled solution, thus increasing its solute concentration, the apparent freezing-point depression d is larger than the true depression D which is calculated with the equation:

$$D = d - \frac{du}{80}$$

where D is the true depression, d is the observed depression, and u is the undercooling (all in degrees centigrade).

Determine the freezing point of 0.25*M* or 0.5*M* sucrose and of an unknown solution furnished by the instructor. Keep a record of the undercooling and correct the apparent freezing-point lowering for the undercooling error.

Calculate the *osmotic value* or maximum osmotic pressure which could be developed by the solution under ideal conditions:

$$P = \frac{D \times 24.0}{1.86}$$

where P is osmotic value in atmospheres at 20°C. Since D is a direct measure of the dilution of water by dissolved substances, many values, such as (a) the vapor tension of the solution, (b) the molecular weight of a nonelectrolyte, (c) the dissociation of an electrolyte of known molecular weight, etc., as well as the osmotic value of the solution, can be calculated from it.

Calculate the molar fraction or molecular percentage concentration of water with the equation:

$$W = 1 - \frac{D/1.86}{55.5 + \frac{D}{1.86}}$$

where W is the molar fraction of water and D is the corrected freezing-point depression.

Questions:

1. How do you account for the fact that 34.2 gm. sucrose in 100 ml. water gives a water "concentration" (molar fraction) of 98 + per cent?
2. Why does lowering the vapor tension of water lower its freezing point?
3. Why is freezing-point depression the most commonly used method of determining osmotic value?

Experiment 54. The Freezing Point of Plant Sap. (I)

The principal difficulties in determining the freezing point, and thus the osmotic value of plant sap, arise in the expression of representative samples from the tissues.¹ To compare methods of expression as well as to make actual freezing-point determinations, collect 150 gm. of some plant material, protecting it carefully against evaporation which would raise the osmotic value of the remaining sap. Divide the sample into three lots of uniform material, one of about 20 gm. and two of 50 to 60 gm. Express the sap according to the following outline and tabulate the data obtained by other members of the class for comparison with your own. If necessary, several groups or the entire class may work together on one material, dividing up the different experiments.

a. Rapidly grind the small sample in a mortar until a smooth pulp is formed; then transfer to stoppered test tubes and cool in ice water. Make determinations of the freezing point of two lots of this pulp, remove the thermometer, freeze the pulp in salt and ice for one-half hour, thaw, and redetermine the freezing point as affected by the freezing treatment.

b. Transfer 30 to 60 gm. fresh tissue directly to the cylinder of a hydraulic press and express the sap, collecting it in three samples: (a) the first 3 to 4 ml., (b) the main sample, (c) the last 3 to 4 ml. that can be expressed. Determine the freezing point of each of the fractions separately and compare.

c. Enclose the third sample in a tightly stoppered bottle with inserted thermometer and heat in a 90°C. water bath until the temperature at the center of the bottle reaches 80°C.; then

¹ NEWTON, R., W. R. BROWN, and W. M. MARTIN. The extraction of plant tissue fluids and their utility in physiological studies. *Plant Physiol.*, 1: 57-65. 1926.

cool and express as above. Calculate the molar fraction of water and the osmotic value of each sample. If time permits, compare killing by freezing in a salt and ice mixture with killing by heating.

Questions:

1. Why does the osmotic value of plant sap vary with the pressure used in expressing the sample?
2. Why does killing the tissue before pressing commonly lower the freezing point of the expressed sap?
3. On the basis of effectiveness and convenience, formulate a procedure for expressing representative sap from the tissue you used.

Experiment 55. Isotonic Solutions. (E-I)

The osmotic value of the sap of individual plant cells may be determined with considerable accuracy by the plasmolytic method. The solution of known osmotic value (o.v.) which is just in equilibrium with the cell solution is known as an isotonic solution.

Using cells of *Elodea* or of the epidermis of red onion, or, preferably, the lower epidermis from the *midrib* of *Zebrina pendula*, determine the molar concentration of a sucrose and of an electrolyte solution isotonic with the cell sap. Calculate the apparent percentage ionization of the electrolyte used from the equation:

$$\alpha = 100 \times \frac{\frac{M_s}{M_e} - 1}{n - 1}$$

where M_s is the molality of isotonic sucrose, M_e is the molality of the isotonic electrolyte, n is the number of ions formed by the electrolyte, and α is the percentage dissociation.

Each group will make one or more of the following comparisons: Compare sucrose (mol. wt. 342.2) with

1. Glucose (mol. wt. 180.1).
2. KNO_3 (mol. wt. 101.11).
3. NaCl (mol. wt. 58.45).
4. $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ (mol. wt. 219.1).
5. Potassium oxalate ($\text{K}_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$, mol. wt. 184.22).
6. Potassium acetate ($\text{KC}_2\text{H}_3\text{O}_2$, mol. wt. 98.12).

This experiment illustrates a technique used in permeability and toxicity experiments, as well as in determining the osmotic

value of specific cells. It is interesting historically as the experiment from which the theory of ionization was derived. For a series of determinations a molar solution may be made up and diluted by means of a burette to form solutions varying by $0.05M$ over the range to be covered. The osmotic value of *Zebrina* cells is commonly between $0.1M$ and $0.5M$ sucrose. One milliliter of a $1.0M$ solution with 9 ml. water would be used

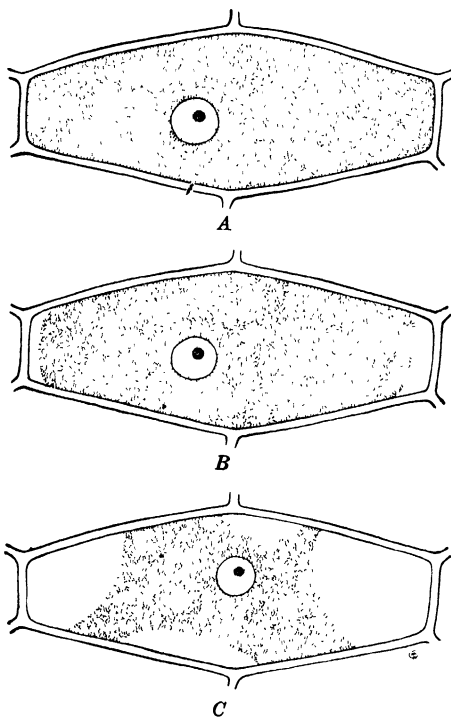


FIG. 22. -Plasmolysis in epidermal cells of *Zebrina pendula* A, normal, B, slightly plasmolyzed, C, strongly plasmolyzed

to obtain a $0.1M$ solution, etc. The cells of onion epidermis may have an osmotic value above that of $0.5M$ sucrose. The solutions should be protected from drying at all times.

To make the determination, place a drop of solution in the bottom of a Van Tieghem cell and a second drop on a clean cover glass. Carefully strip off a small piece of the tissue which is only *one cell thick*; mount at once in the hanging drop and observe for plasmolysis. Try the different solutions until

two are found that differ by $0.05M$, one of which will cause slight plasmolysis (Fig. 22*B*) and the other will not. The isotonic value will then be estimated at some intermediate value from the behavior of the tissue in the two solutions.

Questions :

1. What fills the clear spaces of plasmolyzed cells (Fig. 22*C*)?
2. What are the advantages of being able to determine the osmotic values of individual cells?
3. What advantages does sucrose as a plasmolyzing agent have over an electrolyte like potassium nitrate?

DIFFUSION THROUGH MEMBRANES

Membranes, because of their chemical or physical properties, alter the rate at which substances diffuse through them. Many complicated and obscure relationships are involved, but the final result is that colloidal materials pass through membranes slowly or not at all, solutes diffuse through at speeds depending upon their molecular weight, hydration, electrical properties, etc., and water diffuses at a relatively high rate through common biological membranes. The term semipermeable membrane has been used to indicate a membrane permeable to water but not to dissolved solutes. Since no perfectly semipermeable membrane is known, the term "differentially permeable" is preferable. The following experiments illustrate aspects of differential permeability not already covered under the experiments on diffusion of water.

Experiment 56. Diffusion through Differentially Permeable Membranes—Dialysis. (E)

Prepare four uniform celloidin dialyzing tubes as directed above (page 69) and partly fill with the following solutions:

1. 0.2 per cent safranin-O.
2. 0.2 per cent Congo red.
3. $0.1M$ sucrose solution (mol wt. sucrose 342.2).
4. Starch solution.¹

Use a known quantity of solution and immerse in a known volume of water.² At the end of 48 hr., test the internal and

¹ Starch solution is prepared by rubbing 1 gm. starch with 5 ml. cold water in a mortar and pouring 150 ml. *boiling* water over the paste. Allow to settle in a beaker, and decant the clear liquid.

² A few drops of toluene should be added to starch and sugar solutions to prevent bacterial or fungous growth.

external solutions of preparation 3 for sucrose, using the refractometer, or hydrolyzing the sugar with acid or invertase and determining the reducing sugars with Fehling solution (see page 203). Test the internal and external solutions of preparation 4 for starch using I-KI solution.

Questions :

1. What can you conclude regarding the size of the molecules of the solutes used in this experiment?
2. How is dialysis used in the experimental laboratory?

Experiment 57. Diffusion through a Plant Membrane. (E)

Soak lima beans or *raw* peanuts in water, carefully remove the seed coats from the soaked seeds, and tie them tightly over the end of an 8- or 10-mm. glass tube. Partially fill the tube with molar sucrose solution, set the osmometer thus formed into a small quantity of water in a test tube, and observe diffusion. Make sure that no sucrose leaks out through the wrapping, and determine whether the membrane is readily permeable to sucrose by testing the external liquid with Fehling solution after boiling with a drop of concentrated hydrochloric acid.

Questions :

1. Of what advantage is it to the seed to be covered with a membrane of this type?

Experiment 58. Diffusion from Plant Cells. (E)

Cut red garden beets into 2-mm. slices and wash thoroughly in tap water. Only dark-colored thrifty, freshly harvested beets should be used. Observe the leaching of the red color from the beet slices when covered with various solutions. The following or other combinations may be used:

1. Tap water.
2. Boiling water.
3. Fifty per cent alcohol.
4. Ten per cent NaOH.
5. Ten per cent acetic acid. After the color has diffused from the beets, add alkali to a sample of the extract.
6. Ether and water (cover slices with ether and add water after 3 to 5 min.).
7. Various salt solutions.

Sugar beets may be used, if available, and outward diffusion may be studied with a refractometer.

Questions :

1. What is the advantage of having cell membranes normally impermeable to organic solutes?
2. How do the chemicals used affect the permeability?
3. Why are sugar beets heated before extraction in commercial plants?

Experiment 59. The Permeability of Root Membranes. (I)

Study the growth of a seed plant on dextrose or sucrose agar in the dark to determine how readily these sugars are absorbed by roots. Use vetch, corn, beans, alfalfa, lupines, or other plants.

Prepare two 600-ml. lots of modified Knop solution by mixing 50 ml. of each of the stock solutions used in Experiment 42 with 300 ml. water. Add 7.2 gm. agar to each lot and 6 gm. glucose or sucrose to one lot, and heat on a water bath to dissolve the agar. Divide each medium into four Erlenmeyer flasks, liter size or larger for large seeds and 500-ml. flasks for alfalfa, etc. When small seeds are used, only half as much medium will be required. Plug the flasks with cotton and autoclave at 15 lb. for 30 min.

Weigh out eight lots of 4 to 20 seeds each of the assigned plant. Weigh the seeds to 0.5 mg. and calculate their oven-dry weight from moisture determinations made with parallel samples. Place each lot of seed in a numbered test tube and disinfect by one of the following methods:

1. Soak for 3 min. in 95 per cent alcohol, rinse in sterile water, and soak for 1 hr. in filtered 7 per cent calcium hypochlorite solution.
2. Soak for 20 min. in 10 per cent hydrogen peroxide and transfer at once without rinsing.
3. Shake with alcohol containing 5 per cent of Merko or other organic mercury dust, drain, and drop onto the agar. This method is particularly recommended for corn where pericarp infection is common. All disinfection methods will fail if dead or defective seeds are included.

Decant the disinfected seeds onto the solidified agar using bacteriological technique and grow in the *dark* for 2 to 4 weeks. If contamination appears, remove the seeds, autoclave the cultures, and start with a fresh lot of seeds. At the end of the

experiment wash the plants carefully out of the agar and obtain length of roots and tops, and green and dry weights of roots and tops in the composite samples from each treatment. Correct for the dry matter contained in the original seed and calculate gains or losses as milligrams and percentages.

Questions :

1. To what extent have your plants absorbed sugar?
2. What theory of permeability and absorption best fits your results?

Experiment 60. Diffusion through Perforate Membranes. (I)

a. Your experiments above have shown that organic materials do not readily diffuse through the normal plasma membranes of plant cells. Since organic materials do move readily within the plant, they are assumed¹ to move through the plasmodesms which have been shown to connect the protoplasts of adjoining living cells. Study the diffusion of solutes through small perforations by mixing up two 100-ml. lots of 2.0 per cent agar. To one lot add methyl red and a few drops of 0.1N NaOH solution if needed to bring to the neutral color of the indicator. To the other lot of melted agar, add 0.6 ml. concentrated HCl. Allow the two solutions to solidify in 250-ml. beakers. Bore four holes 1.0 mm. or less in diameter through a thin piece of monel metal or clean copper so that the holes are located at the corners of a 2- or 3-cm. square. The metal membrane should be 8 to 10 cm. across to prevent the two agar blocks coming in contact around the edges of the metal. Remove the two agar blocks from the beakers and press them together with the perforated metal plate between them, using a little warm agar or gelatin to insure a contact between the two blocks. Place the material in a covered dish to reduce evaporation and observe the diffusion of acid from the lower block through the perforations in the metal plate into the upper block containing methyl red indicator. A simpler but somewhat less striking demonstra-

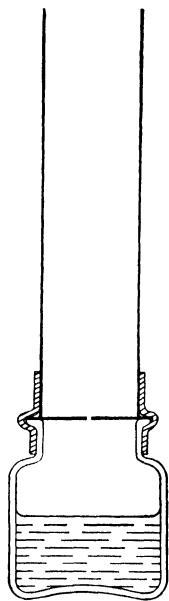


FIG. 23.—Apparatus for measuring diffusion through small openings.

¹ CURTIS, O. F. *Translocation of solutes in plants*. New York. 1935.

tion may be obtained by dissolving 0.5 per cent methylene blue in the lower agar block instead of the HCl, and observing the diffusion of the blue color through the perforated plate. If the agar is not sufficiently transparent for the shape of the diffusion shells to be readily observed, cut away a part of the upper block.

b. Solder thin pieces of brass across the lower ends of two thin brass tubes some 2 cm. in diameter and 10 cm. long. Bore a 0.5-mm. hole in the center of one of the plates and a 2-mm. hole in the other, and attach them with rubber tape to 50-ml. bottles partially filled with water (Fig. 23). Weigh the assembled bottles and tubes to ± 1 mg.; wrap the bottles in cotton to reduce sudden temperature changes and hold in an incubator at 25 or 30°C. for 2 to 4 weeks. Reweigh and calculate the ratio of the diffusion from each bottle to the area and to the circumference of the opening in each tube.¹

Questions:

1. What directions do the diffusing acid molecules take after penetrating through the opening? Why?

2. How does this scattering of the molecules increase the rate of diffusion through a small opening?

3. Why is the tube soldered above the perforated plate? Why is the apparatus kept at constant temperature?

4. Why is diffusion through a small opening proportional to the periphery rather than to the area of the opening?

¹ BROWN, H. T., and F. ESCOMBE. Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants. *Trans. Roy. Soc. (London)* **193B**: 223-291. 1900.

CHAPTER VI

COLLOIDAL PHENOMENA IN PLANTS

INTRODUCTION

Colloidal systems involve small divisions of matter which expose large surfaces. The particles are, however, many times larger than molecules so that the ordinary molecular and ionic chemical reactions of the dissolved substances are largely replaced by physical and chemical reactions dependent upon surface forces. The physical chemist classifies as suspensions, such mixtures of unlike phases as fog (water in air), smoke (carbon in air), potters slip (clay in water), etc.; and as emulsions such mixtures of immiscible liquid phases as milk and mayonnaise (oil in water), etc. A liquid colloidal system is a sol, and a solid or semisolid system a gel.

The protoplasm of plants consists of colloidal systems varying from emulsions (sols) to moderately stiff gels. The solid substances of protoplasm, proteins, dextrans, gums, etc., are characterized by the readiness with which they form colloidal systems in water. They are classed as self-peptizing because no protecting layer is required to separate the phases, as the egg separates the oil and water phases in mayonnaise and prevents the oil drops from coalescing. The self-peptization of typical plant colloids is associated with their ability to adsorb or absorb (sorb) large quantities of water so that the solid and water blend intimately but not completely as they would in true solution. Such colloids are called hydrophilic because of their affinity for water.

Many, perhaps most, of the problems involving the application of colloidal physics and chemistry to protoplasm and plant physiology are imperfectly understood. The experiments presented in this chapter are intended only to illustrate a few of the basic principles of colloidal behavior and to point out their possible relationship to the life processes of the plant.

References:

- BAYLISS, W. M. Principles of general physiology. 4th ed. Chaps. III and IV. New York. 1924
- SEIFRIZ, W. E. Protoplasm. Pp. x + 584. New York. 1936.

THE STRUCTURES OF COLLOIDAL SYSTEMS

Liquid colloidal systems, called sols, consist of a continuous liquid phase and a discontinuous or dispersed phase which may be gas (foam), liquid (emulsion), or solid (suspension). Frequently in industrial colloids a third substance is used to protect the dispersed phase. As already mentioned, many plant colloids are self-peptizing or self-protecting and do not require the third substance. In fact such self-peptizing colloids as albumin, gum arabic, etc., are commonly used as peptizing or dispersing agents to protect less readily emulsified or suspended substances. The tendency of colloidal particles to stay in suspension is associated with their electric charge, and self-peptizing colloids, along with other forms, take up electrical charges from the water or from substances dissolved in it so that the uniformly charged particles tend to repel each other, and thus prevent consolidation or coagulation of the particles.

In gels both the solid and the liquid phases may be continuous and, if we compare a suspension to a lake (liquid) filled with islands (solid), a gel becomes a lake with perhaps a larger number of islands connected by bridges or by fills and culverts so that both land (solid) and water (liquid) are connected.

Experiment 61. Emulsions. (E)

Place a drop of mayonnaise or other oil-and-water emulsion on a glass plate, add a drop of oil at one side, and gently mix the 2 drops together. Repeat with a drop of emulsion and a drop of water. Why does one substance mix with the emulsion so much better than the other?

Observe the emulsion under the microscope by pressing it thin under a cover glass. Compare the oil-emulsion and water-emulsion mixtures. Mix an oil stain such as Sudan III with the emulsion and, after it has stood for a few minutes, dilute with water and observe under the microscope.

Questions:

1. Is the oil or the water phase continuous in your emulsion?
2. If protoplasm were of the mayonnaise type of emulsion what substances could diffuse through it?
3. What would be the advantage of an emulsion structure in protoplasm?

Experiment 62. Phase Inversion in Emulsions.¹ (I)

Clean 16 small bottles or medium-size test tubes and add to each 10 ml. fresh olive oil colored with a few crystals of Sudan III and containing 0.5 per cent oleic acid in solution.

Arrange in four sets of four bottles each and add to the respective sets 1, 2, 3, and 4 ml. 0.1*N* NaOH. To the four tubes receiving 1 ml. NaOH add respectively, 0.25, 0.50, 0.75, and 1.00 ml. 0.1*M* CaCl₂ solution and repeat for the remaining sets. Add water to bring the total volume to 20 ml. in each case and a drop or two of methylene blue solution to give the water a bright blue color. Shake the bottles vigorously, allow to stand for several minutes and record the results. Check the color reactions for the dispersed and continuous phases of each mixture by placing 2 drops of the emulsion on a slide, mixing one with a drop of water and the other with a drop of oil.

To the bottle receiving 2 ml. NaOH and 0.25 ml. CaCl₂, add successive 0.25-ml. portions of CaCl₂ and shake after each addition. After the second addition of CaCl₂ add two 1.0-ml. portions 0.1*N* NaOH. Record results and explain.

Questions:

1. How do you explain the behavior of the different mixtures?
2. How might such a reaction be related to the sodium-calcium antagonism of Experiment 43?
3. Which emulsion would make the best type for protoplasm? Why?

Experiment 63. Suspensions. (E)

a. Dilute India ink 1 + 9 with water and observe under the microscope with dark-field illumination. Add 10 per cent BaCl₂ solution and 10 per cent HCl solution dropwise to two portions of

¹ CLOWES, G. H. A. Protoplasmic equilibrium. I. Action of antagonistic electrolytes on emulsions and living cells. *Jour. Phys. Chem.* **20**: 407-451. 1916.

the diluted ink until the carbon is flocculated out. Again observe under the microscope. Compare the action of BaCl_2 and NaCl .

b. Heat 500 ml. distilled water to a vigorous boil and add slowly with constant stirring 2 ml. 30 per cent ferric chloride solution. Test the stability of portions of the iron sol against concentrated HCl and NaOH solution. *Save the sol* for Experiment 70.

Questions :

1. What is a suspension?
2. Why do the suspended particles remain dispersed?

Experiment 64. Hydrophilic Colloids. (E)

Weigh 0.1-, 0.3-, 0.9-, 2.7-, and 8.1-gm. samples of gelatin into 250-ml. beakers; soak with a little cold water and then add boiling water to a total volume of 100 ml. Heat on a steam bath if necessary to dissolve the gelatin. Allow the suspensions to cool and observe their properties.

Test the stability of the liquid preparations to the flocculants used on the India ink.

Questions :

1. How does a hydrophilic sol differ from a hydrophilic gel?
2. Are these colloidal systems more or less stable than the carbon suspensions?
3. List some hydrophilic colloidal systems occurring in plants.

PROPERTIES OF COLLOIDAL SYSTEMS

The reactions of colloidal systems to heat and cold and to salts, acids, and bases can all be extended to observations on the stability of plant protoplasm. Coagulated protoplasm is "dead."

Experiment 65. Coagulation. (I)

Grind rapidly 100 gm. alfalfa stem tips, corn or bean sprouts, or other tissue high in protoplasm with 1 l. water in a ball mill; filter through folded cheesecloth and then through a fluted filter or through paper pulp on a Büchner filter and use at once. Or beat the white of an egg into 1 l. water. Test the effect of time

and temperature of heating, particularly at temperatures of 50 to 65°C.; of freezing; of adding alcohol, acids, bases, and salts upon the coagulation of the proteins in small samples of the protein suspension. Submit whole stem tips or seedlings to conditions which coagulate the protein suspensions, hold in a moist chamber, and observe for injury in comparison with untreated controls.

Questions:

1. What is meant by coagulation?
2. Outline several methods of killing plants quickly for fixing or preserving the tissues.

Experiment 66. The Isoelectric Point of Gelatin. (I)

The dispersion of many colloids in water (hydration) is affected by the hydrogen-ion concentration of the medium and is at a minimum at a point called the "isoelectric point." Determine the approximate isoelectric point of sheet gelatin as follows:

Obtain 1 l. each of approximately normal solutions of HCl and NaOH and dilute to produce 500 ml. each of 0.1*N*, 0.01*N*, 0.001*N*, and 0.0001*N*. Determine the pH of each solution using colorimetric methods or, if available, use the glass electrode. The quinhydrone electrode cannot be used for the more alkaline solutions.

Weigh 2 gm. sheet gelatin into each of 11 beakers or wide-mouthed flasks and cover with 200 ml. of solution as follows:

Beaker	Solution	Beaker	Solution
1	1 <i>N</i> NaOH	7	0.0001 <i>N</i> HCl
2	0.1 <i>N</i> NaOH	8	0.001 <i>N</i> HCl
3	0.01 <i>N</i> NaOH	9	0.01 <i>N</i> HCl
4	0.001 <i>N</i> NaOH	10	0.1 <i>N</i> HCl
5	0.0001 <i>N</i> NaOH	11	1 <i>N</i> HCl
6 (flask)	Distilled water		

The distilled water should be freshly boiled and cooled and this sample should be protected from atmospheric CO₂ in a stoppered wide-mouthed flask.

Allow the gelatin to soak 2 to 5 hr. until the most hydrated samples begin to soften too much to handle. Drain on folded cheesecloth and weigh the gelatin. *Save the liquid* and determine changes in pH. Plot the gain in weight of the gelatin against the final pH of the medium and estimate the approximate isoelectric point of the colloid.

Questions:

1. Why does the dispersion (weight) increase on *either* side of the isoelectric point?
2. At what point on the pH range of plant proteins would you expect the most rapid formation of new protoplasm?
3. At what point the most rapid volume increase by hydration?

Experiment 67. The "Isoelectric Point" of a Plant Tissue. (I)

Soak weighed samples of 5 to 10 gm. of potato disks for 24 hr. in solutions like those prepared for Experiment 66 and determine:

1. The weight changes in the potato disks.
2. The pH changes of the media.
3. The conductivity changes of the media.¹

Questions:

1. What isoelectric point is indicated for potato tissue?
2. Robbins² has interpreted this experiment as indicating absorption of H⁺ and OH⁻ ions, Youden and Denny³ as indicating excretion of buffering salts. Which view do your data support?

Experiment 68. Imbibition. (E)

a. Obtain 12 cubical blocks 2 to 3 cm. on a side, six of some softwood such as white pine, and six of some hardwood such as maple. The blocks should be cut so that they show two radial and two tangential faces. Measure the blocks carefully with calipers, micrometer calipers if available, and record the measurements of the longitudinal (lengthwise), radial (across the annual

¹See LOOMIS, W. E., and C. A. SHULL, *Methods in Plant Physiology*, Chapt. 21, for methods.

²ROBBINS, W. J. An isoelectric point for plant tissue and its significance. *Am. Jour. Botany* 10: 412-439. 1923.

³YODEN, W. J., and F. E. DENNY. Factors influencing the pH equilibrium known as the isoelectric point of plant tissue. *Am. Jour. Botany* 13: 743-753. 1926.

rings), and tangential faces. Calculate the volume of the blocks and cover (weight down) for 48 to 72 hr. as follows:

1. Two of each species with distilled water.
2. Two of each species with 7.5 per cent NaCl solution.
3. Two of each species with 30 per cent NaCl solution.

At the end of the period determine the amount of swelling along each of the faces and the volume of each block.

b. Weigh three lots of 10 or more grains of corn each and cover them with water and with 7.5 and 30 per cent NaCl solutions for 24 to 72 hr. Determine imbibition by weighing. If time permits, determine the imbibitional force of the grains by immersing them in graded salt solutions.¹

c. Weigh out 10 gm. oven-dry cotton and soak in hot water for a few minutes. Cool, press out as much water by hand as possible, and weigh. Press in a hydraulic press at 250 lb. per sq. in. and reweigh. Repeat with 1000, 4000, and 16,000 lb. pressure. Plot percentage of moisture retained by the cotton against the logarithm of the pressure and determine by extrapolation the theoretical pressure to remove all the water.

Questions:

1. Is imbibition a strong or a weak force?
2. Why do flat (tangentially) sawed boards warp when wet?
3. Why do architects frequently ignore vertical timbers when allowing for shrink and swell in wood frame buildings?

Experiment 69. Bound Water. (I)

Colloids, particularly gels, possess the ability to reduce the freedom of water by holding it more or less closely in hydration bonds.

a. *Binding against Pressure*.—Dissolve 1.0 gm. gelatin in 20 ml. hot water and allow to cool. Wrap a portion of the gel carefully in a piece of bladder membrane and press in the hydraulic press at 3000 lb. pressure to determine whether the 95 per cent of water in the gel is readily pressed out. If a poor membrane is used, it will burst under the pressure.

Press a 20-gm. sample of pine needles (*Pinus nigra* needles are excellent) in a hydraulic press at 10,000 lb. pressure and deter-

¹ SHULL, C. A. Measurement of the surface forces in soils. *Botan Gaz.* **62**: 1-31. 1916.

mine, by weighing the residue, the percentage of sap pressed out of the leaves. Steam the press residue or cover it with boiling water for 2 min., re-press, and reweigh. Heating kills the cells and precipitates their colloids. How does it affect the water-holding capacity of the tissue? Dry the press residue to deter-

mine percentage of water held by imbibition in the dead tissues.

b. Binding against Evaporation.—Suspend 10 gm. agar in 100 ml. water by autoclaving and pour the suspension into a straight-sided crystallizing dish to gel. Fill a second dish to the same level with distilled water and a third with 10 per cent copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) solution. Weigh all three dishes, set them in a protected dry place, and determine the rate of water loss by evaporation from each.

c. Binding against Freezing.—Place 5 gm. water in a paraffined paper tray, and a block of 10 per cent agar containing 5 gm. water in a second tray of the same type. Freeze the water and gel in a freezing chamber at -10°C . or in the bottoms of metal cans packed in salt and ice at about -10°C . Place 200 ml. water warmed to about 2°C . above room temperature in a wide-mouthed pint thermos bottle; insert a 0.1 degree or calorimeter thermometer and a metal stirring device through a cork stopper (Fig. 24), and record accurately the temperature of the gently stirred water in the bottle. Add the paper container of ice to

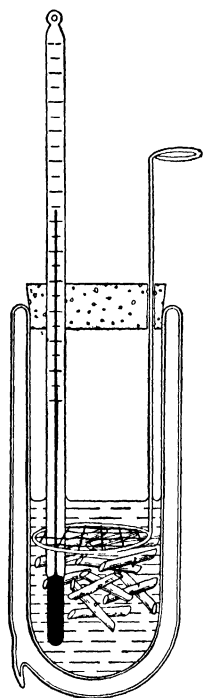


FIG. 24.—Simple calorimeter for determining unfrozen water

the bottle, stopper, stir gently to thaw the sample, and record the lowering of the temperature. Repeat with the frozen gel using a fresh charge of water. Calculate the calories of heat absorbed by each sample (cooling in degrees $\times$ 200 ml. H_2O), and, remembering that 80 cal. are required to melt 1.0 gm. ice, calculate the percentage of water unfrozen in the gel¹ from the difference in the cooling with the two samples.

¹ Detailed procedure and a corrected formula for bound water by the calorimetric method are given in Methods in Plant Physiology. For

Questions:

1. Which exerts the greatest "pull" on water: freezing, drying, or mechanical pressure?
2. How may the water-binding capacity of colloids be advantageous to plants?

Experiment 70. Cataphoresis. (I)

Electrically charged colloidal particles move toward the pole of opposite charge when placed in a direct current field. The sign of the charge is thus determined and, with careful standardization of conditions, the rate of the movement is proportional to the charge, becoming zero at the isoelectric point.

Set up the apparatus as illustrated in Fig. 25, using two or more dry cells in series, or 110-volt *direct* current in series with a variable resistance as a source of electrical current. Fill the tubes half full with a buffer solution of approximately the same pH as the colloid to be tested. Connect the current to the electrode posts and add the colloidal sol very carefully through a pipette to the bottom of the U-tube. The colored sol should form a sharp line with the buffer. Record the position of the colloid on either side, adjust the current to reduce disturbing electrode reactions, and observe migration of the colloidal particles in the U-tube.

The iron sol from Experiment 63, diluted India ink, or other substances may be used.

Questions:

1. Check the electrical charge indicated by cataphoresis with the sensitivity of the colloid to precipitation by acids and bases.
2. Construct a theory of the action of such precipitation. What might happen if too much base or acid were added?
3. How do salts of heavy metals precipitate colloids?

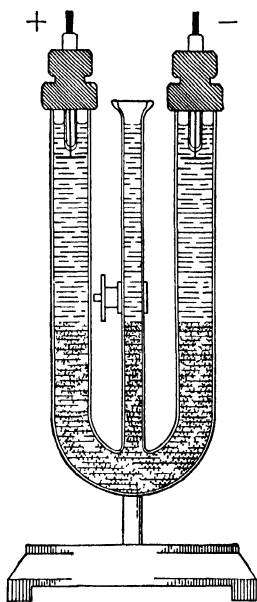


FIG. 25.—Cataphoresis tube. The colloid is pipetted carefully into the bottom and its movement in an electric field observed.

demonstration purposes the corrections for container, specific heat of agar, etc., may be ignored, particularly when the temperature changes are not large.

SURFACE ACTION

Small colloidal particles are estimated to contain a million molecules so that they behave as solids rather than as solutions. On the other hand, the exposed surface area of a 1-cm. cube increases from 6 sq. cm. in the intact block to 6000 sq. m. or about an acre and a half, as the original cube is subdivided into cubes of colloidal dimensions. Each of these billions of tiny cubes is still a solid, but any reaction occurring at or involving surface or interface will be enormously increased by the subdivision of the large cube. The following experiments show some of the properties of interfaces between liquid and air, liquid and liquid, or liquid and solid, and indicate the effects which interfacial action may have upon the structure and chemistry of colloidal systems.

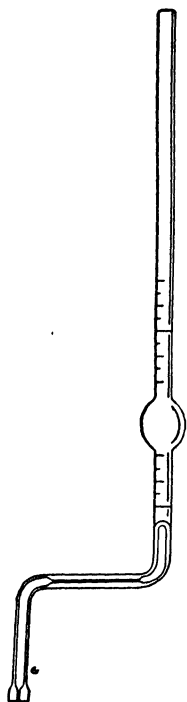


FIG. 26.—Traube stalagmometer. The number of drops formed when the instrument is emptied is inversely proportional to the surface tension of the liquid used.

Experiment 71. Surface Tension. (E)

a. *Demonstrations* of surface tension. Float a clean needle on freshly drawn tap water¹ in a *clean* dish. Place the needle with a pair of tweezers to avoid touching the water with the fingers. Touch an oily rod to the water at one side of the needle. Why does the needle jump? Can you repeat the experiment without clean water? Why?

Drop small crystals of camphor on freshly drawn tap water in a *clean* dish and observe their action. Touch the water with the oily rod and again observe the camphor.

b. *The drop method* of measuring surface tension is convenient for demonstrations. Use a stalagmometer (Fig. 26) or a small pipette; clean the instrument thoroughly, fill it with freshly drawn

¹ Freshly drawn tap water is less likely to be contaminated with oil than is distilled water. Oil, as your demonstration shows, very quickly lowers the surface tension of water.

tap water, and count the number of drops formed when the pipette is held vertically and emptied slowly. Repeat with tap water containing a little soap solution. What holds the drops on the end of the pipette or stalagmometer? What is the effect of the soap? Repeat with other materials or solutions, cleaning the pipette thoroughly before reusing.

Questions :

1. Why does oil stop the movement of camphor on water?
2. Why will soapy water wet leaves or oiled paper better than pure water? Try it.
3. How could surface tension measurements be used in physiological studies?

Experiment 72. Surface Tension at an Oil-water Interface. (I)

Prepare the following solutions in small volume. Use all of the indicated salts in the same volume. The solutions may be conveniently prepared by pipetting 2.0 ml. 0.1*M* NaOH, 30 ml. 1.0*M* NaCl, and (or) 3.0 ml. 0.1*M* CaCl₂ into 200-ml. flasks as indicated and making to volume.

Solution	NaOH	NaCl	CaCl ₂
1	0.001 <i>M</i>	0	0
2	0.001 <i>M</i>	0.15 <i>M</i>	0
3	0.001 <i>M</i>	0	0.0015 <i>M</i>
4	0.001 <i>M</i>	0.15 <i>M</i>	0.0015 <i>M</i>

Very carefully clean a stalagmometer (Fig. 26) or a small pipette. Fill several test tubes with fresh olive oil containing 0.5 per cent oleic acid in solution. Fill the stalagmometer with clean distilled water, dip the tip of the instrument into the oil, and determine the number of drops of water produced in the oil when the instrument is emptied. Repeat using the four solutions prepared above, rinsing out the stalagmometer with each new solution before using.

A drop of liquid is held upon the tip of the stalagmometer by its surface tension and the size of the drops and therefore the

number of drops per unit volume is directly proportional to the surface or interphase tension of the drops. The drops are, of course, larger in oil than in air because less weight has to be supported by the surface film. Larger, and therefore fewer, drops indicate a relatively high interface tension while more drops indicate a low tension.¹

Questions:

1. What is the effect of the sodium and calcium salts upon interface tension in this system?
2. Which solution will form the more stable emulsion? Why?

Experiment 73. Adsorption. (I)

One of the important reactions of colloidal systems is the tendency of materials to become concentrated at the interfaces. The marked effect of soap upon the surface tension of water is due to its concentration at the air-water interface. Similarly substances may become concentrated at a liquid-solid interface. In this case the process is known as adsorption.

Partially fill five 250-ml. Erlenmeyer flasks with dilute methylene blue solution by adding 12.5-, 25-, 50-, 100-, and 200-ml. portions of a 0.05 per cent solution of the dye to successive flasks and adding water to bring the total volume to 200 ml. in each case. Add 0.5-gm. portions of decolorizing carbon (animal charcoal) to the flasks and shake occasionally for 30 min. Filter into clean flasks and observe the colors of the filtrates.

Transfer the filters with the carbon residue to test tubes and wash the residue with three 10-ml. portions of water. Catch each wash water in a separate test tube and hold for color comparisons. Make a final washing with 10 ml. 95 per cent ethyl alcohol and receive in a fourth set of test tubes. If time permits, make further tests of the effect of water and alcohol in removing adsorbed methylene blue by shaking duplicate 0.5-gm. samples of charcoal with a solution from which most of the color will be removed, filtering, washing lightly, and shaking one of the duplicate charcoal residues with successive 100-ml. portions of water and the second with the same volumes of alcohol.

¹ The actual values of interface tension may be estimated from the effective weight of the drops formed in oil, after allowing for the buoying effect of the oil, as compared to the weight of the drops formed in air.

Questions :

1. Why is the color which fades so readily from a nonfast cloth, so hard to remove from a white cloth which may be stained by it?
2. How do plant reactions benefit from adsorption?
3. What relation can you see between the surface tension of water and alcohol, and the completeness with which methylene blue is adsorbed from them?

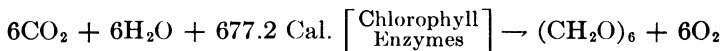
CHAPTER VII

PHOTOSYNTHESIS

INTRODUCTION

The production of carbohydrates by green plants is properly rated as the most important chemical process known to man. This reduction and polymerization of carbon dioxide and water, commonly called "photosynthesis" in America and "carbon assimilation" in Europe, is the foundation of biological and organic chemistry, the source of building materials and energy for plants and animals, and the world's most important source of industrial energy. Not only are we dependent upon photosynthesis in plants for all of our foods and most of our energy, but perhaps 90 per cent of the world's population is engaged directly or indirectly in the production, transportation, processing, or sale of plant-derived materials which are in turn secondary products of photosynthesis.

In its simplest terms, photosynthesis is the production of carbohydrates from carbon dioxide, water, and the energy of sunlight, and we can write the following chemical equation to show the initial and final products of the reaction:



The brackets enclosing chlorophyll and enzymes indicate that these materials must be present, but are recovered unchanged after the process is complete. It is their mode of action which constitutes the complicated part of the photosynthetic process. This equation indicates four groups of factors which may affect the rate of photosynthesis:

1. The supply of CO_2 and water.
2. The energy supply.
3. The physiological factors within the leaf which influence the production and action of the leaf catalysts.
4. The accumulation of the end products.

The water actually chemically combined in photosynthesis represents approximately 0.1 per cent of the water absorbed by the plant and, as long as water is available to maintain life and turgor in the cells, it can hardly be directly limiting for the production of foods. Indirectly, however, it may be seriously limiting, for with deficient water supplies leaves wilt, the stomates close, and CO_2 absorption is reduced. Also the health of the leaves, and thus their assimilation rate, depends upon normal hydration and turgor. Finally, the CO_2 molecules which diffuse into the intercellular spaces of the leaf may be compared to insects which have flown (diffused) in through an open window; they are yet to be caught. The insects may be trapped in some sticky substance exposed in the room; the CO_2 molecules are caught within the leaf in the water which saturates the walls of the mesophyll cells. It is this exposure of wet cell walls, through the stomates, to the open air, which makes transpiration an invariable corollary of photosynthesis in land plants.

We can better appreciate the magnitude of the problem of catching CO_2 when we realize that a well-developed corn plant must absorb all the CO_2 normally contained in 100,000 cu. ft. of air—enough air to fill seventy 12- by 14-ft. rooms, or the equivalent of a four-year gas supply for a family of three or four persons. A maximum crop of corn or sugar cane may absorb more carbon than is normally present in the air above the crop, yet at the end of the season so much CO_2 will have been returned to the air, primarily by decay organisms and secondarily by the respiration of plants and animals and the combustion of wood, coal, and other carbonaceous materials, that the carbon content of the air is maintained near its normal level of 0.03 per cent CO_2 or 0.008 per cent carbon.

We do not know why, when most of the CO_2 comes from decay processes in the soil, this nutrient should be almost entirely absorbed by the leaves rather than the roots, although the rapid movement of CO_2 gas in the air and the short distance between the point of supply in the air and of use in the leaf are factors favoring absorption by the leaves. In the greenhouse, heavy mulches of decaying organic matter contribute to plant growth by supplying CO_2 , and the same relationship may hold to a lesser degree in the field, but in either case the CO_2 is liberated into the air and absorbed through the stomates of the leaves.

Literally thousands of papers have been published on the topic of photosynthesis, but fortunately all but the more recent have been summarized in two monographs. A reading of one or more of the following references before starting the laboratory work of this chapter will assist in understanding and interpreting the experiments performed.

References:

MAXIMOV, N. A. Plant physiology. 2d ed. Chapt. V. New York. 1938.

MILLER, E. C. Plant physiology. 2d ed. Chapt. VIII. New York 1938.

STILES, WALTER. Photosynthesis—The assimilation of carbon by green plants. New York. 1925.

SPOEHR, H. A. Photosynthesis. New York. 1926.

THE FOOD-MAKING PROCESS IN PLANTS

Experiment 74. The Necessity of Chlorophyll. (E)

Choose a vigorous fully grown leaf of *Coleus*, geranium, or other variegated starch-forming plant. A leaf of the common *Coleus* variety which shows a central area of bright red surrounded by red-brown and bordered with normal green is desirable. Outline the leaf and its various colored areas. If the leaf shows red coloring, heat in boiling water for a minute until the red fades, and compare the remaining colored areas with your original outline. Heat the leaf in boiling 95 per cent alcohol to remove the chlorophyll and test for starch accumulations with I-KI solution. Compare the starch pattern with your leaf outline. If a leaf of a solid red *Coleus* variety is available, test it in the same way, heating first in water and then in alcohol. Or test the pure white or yellow leaves of variegated geranium and compare with the partially green leaves.

Questions:

1. How could you prove that the absence of starch storage in these leaves means no photosynthesis?
2. What relation do you find between anthocyanin (red or purple) pigments and photosynthesis?
3. Why do variegated plants never make bad weeds?

Experiment 75. The Necessity of Light. (E)

Shade a portion of a leaf of a starch-forming plant, such as a soft-leaved geranium or *Coleus*, with a Ganong light screen (Fig.

27), or obtain two squares of black paper and two squares of screen wire each 3 by 3 cm. Cut out a small pattern in one of the papers and clamp the two papers, covered by the screen wire squares, to the two sides of a leaf with a test-tube clamp. Expose the cutout paper to the light, and hold the plant in the greenhouse or in a sunny window for 2 days, extract the chlorophyll from the leaves, stain with I-KI, and compare the starch content of the lighted and shaded portions.

Questions:

1. Does the energy of sunlight merely run the "machinery of photosynthesis" or is it included as a part of the product?
2. Why is a ventilated covering used?

Experiment 76. The Necessity of Free Carbon Dioxide. (E)

Place a watered geranium or Coleus plant under a bell jar with a beaker of concentrated sodium hydroxide solution, or of soda lime, to absorb free CO_2 . Leave the plant in strong diffuse light¹ at a moderate temperature for 2 or 3 days and test the enclosed leaves for starch. Compare with leaves from a plant held in a ventilated bell jar.

Questions:

1. Why do we ventilate greenhouses? Would it be a good plan to keep the ventilators closed in cold weather to save heat?
2. Plant breeding, irrigation, and fertilizer experiments indicate that CO_2 is not a seriously limiting growth factor under average field conditions. How do you explain the efficiency of plants in absorbing the very dilute CO_2 of the air?

Experiment 77. The Liberation of Oxygen in Photosynthesis. (E-I)

Place a quantity of Elodea in a large battery jar and cover with an inverted funnel and test tube as illustrated in Fig. 28.

¹ Avoid direct sunlight which will cause overheating of the plants.

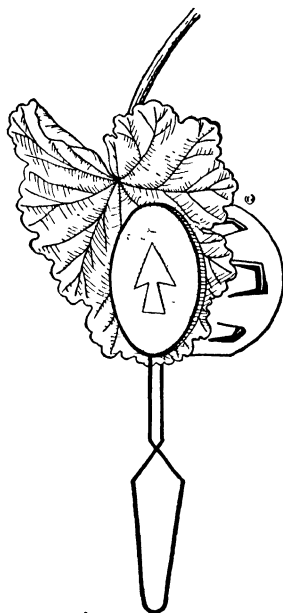


FIG. 27.—Ganong light screen. The ventilated box at the bottom permits the entrance of CO_2 to the leaf while light reaches it only through the cutout in the tin-foil backing of the top glass.

The battery jar should be deep enough and the stem of the funnel short enough that the test tube can be moved without lifting the lower end from the water. Fill the inverted test tube with water and place the apparatus in continuous light for 2 or 3 days, until several milliliters of gas have been collected. Test the gas for oxygen with a glowing splinter, or transfer it to a gas analyzer

(Fig. 39, page 140) and analyze for carbon dioxide, oxygen, and (by difference) nitrogen.

When sea water is available, the experiments of Harvey¹ may be repeated, or plants may be sealed into mixtures of nitrogen or hydrogen with 5 or 10 per cent carbon dioxide and the gases reanalyzed after the apparatus has been held in continuous light for 1 or 2 days. Use the container illustrated in Fig. 41, page 144.

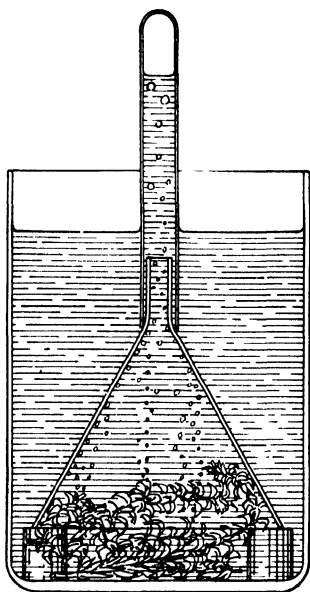


Fig. 28.—Apparatus for collecting the gas liberated by submerged water plants in the light.

Questions:

1. How do we classify reactions in which O_2 is liberated? What energy exchanges are involved?
2. Why do we keep a sprig of water plant such as *Myriophyllum* or *Elodea* in the fish bowl?

THE LEAF AS A FOOD-MAKING ORGAN

Experiment 78. The Structure of Leaves. (E)

Study thin free-hand sections or prepared slides of leaves and note characteristics which will increase or decrease efficiency in the absorption of light and CO_2 and in the transportation of water and carbohydrates. Refer to your studies of stomatal number and opening (Experiments 28 to 30). Make a large-scale diagram of a leaf section showing the movement of the various raw and finished products of photosynthesis.

¹ HARVEY, E. N. Photosynthesis in absence of oxygen. *Plant Physiol.* 3: 85-89. 1928.

Questions:

1. How is the typical leaf adapted to its work of food manufacture?
2. What factor prevents an extreme specialization for light and CO₂ absorption?

Experiment 79. The Chloroplasts. (E)

Study a vigorous healthy leaf of *Elodea* (Fig. 29) under low- and high-power magnifications. Why do leaves appear green? Compare a cross section of geranium, tomato, or other leaf. Notice that the chloroplasts (green bodies) of *Elodea* float in

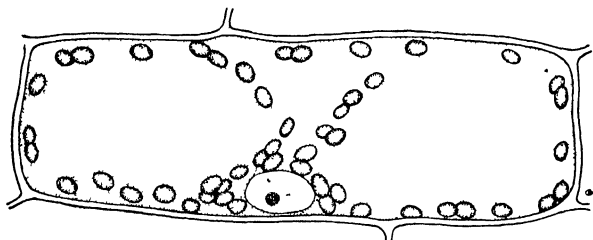


FIG. 29.—Cell from leaf of *Elodea* showing chloroplasts and nucleus

the streaming protoplasm of the leaf and midrib cells without separate motion of their own.

Mount an *Elodea* leaf which has been exposed to good light, place it under the microscope, run a drop of I-KI solution under the slide, and observe the staining of starch granules, at first faint and finally dark purple to black. Granules at the base of the midrib commonly color first, and several minutes may be required to stain starch in the center of the leaf.

Questions:

1. Why can the cells at the lower side of a leaf absorb sunlight and carry on photosynthesis?
2. What is the advantage of having the starch granules deposited within the chloroplasts?
3. What advantages can you see in having the chlorophyll confined to the chloroplasts instead of scattered throughout the cell?

Experiment 80. The Role of Stomates in Photosynthesis. (E)

Carbon dioxide is slightly soluble in cutin and can diffuse through the epidermis of leaves. Early plant physiologists,

who used high concentrations of CO_2 , thought that this was the principal mode of entry. The theory may be tested for normal conditions by coating with wax the upper and lower surfaces of starch-forming leaves such as those of *Fuchsia*, most of the stomates of which are on the lower surface. Tag eight uniform *Fuchsia* leaves and coat the leaves with thin films of vaseline according to the following outline:

1. Two leaves on the upper surface.
2. Two leaves on the lower surface.
3. Two leaves on both surfaces.
4. Two leaves tagged but untreated.

In waxing both surfaces of the leaf, be sure to cover the margins. Considerable care is required, also, to cover between the veins on the lower surface of the leaf. After 48 hr. in a cool, well-lighted greenhouse, remove the leaves, wash with xylol to remove vaseline, extract the chlorophyll by heating first in water and then in alcohol, and stain for starch with warm I-KI solution. Make stomate counts on the upper and lower surfaces of leaves from the plant used and explain your results.

Questions :

1. What is your conclusion regarding the permeability of the leaf to CO_2 ?
2. What will be the effect of wilting on photosynthesis?
3. Is there any advantage in having the larger number of stomates on the lower side of the leaf?

THE MEASUREMENT OF PHOTOSYNTHESIS

Theoretically it should be possible to measure photosynthesis by any of the reacting or end products. The water and energy used cannot be determined readily so that the carbon dioxide used or the carbohydrate produced, and occasionally the energy fixed or oxygen liberated, are the factors used in measuring the photosynthetic rate. The carbohydrate produced would seem to be a logical measure of photosynthesis, but it is subject to at least four errors: (1) Photosynthate is normally moved out of the manufacturing cells, and this translocation is probably most rapid during the time that the translocated materials are being produced and their gradient is greatest. As a result,

corrections made by measuring translocation in the dark are subject to an unknown error from a decreasing translocation rate. (2) The products of photosynthesis are used in respiration, and, like translocation, respiration is probably highest in the illuminated leaf with its higher temperature and sugar content. (3) It is extremely difficult to refer analyses made before and after illumination to the same basis. Dry weight obviously cannot be used, and both green weight and area of tissue are subject to shrinkage errors. (4) Finally, there is the possibility that an oil or an organic acid or some compounds other than those measured may be produced, either as primary or as secondary products.

Dry-weight increments per unit leaf area, sometimes used as a crude measure of photosynthesis, avoid errors due to the type of compound formed, but are subject to translocation and respiration errors, and are particularly affected by shrinkage.

Measurements of carbon dioxide absorption avoid the very serious translocation and shrinkage errors, but tell nothing of the end products of photosynthesis. The respiration error remains, but may be partially corrected for, or may be ignored to obtain a measurement of the net gain of carbon by the leaf.

Experiment 81. A Modified Weight-area Method of Measuring Photosynthesis. (I)

Hold sunflower, bean, corn, tobacco, or other broad-leaved plants in the dark for 24 hr.; cut a 50-sq. cm. sample from one side of a selected number of leaves left on the plants and a second sample from the same number of leaves removed from the plants and held with their petioles in water. Place the plants and leaves in good light for 2 to 8 hr. The leaves held in water will serve as a check on translocation if they are carefully handled. The leaf punches may be cut conveniently with a Ganong leaf punch,¹ or a cork borer of known area may be used and the leaves cut against a piece of cork. Place the leaf punches in a tared² weighing bottle, dry at 100°C. for 24 hr., and determine their dry weight. Recount the pieces and record. It is not essential that you have exactly 50 sq. cm. as long as the area is known.

¹ Available from the Bausch and Lomb Optical Co., Rochester, N. Y.

² Quantitative precautions must be used throughout these determinations and all weighings read to 0.1 mg.

Transfer the dried leaf punches to a 500-ml. Erlenmeyer flask,¹ add 100 ml. 1 + 9 HCl,² and autoclave at 15 lb. pressure for 1 hr. to hydrolyze polysaccharides, proteins, etc. Filter the leaf residue through a tared Gooch filter; wash the flask and residue thoroughly with hot water, dry for 24 hr., cool, weigh, and record as the residual or skeleton weight of the sample. This residue represents relatively stable portions of the leaf tissue whose weight is not directly affected by either photosynthesis or transpiration, and any gains or losses in residual weight over a short period should be due to changes in turgor and consequently to a change in the number of cells and amount of fixed cell materials in a given leaf area.

Take a second set of punches from the remaining halves of the leaves after the plants and cut leaves have been exposed to light for the desired period and determine the dry and residual weight of these pieces. Compare with the respective weights of the first sampling and explain any changes you find in the calculated weights of a square meter of leaf area. Calculate apparent photosynthesis and translocation as grams per square meter of leaf area per hour. How much of the increase in dry weight is due to shrinkage and the inclusion of more cells in the second sample? The simplest method of calculating the shrinkage correction is to divide the initial (morning) residue weight by the later residue weights to determine what fraction of 50 sq. cm. in the afternoon would have occupied the same area in the morning. Multiply the afternoon *total dry weight* by this factor and then subtract the morning total dry weight to obtain corrected increase in dry matter. What translocation rate is indicated by the difference between the weight changes of the attached and detached leaves?

Questions:

1. What have you measured in this experiment?
2. Under what conditions and for what purposes could this method be used to advantage?
3. Is food translocated from leaves during the day or only at night?

¹ If the plants and leaves are exposed to only moderately strong light in a cool (20°C.), humid greenhouse, the dry weights of the punches may be used directly without shrinkage corrections.

² One part concentrated HCl and 9 parts distilled water.

Experiment 82. A Gas-stream Method of Measuring Photosynthesis. (I)

The gas-stream method as modified by Heinicke¹ is convenient and is perhaps the most accurate method available for measuring photosynthesis under approximately normal conditions. The apparatus illustrated in Fig. 30 is set up in duplicate, or larger number, with each gas train attached through a flowmeter to a central vacuum chamber which can be exhausted with a vacuum pump. The flowmeters are calibrated to deliver 100 l. of air an hour, and are controlled by a water- or mercury-filled constant pressure device (see Fig. 43, page 149).

Make envelopes to fit the leaves to be used, from waterproof cellophane sealed with rubber cement. Mount one envelope over a gas inlet and a leaf or shoot so that air will enter the envelope at the base of the leaf and be withdrawn at the top. Set up the second envelope in the same way, but omit the leaf. Expose the leaf to good light. Place 200 ml. 0.1*N* or 0.05*N* barium hydroxide faintly colored with phenolphthalein in each of the absorption flasks, open the valves, and draw normal air through the apparatus at the rate of 100 l. an hour for 1 or 2 hr. If the alkali should become exhausted in either of the absorption towers, as indicated by the fading of the phenolphthalein, record the time and terminate the experiment.

The Ba(OH)₂ solution should be drawn into the absorption tower and the gas stream, finely divided by the fritted glass disk, bubbled up through it. At the end of the experiment, disconnect the absorption flask and tower, let the solution run back into the flask (apply suction if necessary), and wash the tower with about 150 ml. of freshly boiled CO₂-free water, catching the washings in the flask. Disconnect the flask and titrate with phenolphthalein and 0.1*N* HCl. Save the leaves used in the experiment for measurements of leaf area with a planimeter or paper cutouts.

Calculate the carbon dioxide absorbed per square meter of leaf area per hour from the difference of the blank- and leaf-train titrations.

¹ HEINICKE, A. J., and M. B. HOFFMAN. An apparatus for determining the absorption of carbon dioxide by leaves under natural conditions. *Science* **77**: 55-58. 1933.

$$\text{MgCO}_2 = \frac{D_t \times N \times 22}{A_{m^2} \times h}$$

where D_t is the difference of the leaf-train titration and the blank titration; N is the normality of the acid used in titrating the residual $\text{Ba}(\text{OH})_2$; 22 is the normal (0.5M) weight of CO_2 ; A_{m^2} is

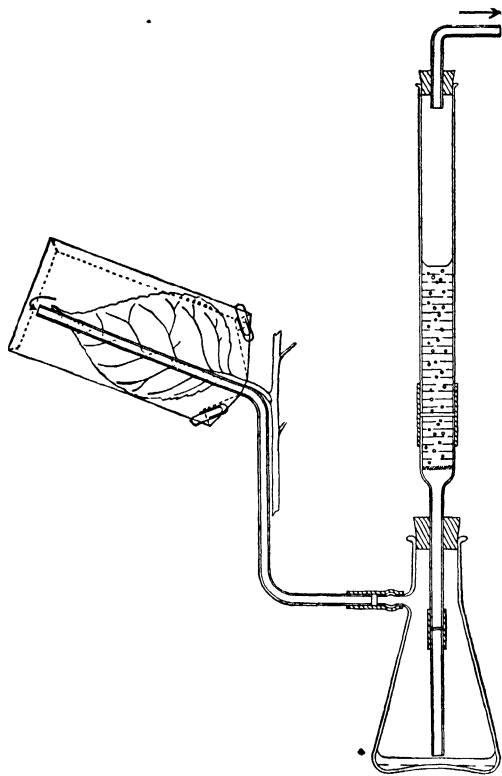


FIG. 30.—Heinicke's gas-stream apparatus for measuring photosynthesis. The apparatus is connected to an aspirator through a flowmeter with safety flasks and pressure regulator, as shown for tower D in Fig. 43, page 149

the area in *square meters* of the leaf used, and h is the time of the experiment in hours. Carbon dioxide absorbed may be changed to equivalent glucose by multiplying by 0.6818 or to equivalent starch with the factor 0.6136.

Questions:

1. What advantages does the gas-stream method have over the dry-weight method for the measurement of photosynthesis? What disadvantages?

2. Why can the respiration correction be ignored in most problems? How should it be made?
3. How much sugar would a plant with 0.5 sq. m. leaf area produce in 11 hr. at the rate found in your experiment?

FACTORS AFFECTING THE RATE OF PHOTOSYNTHESIS

Photosynthesis is dependent upon the supply of CO_2 , intensity and quality of light, and the physiological condition of the leaf cells and plastids. Relative differences in photosynthetic activity and the responses of a plant to varying environmental conditions may be studied by observing the bubbles (mixtures of air and oxygen) given off from many aquatic plants while carrying on photosynthesis. The method is not exact because of the varying proportions of air in the bubbles, differences in the size of bubbles with differences in the size or shape of the vessel from which they escape, escape of gas caused by increased plant temperature, etc. If, however, the same plant material is used for one series of experiments and the proper precautions taken in regard to temperature changes, some interesting results may be obtained, and the method is convenient for demonstrating the principles of photosynthesis. Experiments with land plants should be added whenever practicable.

Experiment 83. Light Intensity. (E-I)

a. (E) Carefully attach a vigorous shoot of *Elodea*¹ to a glass rod with the cut end of the plant stem up. Immerse in tap water and observe the small bubbles given off from the cut stem when the plant is exposed to light. Adjust the size of the bubbles by varying the slant of the cut stem surface until they can be counted conveniently. The number of bubbles emitted in unit time is taken as an approximate measure of photosynthetic activity.

Place a plant at such a distance (2 or 3 m.) from a strong artificial light set up without a reflector (why?) that bubbles are given off slowly; determine the time for 5 or 10 bubbles to be given off, and calculate as bubbles per minute. Reduce the distance between the plant and light by half and again determine the number of bubbles per minute. One or two minutes will be necessary for the plant to become adjusted to the stronger

¹ *Myriophyllum* or similar plants may be used if desired

light, but heating should be carefully watched. Reduce the distance again to one-fourth and one-eighth of the original and record. Plot number of bubbles against relative light intensity, remembering that light intensity varies inversely as the square of the distance. The light intensities may be measured, or the light at the maximum distance may be taken as unity, and the relative intensities of the shorter distances calculated.

b. (I) If time permits, set up three gas trains (Fig. 30), one $\frac{1}{2}$ m. and one 2 m. from a 1000-watt lamp set up without a reflector. Insert uniform leaves attached to potted plants in the two trains, and use the last setup for a check on the normal CO_2 content of the air. Follow the technique used in Experiment 82, allowing the absorption to continue for several hours if possible. Calculate CO_2 absorption as milligrams per square meter per hour and compare with light intensity.

Questions:

1. What does your curve indicate of the method of using light in photosynthesis?
2. What important part do stems play in photosynthesis?

Experiment 84. The Effect of Temperature. (E)

a. Fill four 1000-ml. beakers with water and adjust the temperature of the water with finely chipped ice and warm water to give the four temperatures 10, 20, 30, and 40°C. Hold the beakers at these temperatures and force laboratory air through them vigorously with an atomizer bulb to saturate the water with air. Choose a vigorous sprig of *Elodea* adjusted to bubble satisfactorily, and determine the bubbles evolved per minute when the plant is held for 2- to 5-min. periods in each of the beakers. Avoid disturbing the plant in moving and provide an adequate and uniform light source. The plant should be held at each temperature for a short time before the readings are taken.

b. Fatigue at high temperatures may be studied by holding a sprig of *Elodea* at 45°C. and studying its bubbling rate over a period of time. Try the effect of occasional or continuous stirring and aeration.

Questions :

1. What temperature coefficients are shown by your experiments? What do these tell you about the relative rates of absorption, photochemical reaction, and chemical reactions in your material?
2. What factors contribute to "fatigue" in biological reactions?

THE CHEMICAL PROCESSES OF PHOTOSYNTHESIS

Our knowledge of the reactions involved in photosynthesis is fragmentary and much of it based upon speculation. The temperature coefficient of photosynthesis is ordinarily that of a chemical reaction. We deduce from this that the diffusion and photochemical steps which we know to be involved, and which would be less affected by temperature, are relatively rapid. Experiment 85 should yield data on this problem.

Perhaps the most popular theory of the course of the photosynthetic process involves the production of "active" formaldehyde which is quickly condensed to form sugar or starch. Commercial formaldehyde is not "active" and it may not penetrate the leaf cells readily when supplied from without, but Experiment 86 may still give interesting information on this disputed point.

Experiment 85. The "Dark Reaction" in Photosynthesis. (I)

If the photochemical processes of photosynthesis are relatively rapid and the chemical processes relatively slow, there should be present in the illuminated leaf an accumulation of reduced carbon or its equivalent, which can be combined chemically into sugar without further illumination. Test this hypothesis by measuring the rate of photosynthesis in *Elodea* shoots or in detached sunflower or other large leaves with their petioles in water, illuminated at the same intensity, but one shoot or leaf receiving continuous light and the other darkened for three-fourths or five-sixths of the time.

The experiment should be run for 2 hr., or preferably longer, so that an automatic shading device is required. Attach a black cardboard disk to a small electric motor so that its speed may be controlled. The apparatus illustrated in Fig. 31 is convenient. Four openings to cover one-fourth of the circumference of a 24-in. disk of black cardboard are cut as shown. This disk is rotated in front of the shaded plant material and between it and the light. At 100 r.p.m. the plant is alternately illuminated

for 0.0375 sec. and in darkness for 0.1125 sec. Speeds of 100 to 300 r.p.m. will cover the range of time for the dark reaction given by Emerson and Arnold¹ for temperatures to 20°C. For more rapid alternations, cut six or eight openings in the disk to include only one-sixth of the total area. Do not use a metal disk as it would be dangerous at light speeds.

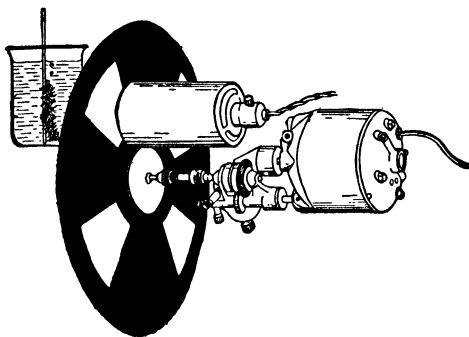


FIG. 31.—Disk for producing flashing light. A disk of cardboard is wired to a small metal plate which is driven by a variable-speed stirring motor.

Expose the check leaves on one side of a 1000-watt electric light in a darkened room. Place the shaded leaves at the same distance on the other side and interpose the revolving disk. Measure photosynthesis by the weight-area method with correction for shrinkage, or count the bubbles given off by a sprig of *Elodea* or *Myriophyllum* in continuous and in intermittent light.

Questions:

1. Would you expect more photosynthesis in a tropical 12-hr. day of intense sunlight or in a northern 16- to 20-hr. day of more diffuse light, assuming that temperature and factors other than light are uniform?

2. From the results of this experiment, would you expect an abnormally high chlorophyll content to increase photosynthesis under normal conditions?

Experiment 86. The Utilization of Formaldehyde by Green Plants. (E)

Hold a number of vigorous shoots of *Elodea* in the dark for 36 to 48 hr. until the chloroplasts are free or nearly free of starch. Divide the shoots into four lots, place two of these in tap water

¹ EMERSON, ROBERT, and WILLIAM ARNOLD. A separation of the reactions in photosynthesis by means of intermittent light. *Jour. Gen. Physiol.* 15: 391-420. 1921.

or dilute nutrient solution, and the other two in the same solution containing 0.02 per cent formaldehyde.¹ Stopper all containers tightly and hold one check lot and one lot with formaldehyde in the dark and the other in diffuse light. Observe the plants for starch accumulation in the plastids and for color and growth, at 1- or 2-day intervals for a week. Transfer to fresh solution at each observation.

Questions:

1. Do your data offer any support for the formaldehyde hypothesis?
2. How does "active" formaldehyde differ from the stable compound?

¹ One-half milliliter of 40 per cent formalin per liter of solution.

CHAPTER VIII

PLANT PIGMENTS

INTRODUCTION

The common pigments of plants may be divided into three groups on the basis of their general properties: (1) the fat-soluble, saponifiable chlorophylls *a* and *b*, (2) the fat-soluble nonsaponifiable carotenoid pigments, such as carotene, lycopene, fucoxanthin, and xanthophyll, (3) the water-soluble red and blue anthocyanins and the water-soluble flavones. The importance of the chlorophylls is obvious. We are interested in any information we can obtain on the formation, constitution, and physiological action of these pigments. Estimations of the chlorophyll content of plants are useful in problems involving photosynthetic efficiency, chlorotic diseases, etiolation, blanching, etc. From a study of the properties of chlorophyll we may hope to arrive at a better understanding of the complex reactions involved in photosynthesis.

The carotenoid pigments have sprung into prominence in recent years as the result of their connection with vitamin A, and methods for the estimation of carotene in food stuffs have become important research tools. Future developments may bring other members of this group into equal prominence, either in connection with animal nutrition, or with their functions within the plant which are at present unknown.

Not enough work has been done on the water-soluble anthocyanins and flavones to establish their physiological significance and consequently they are given only passing attention.

References:

WILLSTÄTTER, R., and A. STOLL. Untersuchungen über Chlorophyll. Berlin. 1913. This work is also available in a translation by F. M. Schertz. Washington. 1928.

PALMER, L. S. Carotinoids and related pigments. New York. 1922.

STILES, WALTER. Photosynthesis. Chapt. III. New York. 1925. Contains a good résumé of the work on chlorophyll.

PROPERTIES OF PLANT PIGMENTS

Experiment 87. The Water-soluble Pigments. (E-I)

a. Cover red leaves and red or blue and yellow flower petals with a little boiling water and observe. Cover thin slices of beets and of carrots with boiling water, and observe fresh sections with a microscope. Which pigments are water soluble?

b. Grind 5 or 10 gm. *Calendula* petals with clean sand or emery and a little acetone, finally adding acetone to make a thin paste. Transfer to a Büchner funnel and wash with 80 per cent acetone, using suction. When you have 50 to 100 ml. of well-colored extract, transfer it to a separatory funnel, add an equal volume of ethyl ether, and water to cause the separation of an ether and a water-acetone layer. The fat-soluble pigments carotene and xanthophyll will be extracted by the ether while the water-soluble pigments will remain in the lower layer. Pour portions of each extract into test tubes and estimate their relative color intensity. Add a few drops of dilute (0.1N) alkali to the water-soluble pigment. Add a drop or two of concentrated HCl. If time permits, repeat with white flower petals and note the effect of acids and alkalis on the water extract.

Questions:

1. List the water-soluble and the fat-soluble pigments common in green plants
2. How are the water-soluble pigments distributed in the plant cell? The fat-soluble pigments?

Experiment 88. The Solubility of Chlorophyll. (E)

Dry several hundred grams of leaves in air. Spread the leaves¹ on a wire tray and dry over a radiator or other source of heat. Complete the drying in a vacuum desiccator or in a vacuum oven at 40 to 50°C. Grind the dried leaves and hold in a stoppered bottle.

Weigh roughly a number of 0.3-gm. samples of the dried leaf powder and place each sample in a test tube. Add 10-ml. portions of the following solvents to the various test tubes,

¹ Willstatter recommends the leaves of nettle (*Urtica* sp.); snapdragon leaves, alfalfa leaves, and tomato leaves are suitable. Avoid woody or gummy materials.

stopper, shake, and observe the solubility of chlorophyll. Use, as available, benzene, chloroform, absolute ethyl and methyl alcohols, pure acetone, ethyl and petroleum ethers, and carbon bisulfide. Use also 80 to 85 per cent ethyl and methyl alcohols and acetone. Estimate the relative green (or yellow) coloring of each solvent and arrange the materials used in the order of their effectiveness as chlorophyll extractors.

Questions:

1. Why is pure acetone used by Schertz for extracting chlorophyll from green leaves, but 80 per cent acetone for extracting dried leaves?
2. Why is acetone rather than the alcohols used to extract chlorophyll in quantitative work?

Experiment 89. The Formation of Chlorophyllide Crystals.

(I)

a. Cover 5 gm. carefully dried nettle-leaf powder from vigorous, active leaves with 15 ml. 90 per cent ethyl alcohol and allow

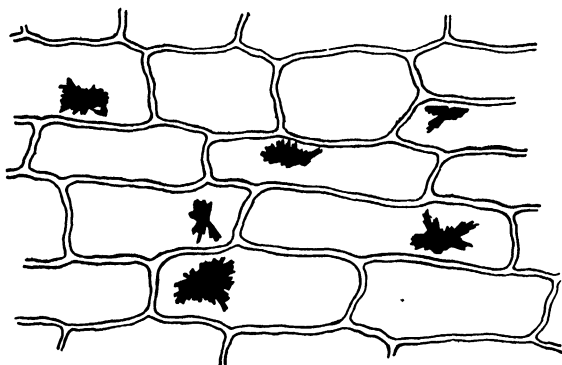


FIG. 32.—Methyl chlorophyllide crystals in the cells of an *Elodea* leaf.

to stand stoppered overnight. Filter with suction and wash the meal with 10 ml. 90 per cent alcohol. Transfer to a small separatory funnel, add 25 ml. ethyl ether, and water until two layers form. Discard the lower layer and wash the ether with a stream of water run down the side of the funnel. Transfer the ether extract of ethyl chlorophyllide to a beaker and evaporate to 2 or 3 ml. on a water bath and away from an open flame. Add 7 to 8 ml. petroleum ether to the warm solution, cover, and allow to stand. The chlorophyllide crystals may be further

purified by redissolving in ethyl ether and recrystallizing from petroleum ether. Observe the crystals microscopically and draw. Dry the crystals with filter paper and observe by transmitted and reflected light.

b. Cover a gram of fresh leaf material with 4 ml. 70 per cent *methyl* alcohol, stopper, and allow to stand. After the leaf material has turned yellow, mount in water and observe crystallized methyl chlorophyllide (Fig. 32). Warm the leaves and crystals in a little methyl alcohol. Elodea, snapdragon, or other thin leaves are suitable for the experiment.

Questions:

1. How are methyl and ethyl chlorophyllides related to chlorophyll?

Experiment 90. The Separation of Chlorophylls a and b. (I)

Shake 50 ml. freshly prepared acetone extract of leaf pigments with 50 ml. petroleum ether in a separatory funnel and add distilled water until two layers are formed. Run off the acetone, wash the ether extract by running a fine stream of water down the side of the flask and discard the washings. Do *not* shake petroleum ether with water or troublesome emulsions will be formed. Extract the petroleum ether with successive 20-ml. portions of 90 per cent *methyl* alcohol until no more color is removed. Chlorophyll *b* is removed in the alcohol together with xanthophyll while chlorophyll *a* and carotene are left in the petroleum ether.

Transfer the xanthophyll and chlorophyll *b* to ethyl ether by adding 50 ml. ether to the alcoholic extract in a separatory funnel and then water until the ether and alcohol separate. Wash the ether to remove alcohol.

Run 2 ml. methyl alcohol saturated with KOH under a few milliliters of each of the extracts in a test tube and observe the color of the ring formed between the solutions and the color after standing 10 min. with shaking.

Saponify the main sample of both extracts by adding 5 to 10 ml. KOH in methyl alcohol to each and shaking for 10 to 20 min. Wash out the saponified chlorophyllides of chlorophylls *a* and *b* with a little water and compare their colors. Use the chlorophyll solutions for Experiment 91.

The orange-yellow solution remaining after chlorophyll *a* is saponified and washed out contains carotene. The greenish-

yellow solution from the chlorophyll *b* extract contains xanthophyll. These solutions may be used for observations of the absorption spectra of these pigments (see Experiment 95 and Fig. 35).

Questions:

1. How do the two chlorophylls differ in appearance?
2. Your ether extracts contained the two chlorophylls in unchanged form. Why were they saponified?

Experiment 91. The Absorption of Light by Chlorophyll. (E-I)

a. Examine with a spectroscope solutions of crude chlorophyll in ether and in alcohol, also the saponified chlorophylls *a* and *b*

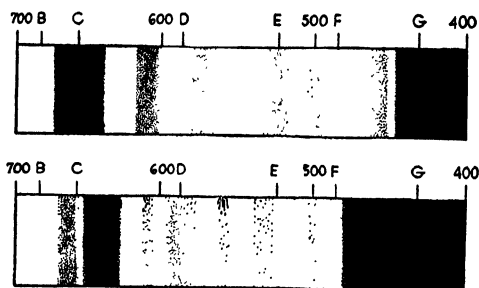


FIG. 33.—Chlorophyll absorption spectra. Chlorophyll *a* above, chlorophyll *b* below. (After Willstätter.)

from Experiment 90. An instrument equipped with comparison prisms and a wave-length scale is preferable. Adjust the concentration of the solutions, the intensity of the light source, and the spectroscope slits to give the maximum sharpness of the absorption lines, particularly the characteristic chlorophyll absorption lines in the red (Fig. 33). Moderate light and solution intensities, with close setting of the spectroscope slit, are desirable.

Plot the spectrum of white light and of the various chlorophyll solutions carefully to scale on cross-sectional paper. Indicate the limits of the various colors of the spectrum by coloring or light shading and the regions of absorption by heavy shading.

b. Remove the air from green and colored leaves or flower petals by weighting them under water and placing in a vacuum desiccator attached to a good pump. Alternately exhaust and replace the air in the desiccator until the material becomes

translucent. Observe the absorption spectra of these translucent leaves and petals when they are placed in front of the spectroscope.

Questions:

1. Why does chlorophyll appear green?
2. How does the spectrum of the leaves compare with that of the various chlorophyll solutions?
3. What differences can you detect in the absorption bands of chlorophylls *a* and *b*?
4. Why do crude (alcohol or acetone) chlorophyll extracts appear to absorb all of the blue and violet light?

Experiment 92. The Substitution of Copper in the Chlorophyll Molecule. (E)

a. Prepare a fresh alcoholic extract of chlorophyll. Add concentrated HCl dropwise until a brownish-green color forms. The HCl breaks down the chlorophyll molecule by removing the magnesium, forming phaeophytin. If this brownish solution is examined with the spectroscope, it will be found to have a new absorption line in the green region. Add a crystal of copper acetate to the phaeophytin solution and heat gently until a bright green color develops. The copper has replaced the magnesium in the chlorophyll molecule. Examine the solution with the spectroscope and save for Experiment 93.

b. Heat leaves in a mixture of 4 parts of water and 1 part of 50 per cent acetic acid saturated with copper acetate, until the green color fades and reappears. Leaves or fruits treated in this way will retain their green color for several years if kept in alcohol or formalin, and the method is used for preserving plant specimens.

Questions:

1. Outline a procedure for producing a fairly permanent *water-soluble* green coloring material from chlorophyll.

Experiment 93. The Stability of Chlorophyll. (E)

Test the effects of light upon the decomposition of chlorophyll in solution. Use fresh acetone-ether and alcohol solutions of chlorophyll and expose them in test tubes to north light and to direct sunlight. Wrap duplicate tubes in heavy black paper to exclude all light and hold with the exposed samples as controls.

Shake two tubes each of the acetone-ether and the alcohol extracts with an equal volume of some purified vegetable oil until the chlorophyll is transferred to the oil. Place one of each of these tubes in each location and compare the rate of decomposition of the chlorophyll in oil and in ether or alcohol. Include also two tubes of the copper-substituted chlorophyll prepared in Experiment 92. The tubes in direct sunlight should be observed at 1-hr. intervals to determine differences in decomposition rates.

Questions :

1. What factors may contribute to the normally yellower color of sun leaves as compared to shade leaves of the same plant?

2. What significance may the protective action of oils have in photosynthesis?

Experiment 94. The Localization of Carotene. (I)

a. Cut thin slices of fresh carrot and treat with a drop of concentrated H_2SO_4 . A deep blue color indicates carotene. Examine a section of carrot microscopically and observe the localization of the orange-red color. If available, observe sections of the green top portion of carrot roots, or expose the tops of carrot roots in the greenhouse, with the roots set in moist sand, until a green color appears and observe sections microscopically to determine the location of the green and yellow colorings.

b. Cover shoots of *Elodea* with a 20 per cent solution of KOH in 40 per cent by volume *methyl* alcohol and hold in a stoppered bottle in the dark for 2 days. Wash the shoots to remove some of the potash and mount a leaf in water or glycerin. Carotene appears in slender, frequently clustered, orange-yellow crystals (Fig. 34). Typically the crystals are grouped as shown in the figure but in scattered areas so that a careful search may be required to locate them.

Questions :

1. Where is the carotene located in plants?

2. What chemical reactions are involved in the method of observing carotene in the leaves?

Experiment 95. The Purification of Carotene. (I)

Grate about 300 gm. fresh carrots, or drain and pulp the canned product, and extract thoroughly with 500 to 1000 ml. acetone and

150 ml. petroleum ether. Transfer the extract to a separatory funnel, add water if necessary to separate the ether, discard the acetone, and wash the petroleum ether extract of carotene and xanthophyll, first with water to remove acetone and then with

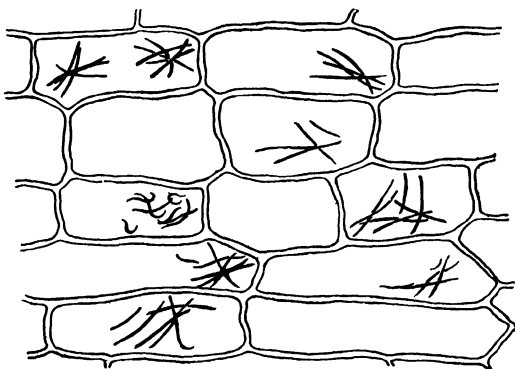


FIG. 34.—Carotene crystals in an *Elodea* leaf.

80 per cent *methyl* alcohol to remove the xanthophyll. Avoid shaking which will form emulsions. Concentrate the petroleum ether to 5 ml. at 50°C in a vacuum and take up in 25 to 50 ml. 95 per cent ethyl alcohol. Carotene crystallizes out on standing in the cold. Filter out the crystals and wash rapidly with a

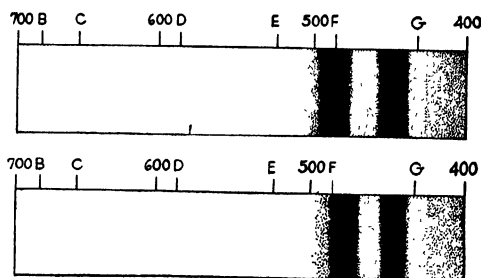


FIG. 35 —Absorption spectra for carotene and xanthophyll. Carotene above, xanthophyll below. (After Willstätter.)

few milliliters of a mixture of two parts of petroleum ether and one part of 95 per cent alcohol.

Examine and draw the crystals and record their color by reflected and transmitted light. Dissolve some of the crystals in petroleum ether and determine the absorption spectrum with the apparatus and method used for chlorophyll (see Fig. 35).

Or compare the absorption spectra of the carotene and xanthophyll solutions from Experiment 90.

Questions:

1. Why has carotene been so extensively investigated?
2. What optical effect gives carotene its yellow color?

THE FORMATION OF PLANT PIGMENTS

Experiment 96. The Relation of Light to Pigment Formation.
(I)

a. *Anthocyanins*.—Observe such plants as are available and determine whether red and purple leaf and stem colors are best developed in shaded or exposed locations. Can you tell whether pigmentation is dependent upon direct sunlight or favored by the normally higher carbohydrate levels of exposed leaves? Sprout rural potatoes or tubers of some pink-skinned variety in the dark and in the light. Is the blue or pink sprout pigment dependent upon light for its formation?

b. *Carotene*.—Germinate oats, broad beans (*Vicia faba*), or white corn in complete darkness and test the sprouts for carotene by extracting with acetone and petroleum ether and then washing the petroleum ether with 80 per cent methyl alcohol according to the outline given in Experiment 95. The presence of a yellow coloring in the washed petroleum ether may be taken as an indication of carotene. Is xanthophyll present?

c. *Chlorophyll*.—Germinate corn or oat seeds in complete darkness until the shoots are 2 to 3 cm. long. Prepare six or more large test tubes with a pad of wet cotton in the bottom of each; place two seedlings in each tube, and plug with dry cotton to retard evaporation without excluding oxygen. Set up the experiment in the morning to permit adequate light exposures and determine the time in minutes for the formation of a visible green color in the plants when exposed: (1) to full greenhouse light, (2) to north laboratory light, (3) to artificial light of normal reading intensity (10 f.c.). Tightly cover duplicate tubes with heavy black paper and place in each location as controls. If possible, determine the light intensity in foot candles at each location.

As an interesting variation of this experiment, expose etiolated plants, in test tubes with a little wet cotton, for periods of 0,

1, 5, 15, 60, and 240 min. to a light intensity of 1000 to 2000 f.c.¹ and then hold in a dark chamber. Observe chlorophyll development in the various plants after 24 hr.

Questions:

1. Why can vegetables be blanched by covering?
2. How are plants benefited by the light response in chlorophyll formation?

Experiment 97. The Relation of Wave Length of Light to Chlorophyll Formation. (I)

If light filters are available, determine the time for visible formation of chlorophyll in light of varying wave lengths. If no filters are available, use colored cellophane or make red and blue filters with sodium dichromate and copper nitrate solutions. A solution of 500 gm. sodium dichromate in 1 l. of water removes violet, blue, and most of the green from sunlight, transmitting red and yellow. A 1.5-cm. layer of the solution will transmit 30 to 35 per cent of the visible portion of sunlight. A filtered $\text{Cu}(\text{NO}_3)_2$ solution of the same concentration transmits the light absorbed by the chromate solution and absorbs the red and orange. A 1-cm. layer will transmit 25 to 30 per cent of the visible portion of the sun's spectrum. The copper solution is expensive; do not waste it. The solutions may be poured into special flasks or double-walled vessels, or they may be placed in large Petri dishes and sealed over light-tight boxes containing the moist plants. Or the tubes may be wrapped in colored cellophanes of known transmission spectra. Amber cellophane will usually screen out blue and violet light. Red may need reinforcing with amber; orange-red is better than a darker purplish red. Blue and green cellophanes must be studied spectroscopically to determine their value. The light transmitted by the various screens should be as nearly equal as possible and the absorption spectrum of each screen or solution should be plotted.

Questions:

1. What light colors are transmitted by the screens you used?
2. What wave lengths of light are responsible for chlorophyll formation?

¹ Under one or two layers of cheesecloth in full sunlight, or 20 to 30 cm. from a 1000-watt Mazda lamp. Use comparable light for all exposures and measure the actual intensity if possible.

Experiment 98. Photosynthesis and Chlorophyll Formation.
(I)

Place etiolated corn or oat seedlings in a small beaker and set the beaker with its contained plants into a small desiccator or other closed container, the bottom of which is covered with barium hydroxide solution. Compare the time for chlorophyll formation in the CO₂-free container with the time in a similar container with normal air.

Questions :

1. Is chlorophyll formation dependent upon carbon assimilation or conditions favorable for this process?

Experiment 99. Oxygen and Chlorophyll Formation. (I)

Displace the water in an inverted bottle with hydrogen from a Kipp generator (Fig. 44, page 155) charged with zinc, or connect two 100-ml. rubber-stoppered bottles with two pieces of rubber tubing. To one bottle add 2 gm. pyrogalllic acid and 10 gm. KOH in 60 ml. water. Connect the bottles and pour the pyrogallol solution from one bottle to the other several times. Admit air carefully to replace the absorbed oxygen and repeat. Drain the solution to the lower bottle, clamp the rubber connections, disconnect the lower bottle, and replace with another completely filled with freshly boiled distilled water. Transfer the oxygen-free air to the clean bottle, again disconnect under water with the clean bottle inverted, insert a pad of freshly boiled cotton and four seedlings, and stopper tightly. Or place the cotton pad and seedlings in the bottle of hydrogen. The bottles must be carefully handled and tightly stoppered to prevent the admission of oxygen. Hold the culture in strong diffuse light and observe rate of chlorophyll formation. Compare with controls in cotton-plugged bottles. If chlorophyll develops in the minus oxygen culture, repeat the experiment taking greater precautions to exclude oxygen.

Questions :

1. What does this experiment tell you about the method of chlorophyll formation?

2. Would you expect chlorophyll to be formed readily in a cell whose food supply had been exhausted? Why?

Experiment 100. Temperature and Chlorophyll Formation. (I)

If controlled temperatures of 0, 5, 10, 15, and 20°C. are available with artificial light of 25 to 100 f.c., determine the time for visible chlorophyll formation at each temperature with uniform light. Compare etiolated corn or melon seedlings with radishes or peas. Be sure that the seedlings are protected from drying.

Questions:

1. How do you explain the observed temperature responses in chlorophyll formation?

Quantitative methods for chlorophyll *a* plus *b*, carotene, and xanthophyll are given in Loomis and Shull, *Methods in Plant Physiology*.

CHAPTER IX

PLANT FOODS

INTRODUCTION

The raw materials of plant food production, which we studied in Chapt. IV, are sometimes called "plant foods" or "plant food elements." Because one automobile manufacturer has his own blast furnaces, we do not say that his cars are made of iron ore while those of his competitor who owns no furnaces are made of steel. Similarly, the fact that plants may manufacture their own foods should not obscure the important point that the foods actually used by the cells of plants and animals are identical. Sugars, oils, proteins, are plant foods just as they are animal foods. The difference lies in the ability of many plants, under certain conditions and in certain tissues, to manufacture these foods themselves. The raw materials of food manufacture we call nutrients, and we define a food as an energy-containing organic compound which can be used, either directly or after digestion, in cell metabolism.

Plant foods are stored in various plant tissues when they accumulate in excess of the daily needs of the plant, and these stores may be drawn upon later to carry the plant through unfavorable seasons, to meet unusually large food uses in reproduction, or to replace leaves or roots. Incidentally these stored foods give plants their economic value as food for man and animals. Try to recall a few important crop plants that are not grown for food stored in roots, stems, leaves, or fruits and seeds, and you will see the importance of studies such as are outlined in the following experiments.

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CURTIS, O. F. The translocation of solutes in plants. New York. 1935. Critical discussion of solute movement in plants.

THE IDENTIFICATION OF PLANT FOODS

The following experiments give simple tests for the more important food materials. For more extensive tests, or for tests of minor food substances and for intermediate products of synthesis and metabolism, the student is referred to Molisch.¹

Experiment 101. Sugars in Plants. (E-I)

Collect several types of plant tissue, if possible from plants showing varying growth responses, and test for sugars.

a. *Macro-tests*.—Grind 5 to 10 gm. of fresh tissue with sand, a pinch of CaCO_3 , and a little ether; transfer to a beaker with 50 ml. water and heat carefully with stirring to 70°C . to increase the permeability of the cell membranes of the tissue. (Heating above 75 to 80°C . will gelatinize starch and bring interfering substances into suspension.) Cool, add three to five drops of a saturated solution of normal (neutral) lead acetate, filter the extract, and use for sugar tests.

Reducing substances give a red precipitate upon heating with Fehling solution. Mix 10 ml. of the sugar extract with 10 ml. of a mixture of equal parts of Fehling A and Fehling B (see page 203), and bring to a boil. A red or orange precipitate indicates a positive test. An estimate of quantity may be made by comparing the amount of precipitate produced by the different extracts.

Sucrose gives a negative test before hydrolysis. To test for this substance add 1.0 ml. concentrated (37 per cent) HCl to 10 ml. of the sugar extract and heat on a 70°C . water bath for 5 min. after the temperature of the solution reaches 67°C . Cool quickly, nearly neutralize with Na_2CO_3 , and test for reducing substances as above. The gain in reducing substances with hydrolysis may be taken as sucrose.

¹ MOLISCH, HANS. *Mikrochemie der Pflanze*. Jena. 1923.

b. *Micro-tests*, for determining the tissues in which the various sugars are held, are made with sections of the plant materials used above. Mount thin sections in a drop or two of 15 to 20 per cent NaOH containing a few crystals of copper tartrate.¹

Fructose gives a very fine yellow-red crystalline precipitate at once and without heating (Fig. 36).

Glucose forms additional crystals on gently warming the slide over a micro-burner or a beaker of boiling water.

Sucrose gives no precipitate until warmed with dilute acid when it shows tests for both fructose and glucose.

None of the micro-tests give cell by cell localization of sugars because of the ready solubility of these compounds, but it is possible to dissect out small pieces of various tissues and by testing these separately, to obtain fairly good pictures of sugar distribution within the plant.

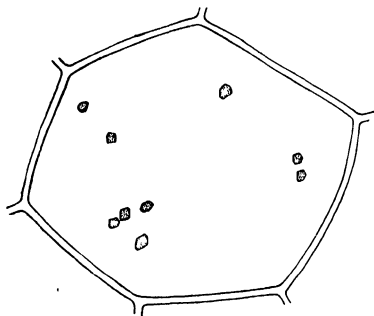


FIG. 36.—Cuprous oxide crystals in plant tissue.

Questions:

1. How common are the three sugars in the materials tested by the class?
2. What advantages do sugars have as storage substances? What disadvantages?
3. Show by chemical equation the change which occurs when sucrose is hydrolyzed.

Experiment 102. The Storage of Starch. (E)

Starch is one of the common forms of stored food and is easily detected by staining with dilute iodine solution.² Mount sections of soaked seeds, tubers, roots, herbaceous and woody stems, etc., in water and add I-KI solution from the edge of the cover glass. Starch stains first violet and then dark blue in clear-

¹ To prepare copper tartrate, dissolve 30 gm. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 300 ml. hot water, and 70 gm. sodium-potassium tartrate in 200 ml. hot water; mix the two solutions, filter out the precipitate, dry in air and then in a desiccator, and hold in a brown bottle.

² Iodine is nearly insoluble in water, but is slowly soluble in potassium iodide solution. Prepare I-KI solution by mixing 0.3 gm. iodine crystals, 1.5 gm. KI, and 100 ml. of water.

cut, granular bodies. Starchy dextrans stain lavender in amorphous masses, and a few substances cause blue staining of the cell walls. Compare the starch content of plants making a weak growth in heavy shade, with those stunted by being pot-bound in good light.

If you did not observe starch grains within the chloroplasts of *Elodea* in your study of photosynthesis, mount a leaf of this plant, which has been exposed to good light, in I-KI solution and study the staining of the starch grains in the midrib cells at the base of the leaf. Where are the starch grains located? Cut thin slices through "greened" potatoes, mount in water, and observe the effect of I-KI when added from the side.

Grate a raw potato and wash a little starch through folded cheesecloth. Cover about 0.25 gm. of starch with 50 ml. H_2O ; add 5 ml. concentrated HCl , and boil under a reflux for 2 hr. or autoclave at 15 lb. for 30

min. Cool, nearly neutralize with Na_2CO_3 , filter, and test for reducing sugars with Fehling solution.

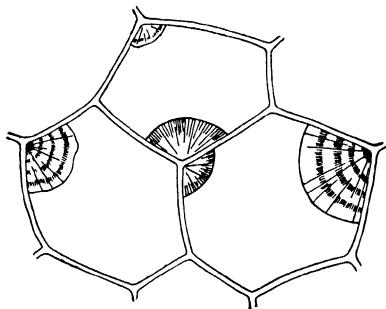


Fig. 37.—Inulin crystals in tuberous root of dahlia.

Questions:

1. Where are starch grains formed: (a) in the leaves, (b) in the potato?
2. What are the advantages of starch as a storage form?
3. Show the changes occurring when starch is digested.

Experiment 103. The Storage of Levulosans. (E)

Recent experiments on the commercial production of fructose from the common artichoke (*Helianthus tuberosus*) for the use of diabetic patients have focused attention upon these storage carbohydrates characteristic of many of the Compositae.

Mount thin sections of dahlia roots in alcohol and observe first the formation of a milky precipitate and then the production of spherocrystals soluble in warm water or dilute acids. Well-developed crystals may be formed by steeping slices of an inulin-containing tissue in 95 per cent alcohol for several days or longer (Fig. 37). Test for solubility in hot water.

Questions :

1. How does inulin differ from starch in structure and reactions?
2. What glucose polymers are comparable to levulin?

Experiment 104. Cell-wall Materials. (I)

a. Cellulose.—Mount sections in I-KI solution and observe any blue color produced by starch, amyloid, etc. Add a drop of 75 per cent H_2SO_4 from the side of the cover glass. Cellulose membranes swell and become blue. Compare young and older, lignified cells.

b. Pectic Substances.—Pectic substances stain red with ruthenium red (1 to 10,000). Cover for 20 min. and wash. Keep the stain in the dark and make up fresh as needed; it is expensive and should not be wasted.

Methylene blue stains pectic substances violet; other membrane substances blue or green.

Study fruit tissues and such stems as are available.

c. Lignin.—Place sections on a watch glass in 1 per cent potassium permanganate solution for 3 to 5 min. Dip in water and hold in dilute (1 + 20) HCl for 2 min. Wash and mount in 2 per cent NH_4OH . Lignin is brown to red.

Or soak for a minute in a 2 to 5 per cent solution of phloroglucinol in alcohol or water, and mount in a drop of concentrated HCl. Lignin is red to red-violet.

Compare young and old tissues and strongly “differentiated” stems with those relatively undifferentiated.

Questions :

1. What role do these substances play in the plant?
2. Are any of them digested by animals? By green plants?

Experiment 105. Plant Proteins. (E)

Sections to be stained for proteins should first be washed in warm 95 per cent alcohol to fix proteins and remove soluble substances.

a. Eosin.—Place sections in *dilute* eosin solution for 10 min., wash, and mount in glycerol. Proteins stain red. Look for leucoplast staining at the surface of starch grains. If membranes stain, use a more dilute solution of eosin.

b. Biuret Reaction.—Treat sections for 30 min. with 5 per cent CuSO_4 solution; wash thoroughly with water and mount in

50 per cent KOH or NaOH. All substances containing a biuret group (CONH_2) stain red to blue-violet.

c. *Millon Reagent*.¹—Mount sections directly in reagent; if necessary, warm gently. Proteins containing tyrosin stain vermilion red. Many aromatic compounds also stain, but most of these are removed by preliminary washing with alcohol.

Study proteins in seeds and meristematic tissues. Older vegetative tissues commonly contain so little protein as to give unsatisfactory tests. Leaves are better than stems.

Questions:

1. What types of tissues are high in proteins?
2. Amino acid formation is limited to plants and it is only certain seed proteins that are "incomplete." Why do we not make more use of plants as a source of protein in human diet?

Experiment 106. Oils and Waxes in Plants. (E)

a. Study sections of gladiolus, onion, citrus, lettuce, dandelion, or other leaves; also onion scales and fatty seeds. Place sections in a solution of Sudan III (0.5 per cent in 70 per cent ethyl alcohol), and allow to stand for 20 min. Wash with 50 per cent alcohol and observe for red stained oil drops.

b. Mount cross sections of older monocot roots in Sudan III and observe suberization of endodermal and epidermal layers.

If tissues containing stored oils or wax-impregnated cells are treated with concentrated H_2SO_4 , all of the cell constituents except oils and wax-protected tissues are dissolved. H_2SO_4 makes a particularly good demonstration when used after Sudan III on fatty seeds.

Questions:

1. What advantages does oil have as a storage food?
2. How are plant oils changed commercially into compounds such as lard substitute?

THE MOVEMENT OF FOODS IN PLANTS

The mechanism of the translocation of foods in plants is one of the most interesting unsolved problems of plant physiology.

¹ Dissolve 1 ml. mercury in 9 ml. concentrated nitric acid (under hood) and dilute with an equal volume of water. Add a small crystal of KNO_3 to old Millon reagent to restore its activity.

The experiments of Curtis¹ and of Maskell and Mason² have established the phloem as the tissue normally concerned with both upward and downward movement of plant foods. Their results indicate that under some conditions foods move along a positive gradient, that is, from points where they are manufactured or formed in digestion and are, therefore, high in concentration, to points where they are used and are, therefore, low in concentration. Movement is, however, much more rapid than could be expected on the basis of unaided diffusion and it is stopped by conditions interfering with the vital activities of the phloem. Moreover, Phillis and Mason,³ and Loomis⁴ have shown that sugars may move at times against steep concentration gradients. It should add to the interest of the experiments in this section to know that many of them are capable of yielding new knowledge on controversial and unsolved problems.

Experiment 107. Translocation from Germinating Seeds. (I)

Sprout 60 to 100 beans or peas or the same number of grains of corn in moist sphagnum and transfer to paraffined wire screens over a mixture of nine parts distilled water and one part boiled, cooled, and filtered tap water. This mixture is used to obtain a low mineral content in a nontoxic or only slightly toxic solution. Allow the seedlings to grow in complete darkness until they begin to show evidences of starvation. Be sure to save the shriveled cotyledons of the bean plants.

Soak a second lot of bean or pea seeds until the seed coats can be removed and determine the dry weight of an average embryo (less testa). If corn is used, dry the entire fruit without removing the pericarp.

¹ CURTIS, O. F. Studies on solute translocation in plants. Experiments indicating that translocation is dependent on the activity of living cells. *Am. Jour. Botany* **16**: 154-168. 1929. Also earlier papers.

² MASON, T. G., and E. J. MASKELL. Studies on the transport of carbohydrates in the cotton plant. II. The factors determining the rate and direction of movement of sugars. *Ann. Botany* **42**: 571-636. 1928.

³ PHILLIS, E., and T. G. MASON. Studies on the transport of carbohydrates in the cotton plant. III. The polar distribution of sugar in the foliage leaf. *Ann. Botany* **47**: 585-634. 1933.

⁴ LOOMIS, W. E. The translocation of carbohydrates in maize. *Iowa State Coll. Jour. Sci.* **9**: 509-520. 1935.

As soon as the seedlings have made their maximum growth upon the stored food of the seed, separate cotyledons or endosperm from seedling and determine separately the average dry weight of each and the gains or losses per seed and per seedling.

Arrange your data to show the percentages of the dry matter originally present in the seed that are: (1) translocated to the seedlings, (2) left in the seed, (3) lost by respiration or otherwise.

Questions :

1. Why cannot all of the dry matter be removed from endosperm or cotyledons?

2. Why is the dry weight of the germinated seed and seedling less than that of the original seed?

Experiment 108. Translocation from Leaves. (I)

Choose several seedling kidney bean plants on which the first pair of simple leaves is well developed, but which do not yet show extensive development of the plumule bud. Expose these plants to bright sunlight until the leaves show heavy starch accumulations on test with iodine. Treat one petiole of each pair of leaves by one of the following methods, leaving the second petiole and leaf intact as a check. Try:

a. Cutting through one petiole and holding the cut leaf in water for comparison with the check leaf.

b. Vaselining one petiole heavily to reduce the rate of gas exchange and slow down respiratory activity in the petiole.

c. Killing a section of one petiole by steaming (compare Experiment 14). Support the leaf with sticks and string before treating, and kill the petiole section with a jet of steam piped from a flask of boiling water. Avoid heating any other portion of the plant.

d. Anaesthetizing a section of one petiole by slipping a short piece of 12- to 15-mm. tubing over the leaf, supporting the tube with a stand, and plugging it with split corks. Enclose a piece of cotton wet with ether and seal with vaseline.

Hold all the plants in the dark in a moist chamber at 20°C. for 24 hr.; remove the chlorophyll from the leaves with alcohol and stain them with I-KI to show starch. Marked loss of starch from a leaf is taken as evidence of translocation, and the abnormal

retention of starch in a leaf as evidence of interference with translocation.

Questions:

1. What theory of translocation best fits your results?
2. How would each of the treatments affect translocation according to this theory?

Experiment 109. Translocation and Growth.¹ (I)

A number of experiments upon the effect of interrupting the phloem upon growth may be performed as time permits.

a. Study the effect of ringing upon the growth of young apple, orange, or other fruits. Measure the circumferences of 10 to 20 rapidly growing fruits, tag half of them as controls, and isolate the others from any phloem connections with the leaves by defoliating the fruiting twigs and carefully scraping away the phloem from a 1-cm. strip at a point below the fruit. If desired, the twig may be ringed above and below the fruit and leaves left at the tip of the twig, but separated from the fruit by a phloem ring. Cover all rings with melted paraffin; observe at intervals to be sure the phloem is not replaced by new growth, and compare the growth rates of the control and ringed fruits.

b. The effect of ringing upon apical growth may be observed by measuring the elongation of four sets of shoots during early spring growth. Treat the four sets as follows: (1) controls, no treatment except to measure growth in length; (2) all leaves removed as fast as formed, measuring as before; (3) the phloem scraped away carefully at a point 6 to 10 cm. from the tip of the shoot and the wound covered with hot (95°C.) paraffin; (4) the phloem removed as in (3) and leaves continuously removed as in (2). Ringing must be carefully done and twigs on which the rings callous over must be discarded.² Record the growth in millimeters over a period of 2 to 4 weeks of the twigs receiving each treatment.

Questions:

1. Explain the effect of cutting the phloem upon growth.

¹ CURTIS, O. F. Translocation in plants. Chapt. II. New York. 1935.

² CURTIS, O. F. The upward translocation of foods in plants. I and II. *Am. Jour. Botany* 7: 101-124; 286-295. 1920.

CHAPTER X

RESPIRATION

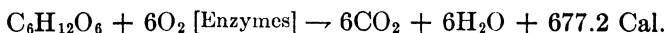
INTRODUCTION

The production of foods by green plants is of such fundamental economic importance and seems to differentiate these organisms so sharply from animals that we sometimes forget the many basic similarities of all forms of life. Any characterization of the living state is based upon the ability possessed by all living organisms to liberate and utilize energy. Moreover, the continuance of life depends upon a constant supply of energy to every living cell—in the dormant kernel of wheat as well as in the straining athlete. Scientists discredit reports of the finding of living seeds thousands of years old, for they know that if the energy-producing machinery of the seed is slowed down too much it will stop completely and the seed will be dead, and that, if it is maintained at a high speed, the food supply will be exhausted and the seed will starve.

The process by which energy is made available for the use of plant or animal cells we call respiration. In its basic outline, respiration is the reverse of photosynthesis. We said that photosynthesis can be represented by the equation:



Conversely, respiration may be represented empirically by the equation:



The enzymes of the second process are different; the direction of the action is reversed and, most important, the energy which was originally the radiant energy of sunlight has now become usable chemical energy, available alike to roots and tops, by day or by night.

Our empirical and ideal respiration equation calls for the utilization of a hexose sugar as the source of energy. Many

other substances may be used, either directly or after being changed to hexoses. Our equation requires the consumption of six mols of free oxygen and the production of six mols (an equal volume) of carbon dioxide. Actually, combined oxygen may be used instead of free oxygen, and the ratios of the two gases may vary with the compounds being oxidized and with the various side reactions which may be proceeding simultaneously within the tissues. The quantity of energy liberated will vary also with the energy content of the oxidized compound.

As with photosynthesis, the "enzymes" bracket covers the unknown factors. It is sufficient to note here that the sugars are exceedingly stable compounds under ordinary conditions and no enzymes are known which will oxidize them directly. Molecular oxygen too is a relatively inert compound, and much of the mechanism of respiration consists of changing both the sugar and oxygen molecules into more reactive forms.

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DEMONSTRATIONS OF RESPIRATION

Experiment 110. The Production of Carbon Dioxide by Plants. (E)

A rough measure of relative CO_2 production may be obtained by measuring the time for the production of a white precipitate in a barium hydroxide solution. Place samples of plant materials into Mason jars or similar containers and seal with metal lids of the Kerr Mason type or with two-hole rubber stoppers fitted with rubber tubing and pinch clamps. Use such materials as dry grain, soaked grain, tubers or corms, fresh green leaves, etc.; 100 gm. green materials or dry seeds and 100 gm. dry seeds before soaking for the soaked grain samples. Allow the CO_2 formed by the plant materials to collect in the jars for 4 to 16 hr. Place two samples of green leaves in sealed jars containing a

little water, and hold in the dark for 4 to 12 hr. Transfer one of the jars to diffuse sunlight for 2 to 4 hr. before testing both containers for accumulated CO_2 .

Arrange the apparatus as shown in Fig. 38. Fill several medium-size test tubes with clear barium hydroxide solution and stopper until needed. The experimenter draws air from the plant chamber at a *uniform* rate through the barium solution, and the time to produce a clearly visible barium carbonate precipitate is recorded. Run check determinations on the laboratory air and on expelled breath.

The plant containers are shaken to equalize their CO_2 content and a capillary tube is then passed to the bottom of the container through a hole punched in the tin lid or through the stopper as shown in Fig. 38. If the sample is withdrawn carefully, it will not be seriously affected by the air with which it is replaced. The CO_2 content of the gas sample is inversely proportional to the time for a clearly visible precipitate formation. Laboratory air is approximately 0.04 per cent CO_2 .

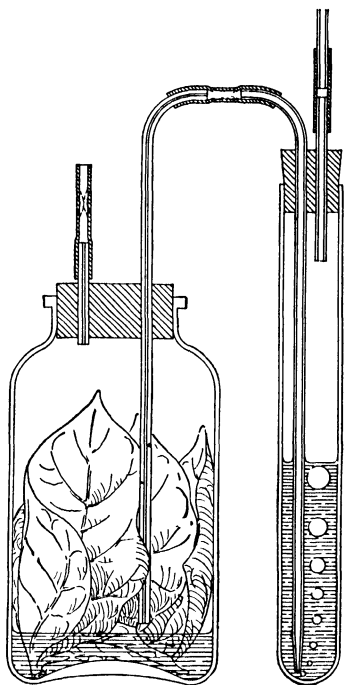


FIG. 38.—Apparatus for demonstrating respiration. Air from the bottle containing the plant material is bubbled through the barium hydroxide solution in the tube.

Questions :

1. Why do dry seeds store better than moist seeds?
2. Do leaves carry on respiration in the light?

Experiment 111. The Production of Heat in Respiration. (E)

When respiration rates are high and particularly when a part or all of the respiration is due to fungi, much of the energy liberated may be wasted in the form of heat.¹ Measure the

¹ GILMAN, J. C., and D. H. BARRON. Effect of molds on temperature of stored grain. *Plant Physiol.* 5: 565-573. 1930.

temperatures reached by nonsterile cultures of oats packed in thermos bottles.

Fill one bottle with air-dry grain, insert a thermometer, and plug with cotton. Soak in warm water (40°C.) for 15 min.

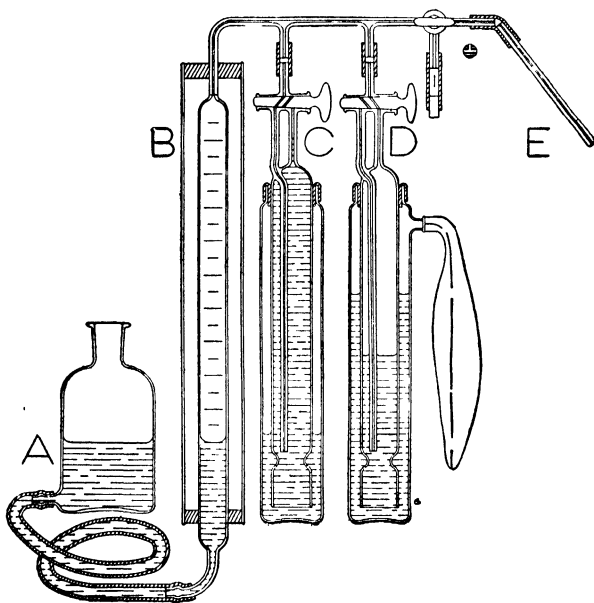


FIG. 39.—Haldane gas analyzer. The gas is measured in *B*, CO_2 absorbed in *C*, and O_2 in *D*.

enough grain to fill two bottles, drain, cool to room temperature, and pack into the thermos bottles, stoppering one tightly with a rubber stopper and plugging the other loosely with cotton. Thermometers should be inserted into both cultures. Record the temperatures of the grain within the bottles and of the laboratory air daily for a week and plot temperatures against time. If a Haldane gas analyzer (Fig. 39) is available, analyze samples of gas from the three bottles at the end of the experiment.¹

¹ The Haldane gas analyzer illustrated in Fig. 39 is very useful in respiration experiments and should be used, when available, by intermediate students or demonstrated by the instructor in elementary sections.

To use the analyzer, add enough 20 per cent NaOH solution to *C* to fill as shown. Add the same quantity of a mixture of one part pyrogallol, five parts stick NaOH, and 30 parts of water, to tube *D*. Remove the air from *C* and *D* (see tube *C* in figure) and close the stopcocks. Drive the air

Record also the odor and condition of the grain from the bottles.

Questions:

1. Why does moisture increase the heating of grain and hay?
2. What is the effect of stoppering the bottle? What use is made of this principle?

Experiment 112. Weight Losses in Respiration. (E)

Use your data from Experiment 107, or, if that experiment was not performed, germinate wheat or corn in sphagnum moss and transfer to paraffined netting over a mixture of nine parts distilled water and one part boiled tap water as soon as the seedlings are large enough to handle. Grow the plants in complete darkness for 2 to 3 weeks and then determine separately the average wet and dry weight of plant and seed residues. Compare with the average dry weight of seeds like those used in the experiment.

Questions:

1. Have your seedlings "grown"?
2. Why do grain dealers allow for a monthly shrinkage in the *dry weight* of their holdings?
3. Do seeds high in fats always yield results similar to those high in carbohydrates? Why?

from the pipette *B* through *E* and close the stopcock to *E*. Now attach *E* to the source of gas to be analyzed, open the cock with notch up as shown, and lower *A* to fill the pipette *B* as shown in the figure. Close the stopcock to *E* (notch to right), raise the leveling bottle slightly to prevent drawing any solution from *C*, and open the stopcock on *C* as shown in the figure to admit the gas at the bottom of the absorption pipette. Raise the bottle and bubble the gas through the NaOH to remove the CO₂. When all the gas has been driven over, turn the cock as shown in *D* and draw the gas back into *B*. Repeat this process until a constant volume of gas is obtained and read the new volume in *B* with the water level in *A* and *B* equal. Loss in volume, when temperature changes are guarded against, represents CO₂. Repeat with the pyrogallol tube to remove the oxygen from the gas. Note that oxygen found is *unused* oxygen; subtract unused oxygen from the original oxygen percentage (percentage in air) to obtain oxygen *used*. The rubber bag attached to *D* serves to protect the pyrogallol solution from the oxygen of the air.

A D'Orsat gas analyzer is used in the same way except that plain stopcocks at *C* and *D* make it impossible to bubble the gases through the absorbing solutions.

Experiment 113. Respiration in the Absence of Oxygen. (E)

Animals or green plants will soon die in the absence of molecular oxygen, but many bacteria are poisoned by free oxygen and carry on respiration by the processes known as fermentation. Other bacteria and molds are able to use molecular oxygen or to live for extended periods on the energy obtained by fermentation.

Fill three fermentation tubes (Fig. 40) with 10 per cent solutions of glucose, sucrose, and lactose, respectively, and inoculate each with a small quantity of yeast. Additional tubes may be used for such other carbohydrates as are available. Observe for fermentation and the production of gas in the back arm of the tube.

Test the gas formed by adding a small piece of solid NaOH to the solution and stirring carefully to avoid introducing air.

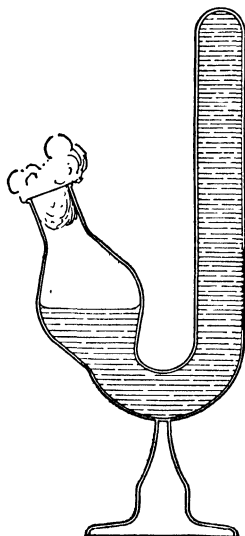


FIG. 40.—Fermentation tube. Carbon dioxide produced by fermentation collects in the back arm of the tube.

Questions :

1. What reaction has occurred in your tubes?
2. How does the energy released by fermentation compare with that liberated in oxygen respiration?

Experiment 114. Respiration in Roots. (E)

Carefully wash out corn or other small fibrous-rooted plants and place the roots in a bottle containing water made slightly alkaline with dilute NaOH solution and colored red with phenolphthalein. Prepare a second bottle in the same way, stopper tightly, and leave without a plant. Allow both bottles to stand in diffuse light and examine the solution after 12 to 24 hr. What has happened? Why? Pour some of the solution from the bottle containing the plant into a white porcelain dish and boil gently for a few minutes. Explain. Blow through the solution until it changes color and boil again.

Questions :

1. What acid is produced by the roots?
2. Would this acid be of any assistance in mineral absorption by roots?

THE MEASUREMENT OF RESPIRATION

Carbon dioxide production is the most common measurement of respiration although Experiment 115 will show that the consumption of oxygen also should be known for an accurate interpretation of the results. Determinations by the Warburg method are sometimes calculated from oxygen consumption alone. Loss in total dry weight is a possible measure for nonfatty tissues, but it is less sensitive than the gas methods and is not commonly used.

Experiment 115. The Respiratory Quotient. (I)

The respiratory quotient is the ratio of volume of CO_2 produced by a respiring tissue to the O_2 consumed— CO_2/O_2 . The respiratory quotient in combination with the CO_2 production in milliliters of CO_2 per gram dry sample per hour, or other units, gives a fairly adequate picture of respiration rates and processes.

Seal duplicate 50- to 100-gm. samples of plant material, whose moisture content is known, into mercury-sealed respiration chambers (Fig. 41). Carbon dioxide is lost through rubber or wax so that a mercury-to-glass seal is required for measurements of the accumulation of CO_2 . The chamber is made by cutting away the top of a *sheet-iron* Mason-jar cover and soldering a sheet-iron ring 4 to 5 cm. wide over the remaining band. Zinc rings cannot be used as they are soluble in mercury. *Solder also* is soluble, and the finished ring must be dipped into hot asphalt to cover all solder, and quickly screwed onto a 1- or 2-qt. Mason jar. The asphalt should form a tight seal between jar and ring without covering the top edge of the jar. A No. 12 stopper is used with capillary-tube connections as shown. Mercury is poured over the stopper after the apparatus is assembled, sealing it against loss of CO_2 . The tubes also are sealed: *A*, by dipping into mercury, as shown and, *B*, by allowing a little mercury to be drawn above the clamp. Or, *B* may be sealed with rubber tubing and pinch clamp alone in less accurate experiments involving respiratory quotients only. If the services of an expert glassblower are available, difficulties due to mercury-soluble metals may be eliminated by sealing flaring glass collars onto wide-mouth liter Erlenmeyers. Arrange stopper and tubing as shown in Fig. 41.

At intervals of 2 to 4, 8 to 16, 24 to 48, and 72 to 96 hr., withdraw 25- to 50-ml. samples of gas from the chamber and analyze for CO_2 and O_2 . To withdraw gas samples, connect a mercury-filled gas-transfer pipette to the tube *B* with a short piece of capillary tubing. Withdraw the desired sample, thus lowering the gas pressure in the chamber and causing mercury to rise in the arm *A*. A reduction of 10 to 15 per cent in gas pressure

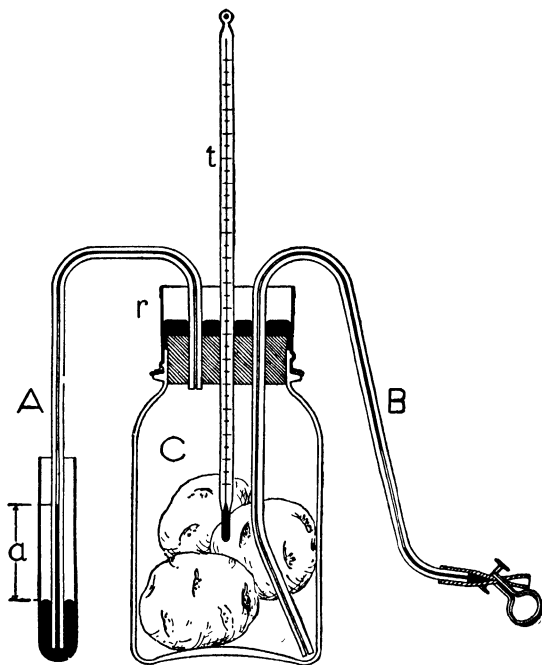


FIG. 41.—Respiration chamber. Mercury seals are required to prevent diffusion of oxygen or carbon dioxide through the rubber connections.

within the chamber will not affect the course of the experiment significantly, but may be reduced by using a 2-qt. instead of a 1-qt. Mason jar. Return the sample to the chamber several times to mix the gases and insure a representative sample. After removing the gas sample, reseal the tube *B* with a little mercury and leave until the next sampling. Transfer the sample to the pipette of a Haldane gas analyzer (Fig. 39) and analyze for CO_2 and O_2 . A gas burette graduated from 1 to 100 per cent is required for the analysis. The respiratory quotient may

be obtained from this analysis alone by dividing the percentage of CO_2 found by the percentage of O_2 *used* (analysis percentage subtracted from percentage of O_2 in air).

To adapt the apparatus to quantitative measurements of respiration (gas analysis method) as well as of respiratory quotient it is necessary to know: (1) the free volume¹ of the container *C* with the sample, stopper, and tubes in place; (2) the temperature of the gas at each sampling; and (3) the pressure of the gas, determined by subtracting the mercury reading in the tube *A* from the barometer, at the time of each sampling. The volume of the contained gas at 0°C . and 760 mm. pressure is then obtained with the equation:

$$V_n = \frac{V_o \times 273 \times (P - a)}{T \times 760}$$

where V_n is the normal volume of gas; V_o is the actual volume of gas within the container, calculated from the change in pressure with the removal of a measured sample of gas, or obtained by displacement; T is the absolute gas temperature at time of sampling ($t^\circ\text{C} + 273$); note that multiplying V_o by the fraction $273/T$ reduces the gas to its volume at 0°C .; P is the barometric pressure at time of sampling in millimeters of mercury; and a is the reading in the tube *A* before withdrawing the sample, in millimeters of mercury above the level of the mercury in the tube. Note that multiplying V_o by $(P - a)/760$ reduces the gas to its volume at normal pressure.

The normal volume of gas (V_n) at each sampling, multiplied by the percentages of CO_2 and of O_2 obtained by analysis, gives the

¹ This is obtained from the change in pressure within the jar upon the withdrawal of a measured volume of gas. Place the sample in the chamber *C*, and assemble the apparatus for the experiment. Withdraw enough air through *B* to produce a small reading a on the tube *A*. Record this reading accurately in millimeters of mercury; withdraw a further 30- to 50-ml. sample of air with a mercury-filled gas pipette and record the *increased* value of a in millimeters. Calculate the volume of free gas with the equation:

$$V_o = \frac{xP}{h}$$

where V_o is the desired gas volume; x is the volume of gas withdrawn, at room temperature and pressure; P is the barometer reading in millimeters of mercury (record); and h is the *change* in a as a result of the withdrawal of sample x . Nearly refill the apparatus with air, draw a little mercury into *B*, and start the experiment.

normal volume of CO_2 produced and of O_2 unused. Subtract the volume of O_2 unused from the volume originally present (from original volume, pressure and temperature, and the percentage of O_2 in laboratory air, or from the volume and percentage at the last reading where a series of determinations is made) to obtain the desired figure of oxygen consumed.

Divide the volume of CO_2 produced by the volume of O_2 consumed to obtain the respiratory quotient. If the quotient only is desired, ignore temperature and volume corrections and divide the percentage of CO_2 found by analysis, by the percentage of O_2 used (normal percentage less observed percentage). If the rate of respiration as well as the quotient is desired, calculate CO_2 produced as milliliters of CO_2 (normal p and t) per gram dry sample per hour. Determine the dry weight of the sample from the percentage of moisture in parallel material, or dry the experimental sample at the end of the experiment.

With this apparatus it is possible to study respiration rates and quotients under conditions of diminishing partial pressures of O_2 and increasing pressures of CO_2 . Data obtained after the O_2 of the container is nearly exhausted should be interpreted in the light of this condition.

Questions:

1. What is the respiratory quotient of your materials in the first periods? After longer periods?
2. At what oxygen percentage does fermentation become a dominant factor in your experiments?
3. Outline an experiment for which the gas analysis technique for respiration measurement would be adapted.

Experiment 116. Osterhout's Method for Measuring Respiration.¹ (I)

This method is a modification of the gas-stream method in which the time required to produce CO_2 to neutralize a given quantity of alkali is measured instead of the CO_2 produced in a given time. The method is adapted to the rapid measurement of changes in respiration rates. Assemble the apparatus as shown in Fig. 42. A rubber bulb fitted with valves at v_1 and v_2 and operated by a rheostat-controlled motor is used to circulate the gas

¹ OSTERHOUT, W. J. V. A method of studying respiration. Jour. Gen. Physiol. 1: 17-22. 1918.

See also KOSTYCHEV, S. Plant respiration. Translated by C. J. Lyon. Pp. 50-51. Philadelphia. 1927.

within the system. The plant material is placed in the container *C*. When the stopcock s_1 is open the CO_2 produced in *C* is carried into the indicator solution contained in *I*, producing an acid color. When s_1 is closed and s_2 and s_3 are open, the CO_2 is absorbed by the sodium hydroxide pellets in *A* and the indicator solution returns to its original color, ready for a second experiment. The rapidity of reading is determined by the quantity of

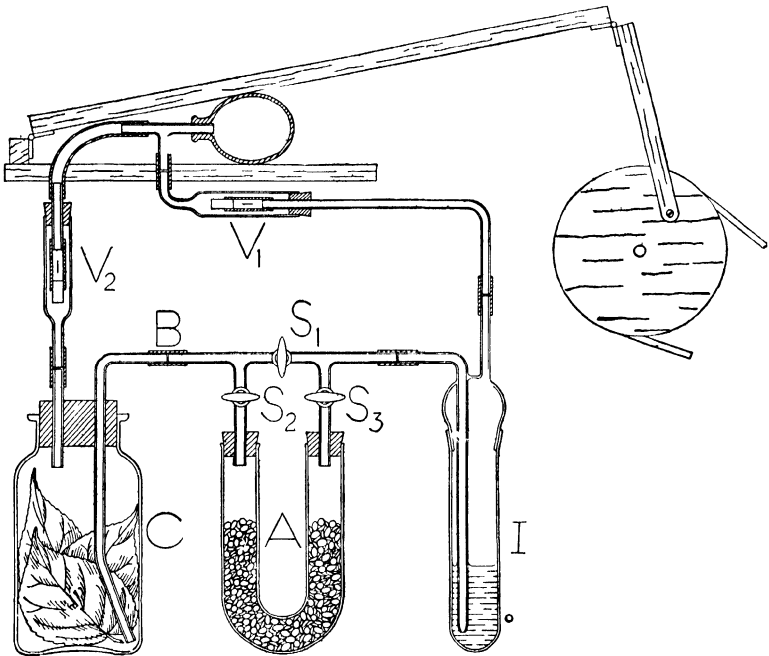


FIG. 42.—Osterhout's apparatus for measurement of respiration.

plant material in *C* and the solution used in *I*. The duration of an experiment is limited only by the oxygen content of the apparatus, and this may be renewed by disconnecting at *B* and circulating the gas with s_1 closed and s_2 and s_3 open. External air enters through *A* and is freed of its CO_2 ; it then sweeps out accumulated CO_2 in the apparatus and restores the O_2 to normal.

Manipulation.—Set up the apparatus and test for leaks. Pipette 10 or 20 ml. indicator solution¹ into *I*, close the apparatus

¹ Made with 10 parts freshly boiled distilled water, one part 0.04 per cent phenol red indicator, and a known volume of 0.02*N* NaOH solution to give

and run with s_1 closed and s_2 and s_3 open. Open s_1 and close s_2 and s_3 and run again; no color change should be observed. A change indicates a CO_2 leak. Place a known quantity of some plant material whose respiratory rate is to be measured, in the chamber C , and again circulate the gas through A for a few minutes with s_1 closed to sweep out CO_2 admitted in opening the chamber. Now, with the motor running: (1) record the time, (2) open s_1 , (3) close s_2 and s_3 , and (4) determine the time for the indicator solution to show a standard color change. Tubes of the indicator showing acid and alkaline colors should be prepared for color standards. Change the stopcocks so that the gas is again drawn through A until the CO_2 is removed from the indicator solution which then recovers its original pink color. Repeat the experiment until you obtain three or more closely agreeing readings.

Study the effect on respiration rate of one or more factors (Experiments 118 to 122). Temperature effects may be conveniently measured by immersing the chamber C in water baths of varying controlled temperatures.

For an approximation of the CO_2 produced in the recorded periods, determine the milliliters of $0.02N$ HCl required to produce the same change in the indicator and calculate CO_2 with the equation:

$$\text{Milligrams } \text{CO}_2 = 22.07T N$$

where T is the titration in milliliters of acid of normality N which gives the standard color change in the indicator. Your readings are the time for this quantity of CO_2 to be produced. Change them to milliliters CO_2 (milligrams/1.977) per gram dry sample per hour for ready comparison. For more accurate standardization of the method, particularly when used with buffered solutions for algae, etc., see papers by Osterhout and his students.¹

Question :

1. What advantages does this method have over that of Experiment 115? What disadvantages?

a reproducible pink color which is on the acid side of the full alkaline color of the indicator. Protect the solution from atmospheric CO_2 .

¹ OSTERHOUT, *loc. cit.*

Experiment 117. Titrimetric Determinations of the CO_2 Produced in Respiration. (I)

This method, sometimes called the "gas-stream method," has the advantages of accuracy and convenience and of permitting a study of the same material over an indefinite period under constant conditions. In the gas-analysis method described in Experiment 115, respiration is carried on in a constantly diminishing oxygen pressure, and in a constantly increasing pressure of carbon dioxide. In addition, the duration of the experiment as an aerobic test is limited by the available oxygen within the con-

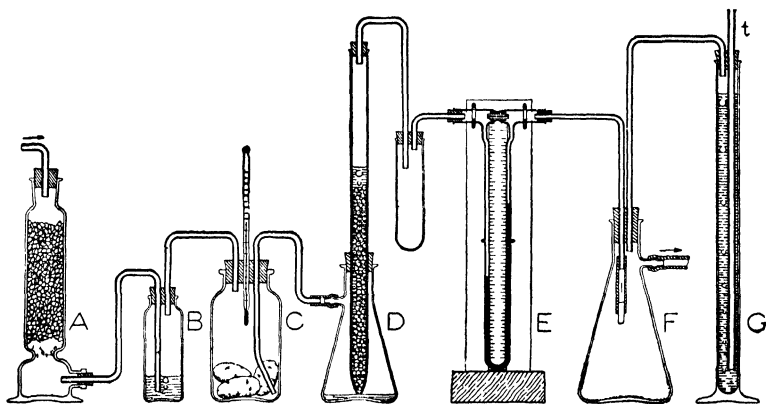


FIG. 43.—Gas train for measurement of respiration. CO_2 liberated by the sample in *C* is caught in the $\text{Ba}(\text{OH})_2$ tower *D*

tainer. The important advantage of the gas-analysis method is the simultaneous determination of both CO_2 and O_2 . For extended studies of plant respiration the two methods, gas-analysis and gas-stream, should be used together to give the most complete picture of the process.

Apparatus.—Set up the apparatus as diagrammed in Fig. 43. The soda-lime tower (*A*) removes CO_2 from the incoming air before it passes through the plant chamber *C*. The CO_2 liberated by the plant material is swept out by the air stream and absorbed in a $\text{Ba}(\text{OH})_2$ tower (*D*). Bottle *B* contains $\text{Ba}(\text{OH})_2$ to serve as a check upon the complete absorption of CO_2 from the incoming air. Make the connections in *C* to withdraw gas from the bottom of the plant chamber to insure removal of the heavy CO_2 . This connection must, of course, be reversed to

measure the respiration of algae, fungi, or other organisms in liquid media.

Several types of CO_2 absorption towers are available. A 20- to 30-mm. tube, 60 cm. long, two-thirds filled with glass beads, and adjusted so that the $\text{Ba}(\text{OH})_2$ solution is drawn halfway up the tube when the suction is applied, is suitable. It is convenient to draw the tube to a small opening at the bottom so that the beads will be retained in the tower. A dry safety flask is placed after the absorption tower to catch any of the standard solution which might accidentally be drawn over. Any solution caught in this tube should be returned quantitatively to the titration flask *D* at the end of the run. A pressure regulator is inserted at *G* to assist in repeating experimental conditions and to guard against excessive suction which might draw over the standard or other solutions. The by-pass tube (*t*) in the pressure regulator should be of 8-mm. tubing drawn to a point at the lower end and fitted to slide in the stopper. It is set near the top of the water at the beginning of the run and is then pressed down until the desired force is exerted on the gas train. It may be necessary, if the resistance of the gas train is high, to use 10 to 15 cm. Hg in the pressure regulator instead of water as shown in the figure. The unequal resistances of different gas trains attached to the same suction pump are adjusted with these regulators. A flowmeter may be inserted between *D* and *F* for regulating rates of gas flow.

The safety flask *F* is fitted with a Bunsen valve (see page 203) to prevent any of the liquids from sucking back. Two or more gas trains, each with its own plant sample, absorption tower, and pressure regulator may be attached to the same aspirator to run duplicate or a larger number of samples simultaneously.

One- or two-quart Mason jars fitted with two-hole rubber stoppers make suitable respiration chambers for small plant samples. Wide-mouthed, 1- or 2-gal. pickle or mayonnaise jars may be used for larger samples of materials showing slow respiration rates. A by-pass tube should be inserted between the plant sample and the flowmeter if it is desired to hold the sample under continuous aeration with occasional determinations of CO_2 production.

Manipulation.—The apparatus is designed to pass a current of CO_2 -free air through the respiration chamber *C* which will carry

with it the CO_2 given off by the respiring plant material. The CO_2 is then precipitated in the $\text{Ba}(\text{OH})_2$ solution as barium carbonate with a partial neutralization of the barium hydroxide. The residual $\text{Ba}(\text{OH})_2$ is titrated and the CO_2 evolved is calculated.

Set up the apparatus with duplicate gas trains, test to insure freedom from leaks, and sweep out the contained CO_2 . Carefully pipette 50 ml. approximately 0.1*N* $\text{Ba}(\text{OH})_2$, colored with phenolphthalein, into the top of the absorption towers and stopper tightly. The indicator serves as a test for excess alkali. If the solution becomes colorless during a run, immediately record the time, stop the gas stream and pipette in 10 to 20 ml. more alkali, wash down the absorption tower, and titrate as directed below.

Place weighed samples of plant material, containing a known percentage of dry matter, into the respiration chambers and immediately stopper and record the time as the beginning of the test. Raise the pressure control tube toward the top of the water or mercury in *G* and start the suction. Adjust the control and absorption tubes until the $\text{Ba}(\text{OH})_2$ solution is drawn up 20 to 30 cm. into the absorption towers and a brisk stream of bubbles is passing through each of the trains. The fresh weight of the sample used may vary from 50 to 100 gm. fresh leaves or moist grain to 2 kg. dry grain or other inactive material. If a titration difference equivalent to 10 to 20 ml. 0.1*N* $\text{Ba}(\text{OH})_2$ is not obtained in 2 hr. it is desirable to use a larger sample or to employ a more dilute (0.05*N* or 0.02*N*) $\text{Ba}(\text{OH})_2$ solution. If the loss of the pink color of the absorbing solution indicates that the alkali is exhausted, stop the run and proceed as directed above.

After the run has continued for 1 or 2 hr. (record time accurately), connect a by-pass tube, if it is desired to continue the aeration of the sample, and disconnect the absorption flask and tower unit. Raise the absorption tower above the level of the liquid in the flask and wash the safety flask and absorption tower thoroughly with small portions of freshly boiled water, catching the washings in *D*. Return any barium solution which may have been carried into the safety tube to the flask *D*. Wash down the outside tip of the tower and the sides of the flask and titrate the residual $\text{Ba}(\text{OH})_2$ (that not neutralized by the H_2CO_3 formed) with 0.1*N* HCl and phenolphthalein until the pink color is just discharged. Subtract this titration from the titration of a

fresh 50-ml. sample of the barium hydroxide solution and calculate the weight of CO_2 liberated in respiration with the equation:

$$\text{Milligrams CO}_2 = D_t \times N \times 22.0$$

where D_t is the difference between blank and experimental titrations in milliliters, N is the normality of acid used for titration, and 22.0 is the normal (0.5*M*) weight of CO_2 .

The CO_2 yield is obtained in milligrams; the result may be divided by 1000 to give grams or divided by 1.977 to change to milliliters of CO_2 at 0°C. and 760 mm. Hg. Note that CO_2 is calculated directly rather than as the carbonic acid which is actually measured.

The apparatus may be modified for gravimetric determination of CO_2 by substituting a potash bulb with suitable calcium chloride drying tubes for the absorption tower.

FACTORS AFFECTING RESPIRATION

The rate of plant respiration is affected by a large number of internal and external factors, particularly by temperature, rate of gas exchange, physiological activity (protoplasm content in part) of the respiring tissue, and available food supply. The following experiments are designed to illustrate some of these effects.

Experiment 118. The Effect of Temperature. (I)

The effect of temperature on respiration may be studied with any of the methods discussed in Experiments 115 to 117. The gas-analysis method (Experiment 115) and the Osterhout method (Experiment 116) are especially adapted to use with various or changing temperatures.

Choose an active material such as leaves or moderately dry (18 to 20 per cent moisture) grain, etc. Fruits and tubers may be used with the gas-analysis method, but they change temperature too slowly for the Osterhout method. With the Osterhout method, a single sample is used and the plant chamber kept in water baths of varying temperature. The tissue should be held with constant air circulation (gas train open) for 15 to 30 min. to allow it to come to the new temperature. Start at a water-bath temperature of 0 to 5°C. with precooled samples (not frozen) and make several determinations until a uniform

respiratory rate is indicated. Raise the temperature $10^{\circ}\text{C}.$ and after a period make a new series of determinations. If possible, continue with temperatures up to $50^{\circ}\text{C}.$ or higher. Plot respiration rate in convenient units against temperature. If time permits, plot the respiration rate at $50^{\circ}\text{C}.$ against time. Occasional renewal of the O_2 supply of the apparatus will be necessary, particularly at the higher temperatures.

To use the gas-analysis method, select as nearly as possible uniform samples, bring the samples to the experimental temperatures, seal in the 1-qt. jars with mercury seals as described in Experiment 115, and hold at the temperatures to be tested. Analyze the gases in the containers held at the higher temperatures after 1 to 4 hr., with successively longer intervals for the lower temperatures, until samples at 0 to $5^{\circ}\text{C}.$ are held for 48 hr. or longer. Determine both CO_2 production and O_2 consumption, and from these the respiratory quotient as well as the production of CO_2 in milliliters per gram dry matter per hour. Plot the latter figure against temperature

Questions:

1. What temperature coefficients (Q_{10}) do you obtain from your data?
2. What temperatures appear to be optimum? How would these be affected by the duration of the experiment?
3. What does the effect of temperature upon plant respiration have to do with the time of planting Irish potatoes? Night temperatures of greenhouses?

Experiment 119. The Effect of Wounding. (I)

The gas-train (Experiment 117) may be used conveniently to study the effect of wounding upon respiration. To eliminate the effect of surface area, select two lots of Irish potatoes, one slightly larger than the other, peel the larger potatoes, and bring the two lots to approximately equal weight. Wash and dry the peeled potatoes and place the two samples in the plant chambers of two assemblies of the gas-train apparatus shown in Fig. 43. Determine the respiration rate of the two lots of potatoes at intervals over several days, if possible, with continuous aeration of the material through a by-pass connection when determinations are not being made. Record the temperature and, if possible, control it at $20^{\circ}\text{C}.$

Plot respiration rates in milliliters of CO_2 per gram dry tissue per hour at various intervals after treating the wounded lot.

Determine dry weights of the samples at the end of the respiration run.

Questions:

1. What can you conclude from your data regarding the nature of the wound stimulus? Is it immediate, indicating gas-exchange acceleration, or delayed, indicating secondary effects?
2. Is stimulation continued after the formation of a secondary periderm?
3. How might cell metabolism rates be affected by wounding?

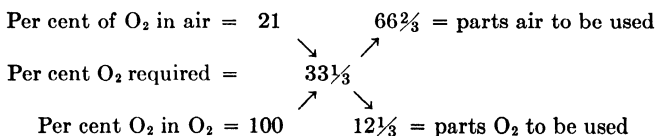
Experiment 120. The Effect of Carbon Dioxide Accumulations.

(I)

The gas-analysis method is convenient for this experiment. Equip several 1-qt. Mason jars with mercury-seal tops and tubes as shown in Fig. 41. If the sample is such (potatoes or apples) that it can be immersed, place it in the jar, fill with water, stopper, and introduce the desired gas mixture through the tube *A* as the water is withdrawn through *B*. When it is undesirable to wet the sample, sweep out the jar containing the material with several volumes of the desired gas mixture. Cover the stopper with a mercury seal, and seal the tubes with mercury to give mercury-to-glass seals at all points. At intervals remove 25-ml. samples of gas through *B* and analyze for CO_2 and O_2 . Run the gas samples back and forth several times to mix the gases within the chamber. Do not forget to record the barometric pressure, gas chamber temperature, and gas pressure *a* when setting up the apparatus and before withdrawing each sample.

Use Irish potatoes, fruits, grain moistened to about 20 per cent moisture, or other materials and study their respiration in air and in air plus 10, 30, and 80 per cent CO_2 . If O_2 cylinders are available, keep the O_2 constant at 20 per cent by mixing CO_2 , O_2 , and air as required.¹ Mix the gases to the desired

¹ To calculate gas mixtures, use the parallelogram method. To obtain 40 per cent CO_2 and 20 per cent O_2 with CO_2 , O_2 , and air: (1) Use 400 ml. CO_2 for each liter. This leaves 600 ml. of which 200, or $33\frac{1}{3}$ per cent must be O_2 . (2) Set up a parallelogram:



By subtracting across the parallelogram and disregarding signs, we find

percentages under inverted water-filled 1- or 2-l. graduates when more elaborate apparatus is not available, generating CO_2 in a Kipp generator (Fig. 44) with marble chips and HCl .

Questions:

1. How do high partial pressures of CO_2 affect (a) the rate, (b) the course (CO_2/O_2) of respiration?

2. What explanations might be given for your results?¹

Experiment 121. The Effect of Moisture on the Respiration of Grain. (I)

Add water to 400-gm. lots of wheat or corn, previously dried to 10 per cent moisture, so as to obtain moisture percentages of approximately 40, 20, and 10 per cent on a dry-weight basis. Hold the moistened grain for 24 hr. with good aeration; determine the actual moisture content of each lot with duplicate 50-gm. samples, and place duplicate 100-gm. samples in mercury-sealed air-filled jars of the type shown in Fig. 41. Record the barometric pressure and the gas pressure and temperature of each jar after determining the free-gas volume as directed under Experiment 115. Hold the jars at a constant temperature of 20 to 25°C. for approximately 4, 16, and 96 hrs., the 4-hr. period for the grain at 40 per cent and the 96-hr. period for the grain at 10 per cent moisture. At the time of sampling, again record barometric and gas pressures and gas temperature. Withdraw duplicate gas samples from each jar and

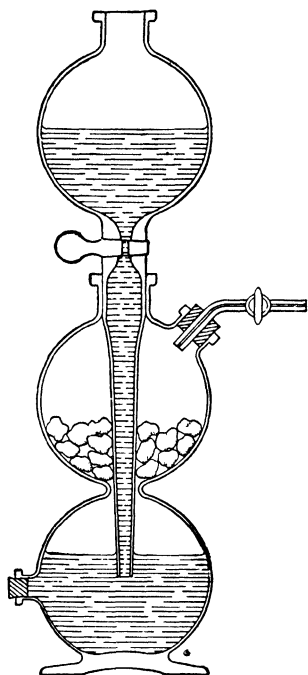


FIG. 44.—Kipp generator. Marble chips (CO_2) or zinc (H) are placed in the central container and 1 + 9 HCl added to come up into the central chamber. When the gas outlet is closed, the acid is forced into the upper bulb as shown, thus stopping the reaction.

that $66\frac{3}{4}$ parts of air and $12\frac{1}{4}$ parts of O_2 will give the desired mixture. Reduce these figures to percentages and calculate the milliliters of each gas to give a total of 600 ml.

¹ Note that a rapid initial drop in the CO_2 content of the mixture may result from solution of this gas in the sample.

determine the percentage of CO_2 and O_2 with a Haldane gas analyzer (Fig. 39). Determine the dry weight of each sample, and calculate the respiratory quotient and the respiration rate in milliliters CO_2 (standard t and p) per gram dry sample per hour. Do not forget that the O_2 used is obtained by difference.

Questions:

1. Why is the respiration of grain increased so rapidly by moisture? List the changes induced.
2. Why is moisture content given such a high weight in grain grading?
3. Would you expect, from your data, that grain with 20 per cent moisture would keep in bulk storage?

Experiment 122. The Effect of Food Supply upon Respiration.
(I)

Vary the food level in two corn leaf samples by holding one lot of plants in darkness and one lot in daylight plus strong artificial light at night, for 48 hr. The material should be collected in the afternoon of a clear day. Quickly enclose duplicate 50-gm. samples of leaves from each lot of plants in respiration chambers (Fig. 41) and determine their respiration rates over a 4- to 6-hr. period by the gas-analysis method.

Dry duplicate 25-gm. samples of the leaves as harvested at 100°C . to determine the percentage of dry matter. Powder the dried leaves, cover 2 gm. leaf powder with 100 ml. warm (50°C .) water and allow to stand with occasional stirring for 30 min., filter; hydrolyze sucrose with invertase and test for total sugars in the extract with Fehling solution (see Experiment 101).

Questions:

1. Why does the respiration rate of Irish potatoes increase as the storage temperature decreases from 5 to 2°C .?
2. Does the increase of respiration with higher sugar represent useful work or is it largely wasted energy?

CHAPTER XI

PLANT ENZYMES

INTRODUCTION

The various condensations, hydrolyses, oxidations, and other reactions, which apparently are brought about with facility within the living cell, may require high temperatures, strong acids or alkalies, or other special manipulations in the laboratory. Still other reactions which appear to be common in the cell have never been duplicated *in vitro*. We assign these differences in the reaction of the living plant to the enzymes produced by the protoplasm. Enzyme chemistry is a modern science, although fermentations which depend upon enzymes formed by micro-organisms have, of course, been known and used since before the dawn of history. Early in the nineteenth century several investigators advanced the idea of catalysis and suggested that cell catalysts, which they called ferments, might be responsible for fermentation. When Pasteur¹ established the relationship of living organisms to fermentation and advanced the hypothesis that fermentation was respiration in the absence of oxygen, the idea of organic catalysts was temporarily abandoned until it was revived again by Buchner.² To distinguish them from inorganic catalysts of the type widely used in industry, enzymes have been defined as the catalysts produced by living cells. They can be further characterized by their usual property of sensitivity to heat, a sensitivity which compares with that of protoplasm since most enzymes are destroyed by temperatures above 60°C.

Enzymes are known and classified almost entirely by their action since very few of them have been crystallized, and probably many of them are so highly colloidal in nature that they must

¹ PASTEUR, L. *Studies on fermentation*. Translated by Faulkner and Robb. London. 1879.

² BUCHNER, E. *Alkoholische Gärung ohne Hefezellen*. *Ber. deut. chem. Ges.* **30**: 117-124; 1110-1113. 1897.

always be separated by differential precipitation and adsorption. Many substances, thought at first to be simple enzymes, such as zymase and diastase have been found to be enzyme systems rather than single catalysts; others, thought to be different, have proved to be identical.

The better known plant enzymes may be grouped as enzymes of digestion, of fermentation, and of respiration. Enzymes of digestion include those which hydrolyze starch to maltose or glucose, proteins to peptids and amino acids, oils to glycerol and fatty acids, and similar reactions. These enzymes are often grouped as the hydrolases. It is commonly assumed, and in some cases apparently demonstrated, that the same enzyme which causes digestion may, under appropriate conditions, bring about the reversed, synthetic reaction. Digestion enzymes have been studied extensively since their products of reaction are easily identified and they are particularly adapted to extracellular study.

The fermentation enzymes form a second group which splits various organic compounds without the addition of water. These also have been studied extensively, particularly in the last decade. Fermentation enzymes obviously are important in the brewing and distilling industries and they are being employed in the production of acetone, butyl alcohol, and other organic solvents. Fermentation reactions are employed in certain food preservations and in the formation of silage, and are used in disposing of sewage and industrial wastes. Organic industrial wastes, which have involved serious disposal difficulties have been made to yield valuable by-products by appropriate fermentations. Among anaerobic bacteria, "fermentations" involving the liberation of hydrogen as the gas or as methane may be classed as respiration. In the higher plants, with which we are concerned, fermentation reactions probably constitute one of the preliminary steps of respiration rather than the important energy-releasing process.

A third group of enzymes is concerned with the oxidation of labile plant compounds by atmospheric oxygen. This group is less well known but obviously is of major importance in the respiration activities of living organisms.

The sensitivity of enzymes to heat, and other reactions indicating their colloidal nature, have led Willstätter to assume that

they are combinations of specific reactive groups, which may be inorganic but apparently are not necessarily so, with specific colloidal complexes. We may think of enzymes as analogous to a wood carver's tools. The usefulness of a tool depends both upon the type of cutting point and upon the kind of handle. Neither the handle nor the point can be used alone. Willstätter assumes that the handle or holder of the reactive group is colloidal and that it is this portion which is sensitive to heat. The reactive group of some enzymes appears to be a metallic ion such as iron, manganese, or copper. Other enzymes may be freed or practically freed of inorganic ions without destroying their reactivity and these are assumed to have an organic reactive group.

The colloidal nature of enzymes, or at least of a portion of the enzyme complex, is indicated by: (1) the production of the Tyndall effect by highly purified enzyme preparations; the Tyndall effect indicates a colloidal suspension rather than a true solution; (2) the readiness with which enzymes may be separated from dissolved materials by dialysis; (3) the tendency of enzymes to be adsorbed on various surfaces when adjusted to characteristic hydrogen-ion values, and (4) the fact that the diffusion velocities of those enzymes which have been studied are in the range of typical colloidal diffusion rates.

References:

WALDSCHMIDT-LEITZ, E. Enzyme actions and properties. Translated and extended by Robert P. Walton. New York. 1929. This is a general review including particularly the work of Willstätter and his students of whom Waldschmidt-Leitz is one.

GORTNER, R. A. Outlines of biochemistry. Pp. 918-953. New York. 1938. A brief but readable summary of enzymes; contains a three-page classification of known, largely plant, enzymes.

BAYLISS WILLIAM. The nature of enzyme action. 4th ed. New York. 1919.

THE PURIFICATION OF ENZYME PREPARATIONS

Enzyme purification depends largely upon the colloidal behavior of these substances. They are freed of soluble materials by dialysis or by precipitation and resuspension or by adsorption upon solid materials such as fuller's earth. The following experiments will illustrate different types of purification. None of the enzyme preparations are assumed to be completely

purified but some of them may represent an activity many times that of the substrate from which they are derived.

Experiment 123. The Preparation of Invertase. (I)

Invertase, the enzyme which hydrolyzes sucrose to glucose and fructose, may be prepared from baker's yeast. Obtain a pound of fresh, starch-free baker's yeast, crumble it into small

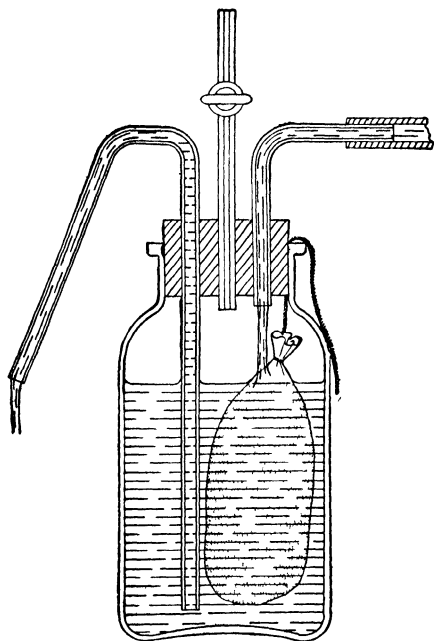


FIG. 45.—Apparatus for dialysis under toluene in running water. The water level in the jar is controlled by the quantity of air present.

pieces, and cover these in a wide-mouth glass-stoppered bottle with 50 ml. toluene. Allow the yeast to stand at room temperature for a week or 10 days. Add saturated neutral lead acetate solution to the mixture until no more precipitate is formed, but avoid a large excess of lead. Centrifuge to remove the precipitate and decant the clear liquid which contains the enzyme. Add just enough potassium or sodium oxalate to precipitate the excess of lead and again centrifuge and decant. With samples of old yeast the enzyme may be thrown down by the lead acetate which does not affect the invertase from

younger more active cells. Transfer the yeast extract to a celloidin dialyzing sac,¹ and hang it in a wide-mouth flask of water as illustrated in Fig. 45. Cover the water in the flask with a layer of toluene and run a stream of water slowly through the flask. The stopper and tubing connections must be airtight so that air pressure in the top of the container will force the water out through the discharge pipe without disturbing the toluene layer. Allow dialysis to continue for 2 or 3 days, then remove the solution, and if necessary, centrifuge and decant a third time. Preserve the invertase solution in a glass-stoppered bottle under toluene and hold in the refrigerator until used.

Questions:

1. Why is the yield of invertase so much higher when the cells are autolyzed than when they are ground?
2. Why is the lead acetate added?

Experiment 124. Malt Diastase. (I)

The enzyme or enzyme system which hydrolyzes starch to maltose or glucose is obviously important in many plant tissues. Germinating barley is rich in the substance classified as secretion diastase and is a convenient source of this enzyme.²

Germinate about 200 gm. plump barley in thin layers at room temperature. Watch the cultures, remove the seedlings when the radicles have attained a length of 5 to 6 mm., and dry the germinating seeds at room temperature. When two-thirds of the seeds have been removed in this way, dry down the entire culture at room temperature and complete the drying in a ventilated or vacuum oven at 40°C. Grind the dried sample finely in a burr mill and then in a ball mill and preserve it in stoppered bottles.

Shake 100 gm. of the malt powder with 250 ml. distilled water previously cooled to 10°C. Hold the sample in the icebox or in a cold bath and shake it periodically for 2 hr. or more. The temperature should be maintained at as nearly 10°C. as possible and should never rise as high as 20°C. Filter the liquid with suction

¹ See footnote, page 69.

² SHERMAN, H. C., and M. D. SCHLESINGER. Studies on amylases. V. Experiments on the purification of the amylase of malt. *Jour. Am. Chem. Soc.* **35**: 1617-1623. 1913. IX. Further experiments upon the purification of malt amylase. *Jour. Am. Chem. Soc.* **37**: 643-648. 1915.

through filter cloth or pulp and mix it with an equal volume of 95 per cent ethyl alcohol; centrifuge out the precipitate, decant the supernatant liquid, and add enough 95 per cent alcohol to make the mixture 70 per cent alcohol by volume. Centrifuge the mixture a second time and *save the precipitate*. The enzyme precipitate should now be transferred to a tared watch glass and air-dried in a refrigerator, the last stage of drying being carried out over sulfuric acid in a desiccator held in the refrigerator. The dried material is a concentrated, but impure, malt diastase, Save it for Experiment 128.

Questions :

1. Why is the grain germinated before drying for extraction of the enzyme?
2. What is the purpose of the 1 + 1 alcohol precipitation?
3. Why are low temperatures used in extraction and drying?

Experiment 125. Pectinase from *Rhizopus*. (I)

The species of *Rhizopus* which cause soft rot of sweet potatoes produce an enzyme which digests the pectic materials of the cell walls.¹ Peel and slice 500 gm. sweet-potato tissue; add a liter of distilled water, boil for an hour, and strain through a cloth. Boil the residue a second time with 500 ml. water, press out the liquid, and filter the combined extracts through cotton wool or filter pulp. Place 150-ml. portions of the sweet-potato extract in 1-l. Erlenmeyer flasks, plug with cotton, and autoclave at 15 lb. for 30 min. Inoculate the flasks with spores or hyphae from vigorously growing pure cultures of either *Rhizopus tritici* or *R. nigricans*. Incubate the inoculated flasks at 37°C. for 3 days; remove the mycelial mats and filter the liquid substrate through cotton wool or filter pulp and hold it in the icebox under toluene.

The mycelium contains intracellular enzymes, while the growth medium contains the secreted or extracellular enzymes. Use the liquid for Experiment 129.

Question :

1. Of what advantage is an extracellular enzyme to an organism like *Rhizopus*?

¹ HARTER, L. L., and J. L. WEIMER. Studies in the physiology of parasitism with special reference to the secretion of pectinase by *Rhizopus tritici*. Jour. Agr. Research **21**: 609-625. 1921.

Experiment 126. Lipase Powder. (I)

The endosperm of castor bean (*Ricinus communis*) contains a fat-digesting enzyme, lipase. Obtain 50 to 100 gm. castor-bean seeds, remove the seed coats, and grind the endosperm. Transfer the powder to Soxhlet extraction thimbles, and extract for 3 hr. with 80 per cent alcohol and then for 12 to 16 hr. with ether. The apparatus should be so arranged that the temperature of the alcohol and ether as they are condensed on the sample does not rise above 45°C. Dry the extracted residue in a current of air at room temperature, grind it in a mortar, and pass it through a 40-mesh sieve. This powder contains proteins and cell-wall residues, as well as lipase, so that it does not represent a concentrated preparation. Keep it in a stoppered bottle in the icebox and use for Experiment 130.

Questions:

1. Why is the double extraction used?
2. What other materials might be expected to be high in lipase?

DIGESTION BY ENZYMES

The splitting of complex compounds such as starch or oil into smaller molecules, usually with the addition of water, is one of the best known types of enzyme action. Probably the same enzyme which digests or hydrolyzes a given compound may, under appropriate conditions, synthesize the same material. A few such syntheses have been successfully performed in vitro. The following experiments illustrate some of the common types of digestions catalyzed by plant enzymes.

Experiment 127. The Hydrolysis of Sucrose. (I)

Sucrose or cane sugar is readily hydrolyzed by acids or by invertase, with the addition of one molecule of water, to one molecule of glucose and one of fructose. Both of these latter sugars react with Fehling solution to produce cuprous oxide and are consequently classified as reducing sugars in contrast to sucrose which is nonreducing.

Dissolve 0.5 gm. c.p. sucrose in 500 ml. water, mix 10 ml. of this solution with 10 ml. of a mixture of equal parts of Fehling A and B,¹ and boil gently for 2 min. To a second 10-ml. sucrose

¹ See page 203 for directions for making Fehling solution.

solution, add one drop of 10 per cent acetic acid and 0.2 to 0.4 ml. of the invertase preparation from Experiment 123.¹ Allow to stand 2 hr. or more at room temperature; add 10 ml. mixed Fehling solution and boil for 2 min. as before. Prepare a third tube in the same way as the second, but boil the enzyme extract before adding it to the sucrose solution. Allow this tube to stand for 2 or more hr.; then add Fehling solution and boil. Compare the volume of cuprous oxide formed in each of the three tubes. A convenient comparison may be made by transferring the liquid and precipitate to conical centrifuge tubes and centrifuging out the cuprous oxide. Rough estimates of reducing sugars may then be made by comparison, particularly if the tubes are graduated for volume.

Questions:

1. Show by a chemical equation the reaction catalyzed by invertase.
2. What commercial and scientific uses are made of invertase preparations?

Experiment 128. The Digestion of Starch. (I)

a. Make up a soluble starch substrate by mixing 20 gm. starch with 100 ml. cold water and pouring the mixture, with stirring, into 650 ml. rapidly boiling water. Boil for 3 min., add 100 ml. 0.6*M* KH_2PO_4 (mol. wt. 136.14), cool, and make to a volume of 1 l. Weigh the diastase preparation from Experiment 124 and add water to make a 1 per cent solution. Pipette 25 ml. of the starch preparation into each of two small Erlenmeyer flasks, warm them to 37°C., and add a few drops of toluene and 1.0 ml. of the diastase solution to one of the flasks. To the second flask add 1.0 ml. boiled diastase, rotate both flasks, incubate them at 37°C., remove drops of solution from each at 5-min. intervals, and test with I-KI solution. Starch gives a bright blue color with I-KI, the more complex dextrans a violet color fading through lavender to pink, and maltose gives no color except the light brown of the iodine. When the flask containing the unboiled enzyme shows no further dextrin coloration by the iodine test, pipette 5 ml. of the solution from each flask into a

¹ A 1 per cent solution of commercial invertase scales (available from the Nulomoline Co., 120 Wall St., New York City) may be used if Experiment 123 has not been performed. Use 0.1 ml., or two drops, of this solution instead of the larger quantity specified for the laboratory preparation.

test tube, add 5 ml. Fehling solution, and boil for 2 min. Transfer the solutions and precipitates to centrifuge tubes, centrifuge, and compare the volume of cuprous oxide precipitate as in Experiment 127.

b. If time permits, determine the diastatic power¹ of your enzyme preparation as follows. Pipette 10 ml. of the starch substrate into each of 10 test tubes. Dilute some of the enzyme preparation carefully to 0.1 per cent (1 + 9). To the first of the 10 tubes, add 0.1 ml. of this diluted enzyme preparation; to the second add 0.2 ml., and so on until the tenth tube receives 1.0 ml. of the 0.1 per cent solution. Shake the tubes, let them stand at 20°C. for 1 hr., add 5 ml. Fehling solution to each tube, shake, and place in a boiling water bath for 10 min. The tube containing the smallest quantity of enzyme in which all the added copper is reduced, as shown by the disappearance of the blue color of the copper ion, is used to calculate the standard value of the enzyme solution. A diastatic power (*D*) of 1.00 is assigned to a solution of which 0.1 ml. produces in 1 hr. enough sugar, under the outlined conditions, just to reduce 5 ml. Fehling solution. The standard value for the diastase preparation is calculated from the equation: $D = 0.1/V$, where *D* is the diastatic activity of the enzyme preparation and *V* is the minimum volume which will produce enough sugar in 1 hr. to reduce 5 ml. Fehling solution.

Questions:

1. What reactions are assumed to be concerned in the digestion of starch by diastase?
2. List some plant tissues in which you would expect to find starch-digesting enzymes.

Experiment 129. The Action of Pectinase. (I)

Cut 1-mm. slices from the central portion of sweet-potato roots and place several of these in 100 ml. of the liquid extracellular enzyme preparation from Experiment 125. The enzyme solution should be warmed to 37°C. before adding the potato disks and held at this temperature during the experiment. Add toluene to prevent growth of molds or bacteria and observe the texture of the potato disks at intervals for several hours. Press

¹ SHERMAN, H. C., FLORENCE WALKER, and MARY L. CALDWELL. Action of enzymes upon starches of different origin. Jour. Am. Chem. Soc. **41**: 1123-1129. 1919.

some of the softened potato tissue thin under a cover glass and observe with a microscope. Compare with thinly cut sections of untreated tissue.

Questions:

1. How does the permeability of fungi differ from that of autophytic (green) plants?
2. How does the enzyme affect the potato tissue? How does this action favor the development of the saprophytic fungus on sweet potatoes?

Experiment 130. The Action of Lipase. (I)

Grind 2 gm. of the castor-bean lipase preparation from Experiment 126 with 16 ml. 0.1*N* acetic acid and allow to stand for 15 min. Wash the residue (on a filter or by centrifuging and decanting) to remove the excess acid and transfer it to a small Erlenmeyer flask. Add 5 ml. castor oil, 10 ml. water, and several drops of toluene; stir to form an emulsion, plug with cotton, and incubate for 16 to 24 hr. at 37°C. Add 25 ml. 95 per cent alcohol, to check the hydrolytic dissociation of the sodium soap formed in titration, and titrate the fatty acids formed in the digestion of the oil against 0.1*N* sodium hydroxide with phenolphthalein as an indicator. Repeat with a second sample, but boil the lipase preparation with a little water before adding the castor oil. Repeat with a third sample but omit the acetic acid treatment.

Questions:

1. Show by equation the action of lipase on oil.
2. What is the effect of the acid treatment?
3. Why is phenolphthalein used as the indicator?

Experiment 131. Protein Digesting Enzymes. (I)

Grind 25 gm. of the seeds of garden pea (*Pisum sativum*) to a fine powder and weigh out duplicate 10-gm. samples. Extract the samples on a filter with several portions of distilled water. Transfer them to 500-ml. Erlenmeyer flasks and make to total volumes of approximately 200 ml. with distilled water. Heat one of the flasks to boiling, add 10 ml. toluene to each flask, plug tightly with cotton, and incubate at 37°C. for 5 days. Filter off the liquid, boil the filtrate, and add five drops glacial acetic acid to the hot liquid, cool, refilter, and determine the nitrogen

in 5- or 10-ml. samples of the filtrate by the Kjeldahl method.¹ The sulfuric acid may be added directly to the liquid sample in the Kjeldahl flask; add one or two drops of paraffin oil and start the digestion slowly until danger of frothing is past. If time permits, determine the nitrogen in 0.1- or 0.2-gm. samples of the pea-seed meal and calculate the percentage of the seed proteins which has been digested.

Questions:

1. What forms of nitrogen would you expect to be present in the filtrate?
2. What is the reason for adding the acetic acid and filtering off any colloidal precipitate formed?

RESPIRATION ENZYMES

The general topic of respiration has been considered in a preceding chapter. It was pointed out that sugars are highly resistant to oxidation but that they are readily susceptible to splitting by fermentation enzymes and that the split products can be oxidized by plant enzymes. The following experiments give demonstrations of enzyme action and quantitative methods for the estimation of activity of some of the oxidizing enzymes.

Experiment 132. Catalase. (E)

An enzyme called catalase which splits off oxygen from hydrogen peroxide is almost universally present in plant tissue. Its action may be demonstrated by grinding 1.0 gm. leaf tissue with 1.0 gm. precipitated chalk until the leaves are reduced to a fine pulp. Add a few drops of water if necessary during the grinding but avoid an excess. After the leaves are finely ground, add more water to a total volume of 20 ml. and rub the leaf and chalk mixture into a smooth suspension. Place 2 ml. of the freshly stirred suspension in one arm of the catalase tube *T* (Fig. 46), and in the other arm place 5 ml. 4 per cent hydrogen peroxide solution to which has been added an excess of calcium carbonate. Connect the tube carefully to the manometer and hold it upright in a water bath at 20°C. until the tube and its contents have come to constant temperature. No mixing of the materials in the two arms of the tube should occur during this preliminary treatment. Open the pinch clamp *C* and adjust

¹ Follow the directions of Experiment 38, but omit the salicylic acid and sodium thiosulfate since no nitrates will be present in the solution.

the level of the water in the burette to the zero mark. Close *C* and then simultaneously tip the catalase tube, to mix the two solutions, and start a stop watch. Stir the catalase-hydrogen peroxide mixture by rotating the tube in the water bath so that

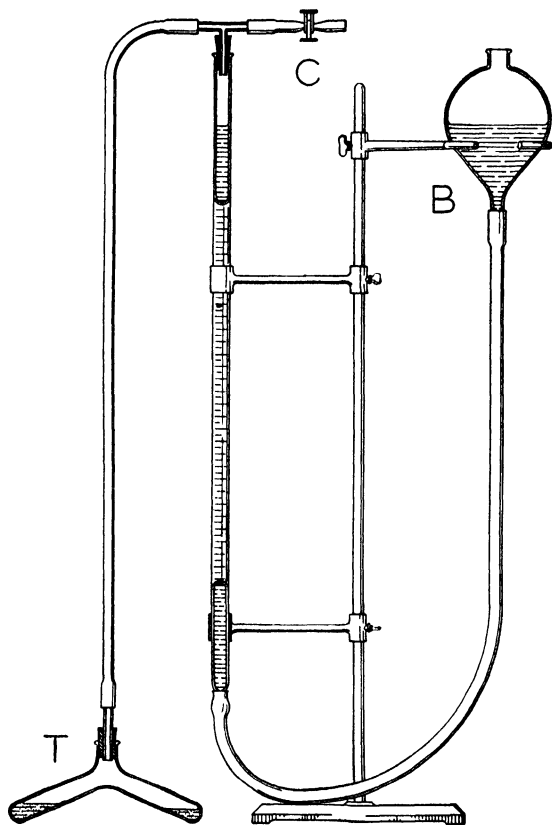


FIG. 46.—Simple catalase apparatus. Oxygen liberated by the action of catalase on H_2O_2 in the tube *T* is measured in the burette.

the solutions run from one arm to the other, and record the time required to liberate a standard quantity (usually 5 ml.) of oxygen, as measured by the expansion of the gas in the burette. The leveling bulb *B* should be lowered during the experiment so that the water level in the bulb remains the same as that in the burette. Repeat using a second 2-ml. sample of boiled enzyme preparation. Some plant materials give more consistent

and reliable catalase measurements if they are allowed to stand overnight in the ice-box after grinding with the chalk.

Compare the activity of the supernatant liquid, after the sample has settled out or been centrifuged, with the activity of the freshly shaken suspension in which solid materials are included in the sample. Compare the catalase activity of several different tissues, including leaf, stem, and storage tissues, as time permits. You will find it interesting also to compare the activity of leaves or growing points in various stages of growth activity.¹

Questions:

1. What types of tissues show high catalase activity?
2. How is catalase fitted into proposed schemes of plant respiration?

Experiment 133. Oxidase. (E)

Cover a few grams of fresh potato tissue with water and grind in a mortar. Filter through cloth, transfer a portion of the filtrate to a white dish, and add a few drops of 1 per cent guaiacum solution in alcohol. Guaiacum forms a blue compound when oxidized. Boil a fresh portion of the potato extract and again test with guaiacum. Add guaiacum to a 4 per cent hydrogen peroxide solution. Is the potato extract a more or less powerful oxidizing reagent than the hydrogen peroxide? Test a number of tissues for oxidase activity as time permits. Use leaves, including onion, pea (*Pisum sativum*), stock (*Mathiola incana*), and violet or pansy, and storage tissues. Test portions of the extracts and save those which do not promptly form the blue guaiacum compound for use in Experiment 134. Compare the activity of finely chopped fresh tissue in case the plant extract does not give a strong reaction with guaiacum.

Questions:

1. What evidence do you have that an enzyme is concerned in the oxidation of the guaiacum solution?
2. Trace the reactions involved in this experiment.

Experiment 134. Peroxidase. (E)

Use the materials which gave a slow reaction with guaiacum or failed to produce a blue color in Experiment 133. Mix a little

¹ KNOTT, J. E. Catalase in relation to growth and to other changes in plant tissue. Cor. Univ. Expt. Sta. Mem. **106**: 1-63. 1927.

KNOTT, J. E. Rapidity of response of spinach to change in photoperiod. Plant Physiol. **7**: 125-130. 1932.

of the finely chopped tissue or a few drops of a water extract with a drop or two of guaiacum and add a few drops of 4 per cent hydrogen peroxide solution. Repeat with boiled extract or tissue.

Questions :

1. How does the “peroxidase” system differ from the “oxidase”?
2. Why is hydrogen peroxide not required for the oxidation of guaiacum by potato tissue?
3. What compound serves as an oxygen activator in these experiments?

CHAPTER XII

GROWTH AND MOVEMENT

INTRODUCTION

The complete development of a plant cell or of an organ such as a leaf may be seen to occur in three distinct although overlapping steps. Meristematic cells divide in the *cell-division* phase of growth. As some of the dividing cells of the root or stem are crowded out of the field of division, or as the leaf reaches a certain size, the enlargement of the cells, which has been previously a secondary factor, suddenly becomes dominant, and we have the phase or period of *cell enlargement* during which the cells may enlarge twenty- to fifty-fold. Toward the end of the period of enlargement changes in the chemistry and morphology of the cell are initiated and continue throughout the life of the cell. These changes do not directly affect the size of the cell and hence are not growth in the strict sense. We classify all of them together as constituting the phase of *cell differentiation*. Cell division and enlargement cannot long continue in plants without a minimum of differentiation, but the course of development may be very different when either the growth or the differentiation phases are dominant. The work of this chapter is concerned particularly with cell division and cell enlargement under conditions of minimum or moderate differentiation. So many of the movements of plants are dependent upon changes in relative growth rates that the two topics, growth and movement, may conveniently be considered together.

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REGIONS OF GROWTH

In contrast to animals, in which growth is commonly generalized, the growth of plants is confined to specific (meristematic) regions, whose development gives these organisms their characteristic forms.

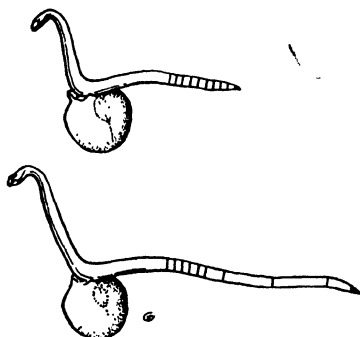


FIG. 47.—Root tips marked to demonstrate region of growth. Upper, as marked; lower, after growing for a few hours.

Experiment 135. The Region of Growth and Movement in Roots. (E)

a. Germinate corn and Windsor bean (*Vicia faba*), scarlet runner bean (*Phaseolus coccineus*), or other large-seeded legume in moist sphagnum. When the radicles are 1 to 2 cm. long, mark them from the tip with a soft striping brush and India ink at 1-mm. intervals (Fig. 47). Record the number

of marks (usually 10), making the first mark directly on the cap. Hold the roots vertically in loosely packed damp moss at 20 to 25°C. and observe and record the growth of each millimeter section at 2- to 4-hr. intervals. Retouch any ink marks which are scattered by growth, placing the new line in the center of the old. Plot the data in histograms showing by vertical lines the hourly growth rate of each of the original 1-mm. sections for each of the periods of observation.

Observe prepared slides showing longitudinal sections of root tips, preferably slides especially prepared by histological technique to show the thin-walled enlarging cells and containing 4 to 6 mm. of the tip of the root. Determine the volume of meristematic and enlarged cells, assuming a cylindrical form. If possible, estimate the volume of the nucleus and of the protoplasm in each cell.

b. Place some of the marked root tips, as prepared for a, in Petri dishes and pack loosely with moist sphagnum. Place the dishes on edge so that the marked roots may be observed through the glass dish in a horizontal position. Mark the position of the root and observe at intervals of 30 to 60 min. for the *time* and *region* of bending in response to gravity.

Questions:

1. What happens during the period of enlargement? Does vacuolation or hydration of colloids appear to be the more important factor in elongation?
2. How is the plant benefited by the location of the growing zone so near the tip of the root?
3. How do roots respond to a one-sided gravity stimulus?

Experiment 136. Regions of Growth and Movement in Stems. (E)

a. Mark the tips of morning glory, sweet potato, or other rapidly growing vines, tomato, asparagus fern (*Asparagus plumosus*), or castor-bean stem tips, etc., at 2-mm. intervals with India ink and soft brush as in Experiment 135. Extend the marks for a distance of 20 to 40 or more mm. from the tip, and observe particularly the marks near the tip where the stem may be difficult to reach and mark because of the surrounding embryonic leaves. Observe growth at 12-hr. intervals, repairing dispersed marks and recording and plotting the data as before.

b. Lay rapidly growing succulent tomato plants and corn plants nearly ready to tassel or just tasseling, both in pots, flat on the bench and observe the time and region of upward (negatively geotropic) bending. Repeat with older, more differentiated (woodier) stems.

Questions:

1. How does the position of the region of growth in stems compare with that in roots?
2. How can asparagus and corn stalks, bamboo shoots, etc., make such rapid growth?
3. Is geotropic bending in the stem limited to regions of active growth as in the roots? How do plants benefit by the observed responses?

Experiment 137. The Growth of Leaves. (E)

a. Plant three pots of peas; germinate one in the greenhouse and two in complete darkness, and compare leaf development.

Observe the dark plants by photographic safe light only. After 5 to 7 days transfer one of the pots from the darkroom to the greenhouse, and observe the subsequent development of the older and of the newly formed leaves.

b. Examine leaf primordia in longitudinal sections of potato, castor bean, or other stem tips. How do the leaves arise? How large are they when they show the first mature cells (protoxylem)?

c. Number the visible leaves of a growing corn or gladiolus plant and record the height of each leaf from a peg driven into the soil in the pot or bench. Hold the plants under good growing conditions, and record the number and height of the leaves on the same plants at 2- to 4-day intervals for 1 to 2 weeks. At the end of the period, dissect the plants and determine the regions of growth. Or mark the central leaf on an onion plant with India ink at 2- to 5-mm. intervals and observe its growth.

Questions:

1. In what ways does light affect the growth of leaves? What responses are involved?
2. Why do grasses withstand frequent clipping or pasturing better than most plants?

FACTORS INFLUENCING THE GROWTH RATE

If we think of growth as primarily cell division and enlargement with differentiation only incidental to the process, the factors affecting growth will be those which influence the supply or utilization of materials for the building of new protoplasm and which affect the rapid vacuolation of newly divided cells. Study the following factors.

Experiment 138. The Effect of the Food Supply. (E)

a. Sprout corn or other seeds until the radicles are 2 to 3 cm. long. Remove the endosperm from half of the seeds. Place the mutilated and normal seedlings together under favorable conditions and observe their growth over a period of 4 to 10 days.

b. Choose four to six thrifty potted corn plants 20 to 30 cm. high. Hold half of them in the dark for 48 to 72 hr. and leave half with greenhouse light. Water both sets of plants liberally; attach auxanometer needles to the central leaves (Fig. 1, page 9)

and observe the growth of the two sets of plants when held under shade in a warm humid house.

Questions :

1. What food materials are used in the building of new protoplasm?
2. How is photosynthate (sugar) involved?
3. From the data of Experiments 138 and 140, explain how plants grown in increasing shade may be first taller and then shorter than those grown in the light.

Experiment 139. The Effect of the Water Supply. (E)

Select four pots of corn that have been allowed to become dry and are just starting to wilt. Attach auxanometer needles to a uniform central leaf on each plant, place them in a warm moist shaded room, water two pots to optimum, leaving two as checks, and record the elongation of the central leaves in millimeters per hour over a period of several hours. Or vary the supply of available water by wetting two pots with 5.8 per cent NaCl solution and compare their growth with that of plants receiving water.

Questions :

1. Why do plants make such rapid growth after a rain, and grow so much taller in well-watered than in dry spots of equal fertility?
2. What basis do we have for the statement that water is probably the most generally limiting factor for plant growth?

Experiment 140. The Effect of Light. (I)

The effect of light upon leaf growth has been observed. The relation of sunlight to water deficit within the plants is demonstrated here. If time and equipment permit, it will be interesting to study the effect of mercury-arc ultra-violet light on plant growth.

Select four corn plants 25 to 40 cm. in height in 6- to 8-in. pots. Run the experiment on a clear, warm day, preferably between May 1 and Oct. 1 to insure good radiation, or the sunlight may be supplemented by the radiation of one or two 1000-watt Mazda lamps with reflectors, at a distance of 60 to 100 cm. from the plants. Attach auxanometer needles to the plants (Fig. 1, page 9), see that the soil in the pots is well moistened, but not flooded, and set up the plants in a warm greenhouse. Shade two plants from the direct radiation of sun or lamps with insulating board and leave two fully exposed. Determine the growth

of the two sets of plants in millimeters per hour over a period of 30 to 60 min.; then reverse the shades quickly and determine the time for a change in the growth rate with the removal and with the addition of shade.

Questions:

1. What important factor in the inhibition of growth by light is demonstrated?
2. What other inhibiting effects are possible?

Experiment 141. The Effect of Temperature. (E)

Germinate seeds of pumpkins and peas until the radicles are 1.5 cm. long. Mark the roots with India ink at a point 1 cm. from the tip. Place 5 to 10 marked seedlings in each of a number of covered dishes, loosely packed with moist sphagnum, and hold the cultures for 16 hr. at constant temperatures varying by intervals of 5 to 10°C. from 0 to 45°C. Record the growth of the roots of the two species in millimeters, and plot growth against temperature. Determine the minimum, optimum, and maximum growth temperatures for the "warm" and "cool" species and calculate the temperature coefficient of growth (Q_{10}) for the different temperature intervals. Be sure that the cultures do not dry out, particularly at the higher temperatures.

Questions:

1. List five plants which you would expect to have relatively high and five to have relatively low optimum growing temperatures.
2. What do your temperature coefficients over the range 10 to 30°C. tell you of the nature of the limiting growth processes in seedlings?
3. Why does growth drop with the higher temperatures?

Experiment 142. The Effect of Oxygen. (E-I)

a. The necessity of oxygen for growth may be demonstrated by removing the oxygen from the air in a 100-ml. bottle by one of the methods described in Experiment 99. Mark the radicles of two or three germinating seedlings with India ink at 1-mm. intervals, and insert them into the oxygen-free bottle while it is held inverted in a dish of water. Stopper the bottle *tightly*, place it with a second, similar bottle of marked seedlings which is plugged loosely with cotton instead of stoppered, and observe growth in the two bottles. The markings on the roots make it possible to estimate growth with considerable accuracy without opening the cultures.

b. Study the effect of flooding upon germination and growth of corn and rice.¹ Fill sixteen 3-in. pots with a loam, old field soil with which has been mixed about 1.0 gm. $(\text{NH}_4)_2\text{HPO}_4$ per kilogram of soil. Treat the seeds with organic mercury dust to reduce seed rots, plant five kernels of corn 2 cm. deep in each of six pots, and lay the same number on top of the soil in the two remaining pots. Plant eight pots with rice in the same way. Treat the cultures as follows:

1 and 2. Water normally for checks.

3 and 4. Water normally until the shoots are 4 to 7 cm. high, then place the entire pot in a gallon jar of water so that the soil is covered with 2 to 3 cm. of water, and hold.

5 and 6. Set in water as for (3) and (4), but at once, before germination.

7 and 8. Cover with water as for (5) and (6), but leave the grain exposed on the surface of the soil and covered only by the water.

Hold the pots in a cool greenhouse, 15 to 20°C.; maintain the water levels and observe the growth of the plants for 4 to 5 weeks. If necessary (yellow color), add 10 ml. of 1.0 per cent $(\text{NH}_4)_2\text{SO}_4$ solution to the water about flooded pots which contain growing plants. At the end of the period, record the height of tops, length of roots (wash carefully from soil), and green and dry weights of tops and roots on growing plants.

c. Try germinating rice seeds (unhulled rice) in freshly boiled and cooled tap water covered with a 2- to 4-mm. layer of paraffin oil to exclude oxygen. Compare with seeds in water not covered with oil and with seeds germinating on moist blotters. At intervals remove seeds from the oil-covered water, hold on moist blotters, and compare their root (radicle) and shoot (plumule) growth at the time of and subsequent to removing them from the anaerobic culture.

Questions:

1. Is oxygen necessary for root growth? In large quantities?
2. Which requires the better oxygen supply, germination or root growth on older plants?

¹ ERYGIN, P. S. Change in activity of enzymes, soluble carbohydrates, and intensity of respiration of rice seeds germinating under water. *Plant Physiol.* **11**: 821-832. 1936.

Experiment 143. The Effect of Auxins. (I)

A number of compounds, some derived from plants and others similar to the plant "auxins," have been shown to increase markedly the plasticity of the walls of elongating cells and thus to prolong or revive the stage of elongation.¹ Recently Hitchcock, Zimmerman, and others² have shown that indolebutyric acid, α -naphthaleneacetic acid, and other compounds have the effect of plant "hormones."

Apply β -indoleacetic acid to various plant parts by mixing 1 part of the chemical with 500 parts of hydrous lanolin (wool fat) and applying the mixture to the petioles, stems, and roots of plants. Apply small quantities of the paste to the upper or lower surface of the midribs of *young* sunflower, tomato, tobacco, or other leaves, and to one side of the young, rapidly growing stems of the same plants. Compare the effect on old leaves and stems and on ink-marked roots growing in damp moss. Hold some of the plants which have been treated on the older stems and compare root formation on the treated stems in comparison with untreated checks. Observe freehand sections of regions showing bending or other responses.

Questions:

1. Why do the leaves and stems bend away from the hormone paste?
2. How do you explain differences in the reaction of younger and older tissues? Of stems and roots?

Experiment 144. The Effect of Rest Period on Growth. (I)

A number of demonstrations of the effect of rest period are possible. If freshly harvested potatoes, either northern or southern grown, of the Bliss Triumph or similar varieties, or freshly harvested gladiolus corms are available, treat them with ethylene chlorohydrin and high temperatures after the methods

¹ THIMANN, K. V. Growth substances in plants. *Ann. Rev. Biochem.* **4**: 545-568. 1935. See also references to Boysen Jensen, and Went and Thimann on page 172.

² HITCHCOCK, A. E. Indole-3-N-propionic acid as a growth hormone and the quantitative measurement of plant response. *Contrib. Boyce Thompson Inst.* **7**: 87-95. 1935.

ZIMMERMAN, P. W., and FRANK WILCOXON. Several chemical growth substances which cause initiation of roots and other responses in plants. *Contrib. Boyce Thompson Inst.* **7**: 209-229. 1935.

of Denny¹ and Loomis.² Clean the corms or cut the potato tubers to 20- to 30-gm. pieces and dip them into 2 per cent ethylene chlorohydrin solution (5 ml. commercial 40 per cent solution plus 95 ml. water), drain, seal in Mason jars, and hold at 18 to 20°C. for 24 to 48 hr. Compare a 5 per cent dip held for 24 hr. Plant 10 to 20 of these treated pieces in the greenhouse with an equal number of untreated pieces. Store a second lot of potato tubers or gladiolus corms at 33°C. with good ventilation for 4 weeks and plant with the first. Compare time for germination, percentage rotting, and growth of the plants from the several treatments. Calculate time for germination both from time of beginning treatment and from time of planting. Which treatments give new plants in the shortest time? Which cause the most rapid germination after planting? Hold gladiolus corms for observations of flowering.³

Questions:

1. List some of the tissues which show a rest period.
2. How may plants be benefited by possessing a resting period?
3. What theories have been suggested to explain rest period?

Experiment 145. The Grand Period of Growth. (E-I)

Your growth data from Experiment 135 should show that successive sections of the root tip grow first slowly, then rapidly, and finally slowly until they stop elongation. Such changes constitute a grand period of growth and a curve resembling that obtained for a single root section may be obtained for an entire plant.

a. A fair number of plants (10 to 30) growing under favorable conditions should be used for growth curves and for these reasons the experiment is not well adapted to the greenhouse. Fairly good growth curves may usually be obtained from spring oats or

¹ DENNY, F. E. Second report on the use of chemicals for hastening the sprouting of dormant potato tubers. *Am. Jour. Botany* **13**: 386-396. 1926.

² LOOMIS, W. E. Temperature and other factors affecting the rest period of potato tubers. *Plant Physiol.* **2**: 287-302. 1927.

LOOMIS, W. E. Forcing gladiolus. *Proc. Am. Soc. Hort. Sci.* **30**: 585-588. 1933.

³ For a list of forcing varieties see: VOLZ, E. C., and C. G. KEYES. Gladiolus forcing: effects of exposure to high temperatures before planting. *Proc. Am. Soc. Hort. Sci.* **30**: 583-584. 1933. Or use Alice Tiplady variety.

barley grown in April, and this material is recommended. Record the maximum height (to tip of stretched leaves or head) of 20 oat plants at weekly intervals from germination until increase in height ceases and plot *rate* of growth in centimeters per week against time. Rate of growth gives a rising and falling curve, whereas total height of plant of course does not drop. When space is available, more advanced students may wish to grow enough extra plants (120 to 150) that 10 can be cut each week for determinations of green and dry weights. Compare rate of increase in height with rate of increase in dry weight per plant.

b. If feasible, measure the length of 5 or 10 pine, lilac, or other shoots at daily intervals from the time new growth starts. Plot the average daily growth of the shoots in millimeters.

Questions:

1. What factors contribute to the grand period of growth of a root tip cell? An oat plant?
2. How does the growth curve differ when increments of dry weight are plotted rather than height?
3. How does the growth curve of pine shoots differ from "normal"?

MOVEMENT IN PLANTS

Plant movements are so nicely adjusted to their environment that we commonly overlook their importance. Roots consistently turn down, that is, they are positively geotropic, and stems turn up until we take these responses for granted, and do not consider that they are indeed fortunate adaptations. If there were a widespread failure in the normal geotropic responses of plants, we can imagine that the problem would become one of primary economic interest instead of remaining purely academic.

Partly because of the lack of an urgent economic stimulus to research on the topic, but more because tropisms involve complex, protoplasmic responses that are difficult to break down into their physicochemical components, we have little concrete information on the physiology of movement in plants. Even the hormone theories, the most successful attempts at an explanation of movement, leave largely unsolved such important questions as how and why the hormones are formed, why they move to one side of a stimulated plant organ, and how they act. Your experiments on movement will necessarily, therefore, be confined mainly to observations of stimulus and response, but they should

not be the less interesting for involving many difficult and unsolved problems.

Plant movements are classified as tropic, nastic, or taxic. In tropic response the direction of movement is determined by the stimulus, as in geotropism. The direction of nastic movements is controlled by the structure of the organ as for example the sleep movements of *Oxalis*. Taxic movements are confined to free-swimming organisms such as bacteria or gametes which may move toward or away from a stimulus.

Experiment 146. Geotropism. (E-I)

Germinate several small pots of oats or corn in sand in complete darkness and when the seedlings are 3 to 4 cm. high use them for Experiments 146 and 148.

Mark the position of several seedling shoots of corn or oats with pieces of wire, lay the pots on their sides in a dark moist chamber, and determine the time for noticeable movement of the tips. Rotate the pots through 180 degrees, adjust the wires to the new position of the plants and determine: (1) how long the tips continue to move in the original direction; (2) how long before movement is reversed; and, (3) the region of movement. Record the temperature. Compare with the bending of roots observed in Experiment 135.

To determine the presentation time of the geotropic stimulus, mark the position of the seedlings in five pots with pieces of wire. Lay all the seedlings horizontally in a dark moist chamber and set pots upright after periods of 5, 10, 20, 40, and 60 min. Observe carefully and determine: (1) the shortest horizontal exposure which will subsequently cause measurable bending—presentation time; (2) the elapsed time between the beginning of the stimulus and measurable response—the reaction time. Does horizontal exposure above the minimum time increase the speed of reaction or do all of the plants show the first evidences of bending at approximately the same time?

Questions:

1. How do the behavior and the reaction time of geotropically stimulated stems compare with the behavior and the reaction time of geotropically stimulated roots?
2. What advantages does the plant derive from geotropism?
3. What type of experiment could be used to determine whether the region of stimulus corresponds to the observed region of response?

Experiment 147. The Nature of the Geotropic Stimulus. (E)

Germinate corn or oats in loose moss and, when plumule and radicle are 1 to 2 cm. long, carefully attach several seedlings, some upright and others inverted, to wood blocks which are fitted into large centrifuge cups (Fig. 48). Place a little moist cotton in the cups, stopper them after the plants are in place, and centrifuge at a moderate speed for 6 to 12 hr.

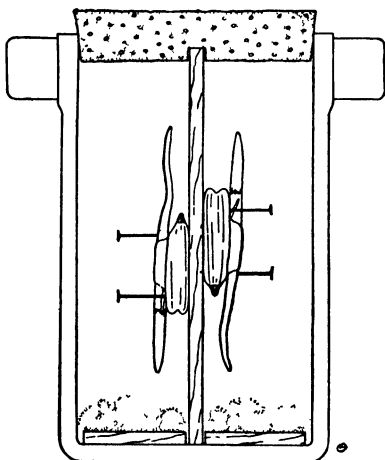


FIG. 48.—Corn seedlings attached for showing the effect of centrifugal force on growth. The seedlings are pinned securely to the central stick and a little moist cotton is placed in the bottom of the stoppered centrifuge tube.

Observe the seedlings after 2 and 4 hr. and at the end of the experiment.

Questions:

1. Upon what force do plants depend for their sense of up and down?
2. What mechanisms for geotropic perception in plants have been proposed?

Experiment 148. Phototropism. (E)

a. Sprout corn or oats in small pots of sand in a photographic darkroom. Cover the pots with tight-fitting, black paper caps as a further protection against light. When the plants are 3 to 4 cm. high,

handle them in photographic safe light and treat as follows:

1. Mark the position of three or more seedlings with copper wire as checks.
2. Cover 7 mm. of the tips of three seedlings with a closely fitted cap of rolled tinfoil to exclude all light from the tip.
3. Cover three or more plants with a heavy coating of vaseline to reduce the supply of oxygen to the growing cells.

Insert wire markers by all plants, water the pots, and place them in a black-lined box with an opening some 10 by 10 cm. exposed to good north light. Observe the time for the differently treated plants to respond to the unilateral lighting.

b. Place rapidly growing geranium, bean, or other long-petioled, potted plants in a black-lined box open to north light

and observe the time for a measurable phototropic response in leaf position. Select several evenly matched and evenly exposed pairs of petioles as the experiment is set up, vaseline one of each pair of these petioles heavily and observe the effect on phototropic response.

Cover one-half of each of two nearly mature leaves with black paper tightly clipped on; mark the position of the leaves carefully, expose to good light, and observe any changes in leaf position due to bending of the petiole.

Questions:

1. Are the growing cells affected directly by weak light? How might direct sunlight differ in its action?
2. How are leaves spread so that the maximum amount of light is intercepted?
3. What is the survival value of phototropism?

Experiment 149. The Relation of Auxins to Phototropism. (I)

The bending of the oat coleoptile under the influence of light has been shown to be associated with the unequal distribution of certain chemical materials within the plant as the result of the unilateral light stimulus.

Grow oat seedlings in six or eight small pots in *complete* darkness until the coleoptiles are 3 to 4 cm. high. Remove one or two pots to a darkened room and illuminate the plants from one side with a 60-watt lamp at 30 cm. for 10 min. Return to the darkroom and work with photographic safe light only. Cut 2 mm. from the tips of the coleoptiles of plants which have been kept in darkness, then seal 2 mm. of the tips of the treated plants onto the untreated stumps with small drops of warm, 5 per cent gelatin. Hold the plants in a dark chamber kept at 22 to 25°C. and 90 to 95 per cent humidity for 2 to 3 hr. and observe their response. For controls make the same transfer with tips which have not been illuminated. This is the experiment with which Boysen Jensen¹ first demonstrated the presence of hormones in plants.

Other experiments in which the hormones from illuminated and control coleoptile tips are allowed to diffuse into agar blocks and these blocks are then applied to one side of an oat coleoptile

¹ BOYSEN JENSEN, P. Growth hormones in plants. Translated by George S. Avery, Jr., and Paul R. Burkholder. New York. 1936.

stump, may be performed as time permits. See Boysen Jensen for details of methods.

Questions :

1. How are your results to be explained?
2. Are cell division and enlargement, or enlargement only, involved in the usual bending of oat coleoptiles?

Experiment 150. Hydrotropism. (E-I)

- a. Fold large filter paper squares and cover the *outside* of two, 10- to 12-cm funnels. Fold the paper over the top of the funnel, fill the funnel with moist cotton, and arrange germinating corn or other seeds around the margin as shown in Fig. 49. Set the funnels in bottles of water. Place one funnel under a bell jar with wet sphagnum to maintain a humidity near saturation and place the other at a humidity of approximately 70 per cent. If the seedlings are properly placed and the humidity of the surrounding air is not too low for growth, the roots will grow over the edge of the funnel and then in and down or straight down, depending upon the humidity and the presence or absence of a hydrotropic response. Determine the difference between air temperature and the temperature of the moist paper over the funnel under the two conditions.

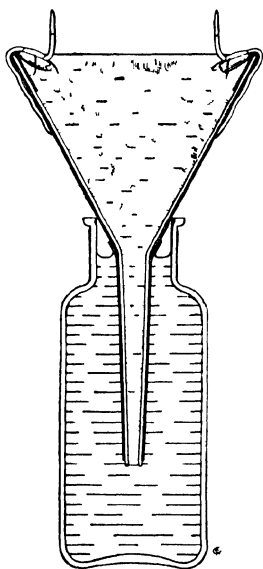


FIG. 49 — The hydrotropic response of roots in air. The seeds should be placed so that the radicles will grow over the edge of the cotton-filled funnel, and will then come in contact with the moist paper on the outside.

- b. Air-dry several kilograms of a loam soil and moisten a little of it carefully until it will barely hold together when pressed in the hand—on the dry side of the gardener's "crumbling test." Pack a test tube half full with the dry soil, fill with the moist soil, mark the intersection, and observe for 1 or 2 days or longer. The moisture should not spread by capillarity from the moist to the dry soil. Ordinarily moisture percentages of one-half and one and one-half times the wilting percentage are approximately correct for the dry and

moist soils, respectively. If the test is satisfactory, moisten half of the soil to the same consistency. Tip a large flat battery jar on one corner and fill half with the dry soil and half with the wet as shown in Fig. 50. Plant germinating seeds of corn, peas, cowpeas (*Vigna*), sunflowers, squash, etc., in the moist soil above the upper end of the dry-soil segment as shown.¹ Cover the jar and hold it in the moist chamber for a week. Remove the seedlings

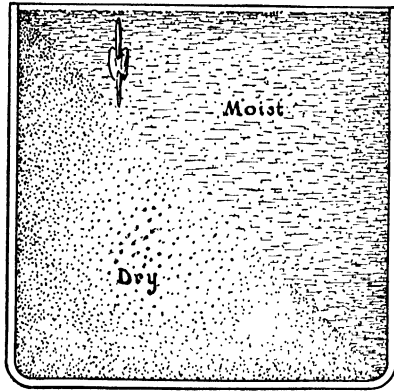


FIG 50.—Arrangement of moist and dry soil and seedlings for studying hydro-tropic responses in soil.

carefully and determine the response of the primary and secondary roots to a steep moisture gradient.

Questions:

1. What tropisms are shown by the seedlings in the funnel? From the response of roots in soil, would you say that moisture or temperature was responsible for the roots bending toward the moist surface in part *a*?

2. How common does hydrotropism appear to be among plants growing in soil?

Experiment 151. The Hydrotropic Stimulus. (I)

Hydrotropic responses may be due to liquid water contact with one side of the roots, to water vapor gradients, or to unknown factors effective over longer distances. If roots are to "seek out" water supplies, rather than simply to make their greatest growth and therefore to develop their greatest absorbing area in the regions of favorable soil moisture, some long-distance

¹ LOOMIS, W. E., and L. M. EWAN. Hydrotropic responses of roots in soil. *Botan. Gaz.* 97: 728-743. 1936.

stimulus is required. Perform the following experiment to determine whether roots will grow through dry soil to a moist layer beyond.¹ Divide a narrow box, about 15 by 15 by 40 cm., into four parts by partitioning with 14-mesh screen wire and coating the wire thoroughly with a vaseline (30 per cent) and paraffin (70 per cent) mixture to form *water-tight* partitions. Fill the end compartments and one of the inside compartments carefully with moist soil. Fill the remaining inside section with soil below the wilting percentage and avoid wetting this section when watering the others. Plant corn, beans, or other large-rooted quick-growing plants in the moist inside compartment.

After 2 to 3 weeks observe the penetration of the plant roots through the screens directly into the wet soil on one side and through the dry soil toward the second moist compartment on the other.

Questions:

1. Would you expect roots to grow through a dry subsoil to the water table below?

2. How do you account for the fact that alfalfa roots can be found penetrating a dry subsoil and growing in the moist soil below?

3. What appears to be the determining factor in the distribution of roots between moist and dry soil?

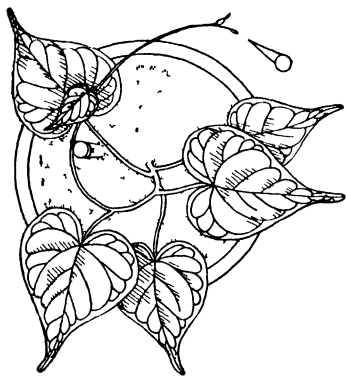


FIG. 51.—Plant arranged to observe twining movements. The bending of the tip past the marker is estimated in degrees of arc.

Experiment 152. Twining. (E)

Obtain a potted twining plant, morning glory (*Ipomoea purpurea*) is excellent, and fasten an active tip loosely and carefully to the top of a short stake so that the vine tip projects 10 to 12 cm. above the stake and is free to move in the air (Fig. 51). Study the effect of moisture supply on the rate at which the tip rotates. With the

soil moderately dry, hold the plant in sunlight in dry air and record rate of rotation. Water the plant, move to a warm moist chamber and again record movement. Express rotation

¹ HENDRICKSON, A. H., and F. J. VEIHMEYER. Influence of dry soil on root extension. *Plant Physiol.* 6: 567-576. 1931.

as time to make a complete revolution, calculated from the average time for the tip to revolve 1 degree about a central axis.

Observe several twining plants and determine which twine clockwise and which counterclockwise when viewed from the top.

Questions :

1. What evidence do you have that twining is a growth process?
2. What part of the stem is involved?

Experiment 153. Shock Movements.¹ (I)

Select two vigorous well-watered *Mimosa* plants and carefully estimate the length of the petiole of a thrifty leaf and the length of one of the subpetioles on each. The leaves must not be jarred or touched during the measuring.

Set a plant in sunlight in a warm dry part of the greenhouse and after 15 min. stimulate one of the tip pinnae by pinching with a pair of fine forceps or touching with the flame of a match. Record the time required for the stimulus to travel the length of the subpetiole, as measured by the closing of the pinnae, and, separately, the time required to travel the length of the petiole, measured by the drooping of the entire leaf (Fig. 52). Record temperature and humidity.

Place the second plant in a shaded moist chamber with a temperature 10 to 15°C. below that used for the first plant. Stimulate as before and record the rate at which the stimulus moves back through the leaf and petiole. Record temperature and humidity. Allow the plants to stand for 20 to 30 min. until recovered, then repeat the experiments with plants reversed.

Questions :

1. What is the mechanism of movement in *Mimosa*?
2. How is the rate of transmission of the stimulus affected by the water deficit within the plants? What method of stimulus transmission is suggested by this relationship?

¹ For a discussion of movement in *Mimosa* and for suggestions for additional work, see: WALLACE, R. H. Studies on the sensitivity of *Mimosa pudica*. I, II and III. Am. Jour. Botany **18**: 102-111; 215-235; 288-307. 1931.

Experiment 154. Sleep Movements. (E)

A number of plant movements are stimulated by temperature, humidity, or light changes. Changes due to light and called "photonastic" or "sleep" movements are the more common.

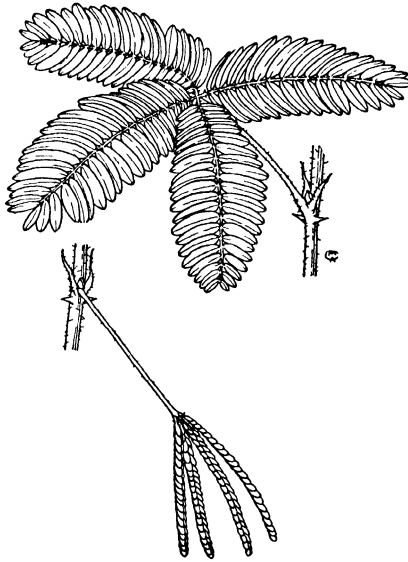


FIG 52 —Mimosa leaves; normal above, stimulated below. The stimulated leaflets have closed, and the petiole has dropped sharply at the point of attachment to the stem

Observe and record the time for the closing of *Oxalis* or *Mimosa* leaves when the plants are placed in the dark and the time to open when again exposed to light.

Hold potted, flowering dandelion plants in light and darkness at temperatures of 10°C. and 25°C., and observe for closing of the flowers.

Questions :

1. What causes *Oxalis* leaves to close in the dark?
2. Is the closing of dandelion flowers at night a response to light, temperature, or both?

CHAPTER XIII

GROWTH-DIFFERENTIATION BALANCE AND GROWTH CORRELATION

INTRODUCTION

We stated in the last chapter that the development of any plant cell or tissue may be divided into three phases: (1) cell division, (2) cell enlargement, and (3) cell differentiation. The last term is used here to cover the sum of the chemical and morphological changes occurring in maturing or matured plant cells. Cell division and cell enlargement together constitute growth in the limited sense of increase in size, and growth-differentiation balance is the balance between the production of new cells and the differentiation of those previously or currently produced. Cell differentiation obviously is dependent upon previous cell formation or "growth," and cell formation in the higher plants cannot continue under normal conditions without at least a minimum differentiation of absorbing, photosynthetic, and food transporting tissues. Our term "balance" is thus a question of relative emphasis rather than of the presence or absence of a given developmental phase, and we speak of plants as on the growth side of the balance when they are producing succulent, little-differentiated tissues, and as on the differentiation side when they are producing the woody, gummy, or acrid tissues typical of the stunted plant. It is understood, of course, that genetic differences are excluded and that our discussion refers to variations and correlations among genetically similar plants.

The internal factors especially concerned in each phase of plant development have been classified as follows:¹

I. GROWTH FACTORS

A. For Cell Division.

1. *Protoplasm*.—The building of new protoplasts depends upon: (a) supplies of sugars, nitrogen, sulfur, and phosphorus; (b) the catalysts and

¹ Loomis, W. E. Growth differentiation balance vs. carbohydrate-nitrogen ratio. Proc. Am. Soc. Hort. Sci. **29**: 240-245. 1932.

enzymes necessary for protein synthesis, including probably potassium, iron, boron, manganese, and other elements, alone or in organic combinations; (c) the necessary electrical and hydration conditions for the condensation reactions involved in protein synthesis.

2. *Moisture*.—Meristematic cells require liberal supplies of water and do not compete effectively for the excess quantities necessary for rapid cell division.

3. *Warmth*.—Cell division involves many biochemical changes which are accelerated by rising temperatures over a limited range.

4. *Energy*.—Respirable sugars and available oxygen are necessary to meet the high energy requirements of meristematic tissues.

B. For Cell Enlargement.

1. *Moisture*.—Cell enlargement is largely the result of the absorption of water by immature cells.

2. *Wall Plasticity*.—Increased sugar supplies, which should increase water absorption, usually check enlargement instead, because of the stimulus to rapid thickening and loss of plasticity of the cell walls. The action of growth hormones depends, in part at least, upon their property of increasing plasticity.

II. DIFFERENTIATION FACTORS

1. *Sugars*.—The maturation of enlarged cells is primarily a series of chemical processes for which available carbohydrates serve as the principal raw material. These changes may occur: (a) in the cell wall, as secondary thickening, lignification, suberization, cutinization, etc.; (b) in the chemical composition of the cell, as the accumulation of alkaloids, oils, gums, etc.; (c) in the structure of the protoplast, as seems probable in cold and drought resistance, and possible in dormancy; or (d) in the type of development as in the change from vegetative buds to flower buds or from somatic cells to spore mother cells.

2. *Warmth*.—Plants grown at lower temperatures are likely to show more food storage and fewer of the chemical changes typical of differentiation, although cool-weather plants may differentiate flowers at low temperatures,¹ possibly because of the higher sugar content of these plants under these conditions.

In using the growth-differentiation outline, the external factor or factors must first be reduced to their internal effects, since any plant reaction is directly related to the physical and chemical conditions within the reacting cells. To determine the result of a given external factor it is necessary to know the net effect of the condition upon protoplasm building materials and processes, upon the water supply, the carbohydrate supply, and the tem-

¹ THOMPSON, H. C. Relation of temperature and length of day to type of growth in celery, cabbage, and beets. Proc. Am. Soc. Hort. Sci. 29: 476. 1932.

perature, all *within the plant*. Variability in many nongenetic plant responses, particularly variations involving the relative development of gums, resins, alkaloids, oils, etc., or the balance between vegetation and fruiting in long-day plants may be predicted or explained upon the basis of growth-differentiation balance.

A second important factor in plant development is correlation. Developing fruit has been shown¹ to possess the ability to suppress vegetative growth in tomato, presumably by its greater competing power for organic nitrogen, although some writers assume the production of specific inhibiting hormones. The suppression of lateral buds in favor of the terminal, particularly when organic foods are deficient, is a phase of the same problem, as are many other interactions in which the activity of one part of a plant directly affects the development of another part. Growth-differentiation balance is primarily the resultant of external factors, whereas correlation is primarily internal, although its expression is affected by external conditions.

References:

MAXIMOV, N. A. Plant physiology. 2d ed. Chapt. XI and XII. New York. 1938.

MILLER, E. C. Plant physiology, 2d ed. Chapt. XIV. New York. 1938.

KRAUS, E. J., and H. R. KRAYBILL. Vegetation and reproduction with special reference to the tomato. Oregon Expt. Sta. Bull. **149**: 1-90. 1918. Includes a good survey of growth-differentiation balance in fruit-tree development.

MURNEEK, A. E. Physiology of reproduction in horticultural plants. II. The physiological basis of intermittent sterility with special reference to the spider flower. Missouri Agr. Expt. Sta. Research Bull. **106**: 1-37. 1927.

GROWTH-DIFFERENTIATION BALANCE

Experiment 155. The Differentiation of Vegetative Tissues.

(I)

Supporting tissues of the stem come under our heading of differentiation and should therefore be increased by factors increasing the average sugar content of the plants.

¹ MURNEEK, A. E. Effects of correlation between vegetative and reproductive functions in the tomato (*Lycopersicon esculentum* Mill). Plant Physiol. **1**: 3-56. 1926.

Fill six 2- to 4-gal. jars with a mixture of composted loam and sand, plant with sunflower seeds and grow for Experiments 155 and 156. As soon as the seeds are well germinated, thin to two or three plants per pot, and treat as follows:

Hold three pots in full light with moderate watering.

Hold three pots under a tight shade of heavy muslin, or three layers of cheesecloth, with optimum watering. Add 1.0 gm. NaNO_3 in 500 ml. water to each of the shaded pots at weekly intervals.

Grow the plants for 6 to 10 weeks, preferably in the months of March, April, and May. Record rates of growth, number of branches, and number of flowers or number of flower buds produced and take notes on color, succulence, etc. At the end of the experiment, determine the relative accumulations of carbohydrates in the plants from the different treatments by testing sections of the stems in I-KI solution. Use the central axis at a uniform distance from the tip of the plant. Do not throw away any tissue.

Determine separately the green and dry weights of leaves, stems, and of the roots after washing them carefully from the soil. Include underground portions of the stem with the stems rather than with the roots. Weigh and dry the basal 10 to 15 cm. of the main stems of each lot of plants separately for estimations of differentiated tissues. Grind these dry stems finely in a burr mill, weigh out duplicate 1.0- to 2.0-gm. samples to ± 0.2 mg., transfer to flasks with 100 ml. $1 + 9 \text{ HCl}$,¹ and autoclave at 15 lb. for 1 hr. Transfer the residue quantitatively to tared Gooch crucibles, previously dried and weighed to ± 0.2 mg.; wash the flask, residue, and Gooch crucibles thoroughly with hot water and suction. Dry the precipitate to constant weight at 100°C . and calculate the percentage of the green and dry weight of the basal stems which is not hydrolyzed by the acid. This is, roughly, cellulose and lignin. Calculate the percentage of dry matter in each of the tissues and tabulate all of your data on the basis of diminishing starch content of the stems as shown by the I-KI test.

Questions:

1. What plant responses are positively correlated with high carbohydrates in your material?

¹ One part concentrated acid and nine parts water by volume.

2. Why are plants grown in dry exposed locations more woody and more resistant to drying than those grown under more "favorable" conditions?

Experiment 156. The Balance of Roots and Tops. (I)

Root growth has the same requirements as shoot growth, but the roots are dependent upon the tops for their food supply and may not receive enough sugar and protein at times when the shoot is using these materials rapidly or manufacturing them slowly.

Tabulate green and dry weights of roots and tops of the plants grown in Experiment 155. Calculate the top-root ratio on the two bases. Also express the weight of the roots as a percentage of the weight of the entire plant. Arrange the treatments in order of decreasing starch content.

Questions :

1. Which plants show the relatively larger root systems? How do you explain the response?

2. Why do plants growing in dry soil have a relatively well-developed root system?

3. Which may be expected to withstand dry weather better, a plant which has been kept closely clipped or one allowed to grow naturally? Why?

Experiment 157. Etiolation. (E-I)

Plant six or more 6-in. pots with 20- to 30-gm. pieces of non-dormant Irish potatoes. Grow two pots in a photographic dark-room, watering and observing only by photographic safe light.¹ Cover two pots in the greenhouse with frames and a double layer of muslin, and leave two exposed on the greenhouse bench. Allow the plants to grow with normal watering for 3 to 4 weeks. Measure the average length and diameter of internodes and the average size of leaves on the plants at the different light intensities. Cut sections from the stems at two or more corresponding levels and measure stem diameter and average diameter of cells with an eyepiece micrometer. Stain the sections with I-KI and observe for starch. Save the pieces of the plants and determine the green and dry weights of the shoots from the different treatments.

If time permits, determine the fiber content of the dry plants by the method of Experiment 155. Calculate grams of fiber

¹ Etiolated plants may be fully as sensitive to light as photographic film and must be protected with the greatest care.

per gram of green weight. It will also be interesting to wash out the roots and determine their green and dry weight and the top-root ratios for comparison with Experiment 156.

Questions:

1. How will heavy shading normally affect the growth-differentiation-balance factors of proteins, water, and sugar?
2. How does it affect cell size, internode length, and degree of differentiation in your experiments?
3. How does the gardener's practice of blanching endive, by tying up the leaves, affect the "quality" of the central leaves?

Experiment 158. Hardening.¹ (I)

Obtain 12 pots of thrifty, rust-free winter oats.² Grow six pots for a week at a temperature of 5°C., and water sparingly. Grow the other six pots at 20 to 25°C. under muslin shade, and water normally. At the end of the week, water all pots and transfer to a freezing chamber at -5°C. Remove three plants of each lot after 2 hr., and the remainder after 24 hr. Return the frozen plants to the greenhouse and observe for injury and recovery.

Questions:

1. Why does hardening increase frost resistance?
2. Why is alfalfa subject to winter injury following late fall pasturing or cutting?

GROWTH CORRELATION

In its broadest sense, correlation is the ability of one part of the plant to affect the development of another. Thus Experiment 156 on root development represents a phase of correlation, in that roots are dependent upon the leaves for growth materials. Competition for foods is probably an important phase of all correlation. Two theories have been advanced to explain competitive ability: one based upon the action of chemical stimulators and inhibitors, and the other upon an electrical transmission of a cell activity stimulus. Polarization of the phloem by either chemical or electrical stimuli is a possible mode of action.

¹ DEXTER, S. T. Effect of several environmental factors on the hardening of plants. *Plant Physiol.* 8: 123-139. 1933.

² Other winter annuals such as winter wheat, cabbage, hardy mustards, etc., may be used. Run preliminary experiments to determine correct freezing exposures.

Experiment 159. Polarity. (E)

The tendency of a plant organ to retain its original directional habits, producing leaves at the apex and roots at the base is called "polarity."

a. Cut dormant willow twigs¹ into 40-cm. sections of uniform diameter and treat as follows:

Place two, tips up, as checks. (Tag or mark with India ink.)

Place two, tips down, as checks.

Ring four carefully through the cambium removing a 7-mm. ring of bark from the center of the twig, scrape away the cambium, and place two upright and two inverted.

Pack the cuttings in damp moss and place in cans or wrap in waterproof paper, and keep upright in a warm place until growth starts. Observe root and bud growth and their relation to the tip and butt of the twig.

b. Cut horseradish, dandelion, or dock roots into 10-cm. pieces with a square cut at the "upper" end and a slanting cut at the "lower" end of each piece. Invert half of the pieces and pack and hold them all in moist sphagnum as for part a. Observe sprout and root production, and then use for Experiment 163 on regeneration.

Questions:

1. How would you explain polarity?
2. Upon what tissues does it depend?
3. In what group or groups of compounds would you look for the determining factor, assuming that a chemical control is responsible?

Experiment 160. The Reversal of Polarity. (E)

To show that polarity is dependent upon physiological processes within the plant, pack several dormant willow cuttings 30 cm. long into a metal box with damp moss. Turn half of the cuttings tip up and half base up, and place a 10-watt Mazda bulb inside a wire basket at one end of the box. Hold the box, with the cuttings vertical and the lighted bulb at the bottom, in a cool (5 to 10°C.) room. Measure the temperature of the moss around the cuttings. It should be 5 to 10°C. warmer near the light.

¹ Twigs should be "dormant" in the sense of not growing, but should not be in the resting stage. Willow sprouts cut in February give the best results in Iowa. These may be packed in damp moss or sawdust and held at 0°C. for use before June.

Observe sprout formation on the cuttings and compare with the results obtained in Experiment 159a.

Question :

1. What explanation of apical dominance best fits your results?

Experiment 161. The Rooting of Herbaceous Cuttings. (E)

Obtain three sets of five or six cuttings each of such plants as *Coleus*, *Iresine*, or tomato. Take the cuttings from vigorous plants, and cut them 7 or 8 cm. long with a good tuft of leaves. Take a fourth set of cuttings from similar plants which have been held in bright light for a week and held just above the wilting point to encourage the accumulation of carbohydrates. Artificial light at night is desirable. Do not use plants stunted until the stems are too woody to root readily. Check on starch accumulation by observing sections from the base of the normal and high-carbohydrate plants after staining with I-KI solution.

Set up the settings in a cutting bench or in flats of clean sand according to the following outline:

1. Controls, with all leaves in light.
2. Defoliated and new leaves removed as formed.
3. Normal plants with leaves, covered with light-tight cover.
4. High-carbohydrate plants with leaves, covered with light-tight cover.

Hold in a cool (15 to 20°C.), moist greenhouse with a light shade of lath or muslin, and observe at weekly intervals for rooting. Use some of the rooted cuttings for Experiment 163c.

Questions :

1. What is the objection to removing leaves from cuttings and transplanted plants to reduce transpiration?
2. What substances formed in leaves in the light might increase rooting?

Experiment 162. Stimulation of Rooting by Hormones. (I)

The treatment of cuttings with various growth-promoting substances to increase the rate and amount of root formation is a recent important development in applied plant physiology.¹

¹ HITCHCOCK, A. E., and P. W. ZIMMERMAN. Effect of growth substances on rooting response of cuttings. *Contrib. Boyce Thompson Inst.* **8**: 63-79. 1936.

The mechanism of action of the hormones appears to be indirect,¹ but the effect is frequently striking.

a. Obtain four sets of vigorous herbaceous cuttings of *Coleus* or some similar plant and treat as follows:

1. *Controls*.—Clip the stems of five or six cuttings under water to eliminate possible air plugging of the xylem and hold the shoots in tap water in a diffusely lighted, dry room for 16 hr. Bed the cuttings in clean sand in a cool, moist, lightly shaded cutting bench and observe at weekly intervals for rooting.

2. *Defoliated*.—Hold in water for 16 hr., as for the controls, but cover tightly when placed in the cutting bench to exclude all light for 24 hr., and then defoliate and remove all new leaves as formed. Observe at weekly intervals for root formation.

3. *Hormone Treated*.—Cut the lower ends of the stems of the third set of shoots under water, and hold for 16 hr. in a dry room in diffuse light with the stems in 100 to 200 ml. of a solution containing *one milligram* of indolebutyric acid for each 100 ml. water. The hormone should be drawn into the plant by transpiration so that a moderate transpiration rate is essential to the success of the experiment. Bed the cuttings and observe for rooting in comparison with the tap-water treated controls.

4. *Treated, Defoliated*.—Treat a fourth lot of cuttings like the third, but cover for 24 hr. after placing in the bench to prevent photosynthetic production of root-forming substances while the absorbed hormone is being translocated downward from the leaves where it was carried by the transpiration stream. At the end of 24 hr., uncover, remove all leaves, and keep defoliated. Compare root formation with that of the defoliated controls.

As much as 8 mg. of hormone per 100 ml. H₂O is recommended for cuttings of woody plants which are difficult to root. Two milligrams per 100 is likely, however, to be all that can be safely used for herbaceous plants held in a location which will give a good transpiration rate, and even this will injure the more sensitive plants.

b. Obtain four sets of 10 dormant cuttings each of privet or other easily rooted woody cuttings and treat as follows:

1. *Controls*.—Bed deeply in a sand-filled cutting bench.

¹ COOPER, W. C. Hormones and root formation. *Botan. Gaz.* 99: 599-614. 1938.

2. *Disbudded*.—Remove all buds and bed as for (1). Examine occasionally and remove any adventitious or auxiliary buds found.

3. *Hormone*.—Apply a generous dab of a 1 to 300 paste of indolebutyric or indoleacetic acid in lanolin to the freshly cut upper end of the third set of cuttings. Cut away 1 or 2 mm. of the tops each week and apply fresh hormone paste. Bed and compare bud and root growth with (1).

4. *Hormone, Disbudded*.—Apply hormone as in (3) to a set, of disbudded cuttings and compare with (2).

Questions:

1. Why is the hormone applied at the top of the plant (or carried to the top in the transpiration stream) to induce rooting at the base?

2. What substances other than indolebutyric and indoleacetic acid may be used as growth hormones (auxins)?

Experiment 163. Regeneration. (E-I)

a. The regeneration of sprouts by roots may be observed on the dandelion or other root material from Experiment 159. Mount sections through the region of sprouting and observe the tissues concerned in regeneration and the manner of development of new sprouts.

b. Obtain six stocky tomato plants about 20 cm. high, cut the tops off smoothly 4 to 6 cm. from the tip, and cover the cut surface of the stubs well with vaseline, or cover half with vaseline and half with lanolin containing 1 part of indoleacetic acid per thousand. Remove all lateral buds as they develop from four of the plants, leave two without pruning, as checks, and observe for callusing and the regeneration of sprouts from the cut surface.¹

c. Study the manner of root formation in the cuttings from Experiment 161, or observe prepared slides. Determine where the roots arise and how their vascular tissue is connected with that of the stem.

Questions:

1. List other examples of regeneration.

2. What appears to be the stimulus for regeneration? How is it affected by growing sprouts?

¹LINDSTROM, E. W., and KATHARINE KOOS. Cyto-genetic investigations of a haploid tomato and its diploid and tetraploid progeny. *Am. Jour. Botany* **18**: 398-410. 1931.

Experiment 164. Inhibition.¹ (E)

Germinate garden beans in pots in the light until the plumules have elongated above the cotyledons. Divide into three lots of some 10 plants each and treat as follows:

1. Controls.
2. Remove plumules, cutting in *first internode* between cotyledons and first foliage leaves. If plants are available, cut a few of them in the second internode and compare. Cover the cut stubs with lanolin containing *no* hormone.
3. Remove plumules as in (2) but coat the cut stumps liberally with a mixture of one part indoleacetic acid in 500 parts hydrous lanolin.

Hold the pots in the greenhouse for a week or 10 days and observe for growth of the buds in the axils of the *cotyledons*. Recut the stumps of lot 3 and apply fresh hormone paste at intervals of 3 to 5 days.

Questions:

1. How is inhibition related to polarity (Experiments 159 and 160)?
2. Why is inhibition more pronounced under low-nitrogen conditions when the apical buds produce less hormone?

Experiment 165. Effect of Fruiting upon Growth.² (I)

Obtain eight thrifty tomato plants about 25 cm. high, carefully wash free of soil, and pot in sand, two plants in each of four 8-in. pots. Set the pots in shallow pans, and water two of them with a full nutrient solution (Experiment 42 page 62) diluted 1 + 2 with tap water. Water the remaining pots in the same way with a solution substituted to omit nitrogen.

Grow on the greenhouse bench until the plants have produced one or more fruits each 1 cm. or larger in diameter. Carefully measure the height of each plant, either from a peg driven

¹ MORELAND, C. F. Factors affecting the development of the cotyledonary buds of the common bean, *Phaseolus vulgaris*. Cornell Univ. Mem. **167**: 1-28. 1934.

SNOW, R. The correlative inhibition of the growth of axillary buds. Ann. Botany **39**: 841-859. 1925.

VAN OVERBEEK, J. Auxin distribution in seedlings and its bearing on the problem of bud inhibition. Botan. Gaz. **100**: 133-166. 1938.

² MURNEEK, A. E. Effects of correlation between vegetative and reproductive functions in the tomato (*Lycopersicon esculentum* Mill). Plant Physiol. **1**: 3-56. 1926.

solidly into the pot or from the fruiting cluster. Remove all fruits from one of the plants in each pot, leaving the other as a control, and observe height growth and branching of the plants over a period of 2 to 4 weeks.

Continue watering the pots with the full nutrient and minus nitrogen solutions. The shallow pans under the pots will hold solution and reduce the danger of the plants suffering from lack of water. Flush the pots with tap water every week or 10 days. Remove branches as formed but record the number produced by each plant.

Questions:

1. How does growing fruit inhibit apical growth?
2. Does it have the same effect on branching?
3. How does inhibition appear to be related to the nitrogen supply?

Experiment 166. Cambial Growth.¹ (I)

Obtain eight one-year-old dormant poplar trees in late winter, after they are out of the rest period, and pot them in 8-in. pots. Treat duplicate trees as follows:

1. Controls, allow to grow normally.
2. Ringed, remove a 1-cm. ring of phloem carefully near the middle of the stick. Scrape away the cambium and cover the ring with paraffin heated in boiling water. Remove all buds below the ring, but allow upper buds to grow normally.
3. Remove all buds from entire plant and watch carefully that no new buds form.
4. Remove buds as for (3), make a slanting cut at the top of the stick, and apply liberally a paste of 1 part indoleacetic acid in 300 parts of lanolin. Cut the top at weekly intervals to expose a fresh surface and apply fresh hormone paste.

After 2 to 4 weeks, when new sprout growth on the controls is 10 to 20 cm. long, section the plants carefully with a sharp razor blade and measure new cambial growth with an eyepiece micrometer (see page 40, for directions for standardization). Measure growth above and below the rings on the ringed trees and at varying distances below the point of applying the hormone paste on those treated with indoleacetic acid.

¹LOOMIS, W. E. Translocation and growth balance in woody plants. *Ann. Botany* **49**: 247-272. 1935.

Questions:

1. Why does midsummer defoliation of a tree by insects produce a double ring instead of the normal single ring of wood growth?

2. Why should a limb, removed in pruning, be cut close against the trunk instead of being left with a short stub?

Experiment 167. First Internode Growth in Maize.¹ (E-I)

Germinating grass seedlings show very interesting correlations and inhibitions in their shift from first internode to plumule growth, which can be studied conveniently in maize.

a. Obtain a large glass battery jar, fill it 5 or 6 cm. deep with sand and place 10 corn seeds against the glass at one side and 10 near the center of the jar. Add 6 cm. of sand and plant 20 more seeds in the same manner, placing the seeds so that they are not directly above the first lot. Add 4 cm. of sand, plant a third lot of 20 seeds, cover with 2 cm. of sand, and water carefully to avoid flooding or washing out the planted grains.

Hold the jar in a well-lighted greenhouse, and observe the germination of the seeds planted at the three depths. Compare the development of the seedlings growing against the glass and receiving light with those growing in the center of the jar and protected from light until they reach the surface. As soon as the plants are well up, remove them carefully and record the average length of coleoptile, the average depth of the crown roots, and the average length of the first internode of the epicotyl (mesocotyl).

b. Plant 20 grains of corn in each of three shallow pots filled with sand, and cover about 0.5 cm. deep. Germinate in complete darkness until the plants are 2 to 3 cm. high. Observe the plants with a No. 2 or No. 3 photographic safe light, and do not expose for more than a few seconds. They are as sensitive to light as photographic film.

Cut 2 mm. from the tips of half of the plants in each of two pots, and apply a 1 to 300 paste of indoleacetic acid in lanolin to the stumps. Expose these two pots to 100 f.c. (a 250-watt Mazda bulb with reflector at 60 cm. if you have no light meter) for 24 hr., water, and return to the dark chamber for 2 days. Hold the third pot in absolute darkness throughout, without even removing it for observation by photographic safe light.

¹ INGE, F. D., and W. E. LOOMIS. Growth of the first internode of the epicotyl in maize seedlings. *Am. Jour. Botany* **24**: 542-547. 1937.

At the end of 2 days remove all plants and record quickly, before they are affected by light: (1) the length of the coleoptile; (2) the length of the first internode; (3) the length of the leaves; and (4) the number of roots which have started at the coleoptilar node. Tabulate and average your data.

Questions:

1. Why can grains be planted deeply in dry weather, to insure germination, without affecting the depth of the crown roots?
2. Why do not the plumule and crown roots start rapid growth with the beginning of germination?

APPENDIX

Solutions and Preparations for Plant Physiology Laboratory

Use distilled water for all solutions

1. Acids—Diluting.—First figure of dilution formula indicates parts of concentrated c.p. acid by volume and second, parts of water; thus, 1 + 2HCl, 1 + 9HNO₃, 2 + 1H₂SO₄, etc.

Pour concentrated H₂SO₄ *into* water.

Two per cent HCl = 1 + 20HCl.

2. Acids—Standard.—See Methods in Plant Physiology for preparation of standard acid.

3. Alkaline Pyrogallol Solution.—Three gm. pyrogallol acid, 15 gm. NaOH pellets, and 90 ml. H₂O. *Protect from air.*

4. Barium Hydroxide Solution—N/10.—Dissolve 32.5 gm. Ba(OH)₂·8H₂O in water and make to 1 l.; allow carbonate to settle out, and decant. Standardize against HCl.

5. Bunsen Valve.—Make a 2-cm. slit in the side of a 5-cm. piece of rubber tubing; plug one end with a piece of glass rod, and attach other end to incoming air line within a suction flask or bottle. Valve admits air with suction but prevents backward movement.

6. Celloidin Mixture.—Dissolve 10 gm. celloidin (Parlodion) in 200 ml. of a mixture of equal parts of anhydrous ether and absolute ethyl alcohol; several hours with occasional shaking will be required. If mixture thickens, thin with solvent to consistency of maple sirup.

7. Cobalt Chloride Paper.—Wet a good grade of filter paper with 5 per cent CoCl₂ solution, squeegee, and dry flat on a glass plate. Cut into 2-mm. squares, and protect from atmospheric humidity. Warm to restore blue color.

8. Copper Tartrate.—See footnote, page 130.

9. Diphenylamine Solution.—Dissolve 0.1 gm. diphenylamine in a mixture of 7.5 gm. concentrated H₂SO₄ and 2.5 ml. H₂O.

10. Eosin Solution.—0.1 gm. in 100 ml. H₂O. *Dilute eosin*; add 1 or 2 ml. of the 0.1 per cent solution to 25 ml. H₂O to give a faint pink color.

11. Fehling Solution.—A. Dissolve 34.64 gm. c.p. CuSO₄·5H₂O in water and make to 500 ml. in a volumetric flask; filter if necessary.

B. Dissolve 50 gm. c.p. NaOH in 100 ml. H₂O; boil to clear, *after dissolving completely*. Dissolve 173 gm. sodium-potassium tartrate (Rochelle salt) in 300 ml. H₂O, mix with NaOH, cool and make to 500 ml.

Use equal parts of A and B to make fresh Fehling solution as needed.

12. Gas Mixtures.—See footnote, page 154.

13. Guaiacum Extract.—Grind 1 to 2 gm. gum guaiacum and shake with 100 ml. ethyl alcohol. After several days filter off the extract.

14. Iodine-potassium Iodide Solution.—Dissolve 0.3 gm. iodine crystals and 1.5 gm. KI in 100 ml. H_2O . Keep in glass-stoppered bottle.

15. Indoleacetic Acid Paste.—For 1 + 500 mixture, dissolve 20 mg. β -indoleacetic acid in 2 or 3 drops of alcohol, and mix *thoroughly* with 10 gm. hydroparaffin (wool fat).

16. Methyl-red Solution.—One-half gram in 100 ml. 50 to 70 per cent ethyl alcohol.

17. Magnesium Mixture.—See footnote, page 56.

18. Millon Reagent.—See footnote, page 133.

19. Molar Salt Solutions.—Multiply molecular weight of salt, including water of crystallization, by molality desired (0.5M, 0.2M, etc.), dissolve in water, and make to 1 l. in a volumetric flask. Or use $\frac{1}{10}$ of the indicated weight of salt and make to 100 ml.

20. Molybdate Solution.—See footnote, page 51.

21. Organic Mercury Dust Suspension.—Shake 5 gm. Merco, Semesan, or similar dust in 100 ml. 95 per cent alcohol. Use to disinfect seeds, water-culture corks, etc.

22. Phenolphthalein Solution.—One-half gram in 100 ml. 95 per cent alcohol.

23. Phloroglucinol Solution.—One gram phloroglucinol in 50 ml. 50 per cent ethyl alcohol.

24. Potassium Hydroxide Solution.—For saponifying chlorophyll; saturate *methyl* alcohol with KOH pellets, allow to settle, and decant cleared solution.

25. Potassium Hydroxide Solution.—For carotene test: 20 gm. KOH pellets, 40 ml. *methyl* alcohol, and 60 ml. H_2O .

26. Potassium Permanganate—Standard Solution.—See Methods in Plant Physiology.

27. Potassium Thiocyanate Solution.—Two grams in 100 ml. H_2O .

28. Safranin-O Solution.—One gram in 500 ml. H_2O .

29. Silver Nitrate Solution.—One-tenth gram in 10 ml. H_2O .

30. Sodium Hydroxide Solution.—Forty per cent for Kjeldahl; 400 gm. flake NaOH in 600 ml. H_2O . Dissolve in *pyrex*; when *fully* dissolved, boil to clear, and protect from atmospheric CO_2 .

31. Sodium Hydroxide Solution—N/10.—Dissolve 4.2 gm. NaOH pellets in 50 ml. H_2O , boil to clear, and make to 1 l. in a volumetric flask. Standardize against HCl (see Methods in Plant Physiology).

32. Starch Solution.—See footnote, page 82. Add toluene, and hold in glass-stoppered bottle.

33. Sudan III Solution.—One-half gram in 100 ml. 70 per cent methyl alcohol.

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