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Within the Living Plant

AN INTRODUCTION
TO PLANT PHYSIOLOGY

Within the Living Plant

AN INTRODUCTION

TO PLANT PHYSIOLOGY

By

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To

E. C. M.

Foreword

The material in this textbook has been employed at the University of Pittsburgh in the author's plant physiology course which is offered at the advanced undergraduate level. This course differs somewhat from similar courses in that it is not presented to botany and agriculture students alone, but to students with varied interests including pre-medical and pre-dental students in addition to students majoring in zoology or botany. The subject matter continues to interest these students, and on a number of occasions, has served to orient them to further study in this field.

This textbook has two major objectives: (1) it presents fundamental principles of plant physiology; (2) it then focuses attention on the manner in which plant physiology may affect the everyday life of the individual. For this reason the book should be useful to both professional and amateur botanists, to specialists in other fields of science and to many laymen who are interested in understanding physiological processes within the living plant.

It is believed that those who read this book will find answers to the type of question which has been constantly directed to the author since the opening of this course in plant physiology. These questions have been related to home gardening, to the natural vegetative covering of the landscape, to observations regarding fruits and vegetables on the market, and to the processing and transportation of perishables. All involve fundamental principles of plant physiology which are explained herein.

Progress in the field of plant physiology during the twentieth century has been phenomenal. In this age of specialization advances are being made along specialized lines in various branches of the subject. It should be the purpose of an elementary course to present an over-all picture of the subject and to direct the attention of the student to additional sources of information, most of which are more specialized or more advanced. It is for this reason that a number of titles for collateral reading have been appended at the end of this book. The titles include textbooks, monographs and scientific papers.

Since a college textbook consists of a compilation and organization of knowledge in a particular field of study, original contributions by the author usually constitute a relatively small proportion of the entire subject matter. He must of necessity draw upon the contributions of his

fellow workers throughout the entire world. Credit has been given in a number of instances by including the names of men and women who have contributed to the sum total of knowledge in this field, but it is not possible to list in these pages the names of all who rightfully belong there.

A certain amount of material in this book has been obtained from Plant Physiology, official publication of the American Society of Plant Physiologists. Appreciation is expressed to the Editorial Board and to the contributors of the papers which have been quoted. Photographs have been kindly supplied by research workers in many other institutions. Acknowledgement has been made either in the legend of the specific illustration or here in the foreword. For many of the original photographs which appear in this book the author acknowledges assistance from the following persons: Edward Himchak, William H. Randall, Francis D. Crisley, Alan S. Heilman, all of the University of Pittsburgh; J. R. Winston, U.S. Dept. of Agriculture; The Brock Studio, Beulah, Michigan; Bert E. Roper, Winter Garden, Florida; and W. H. Connery, Orlando Photo Studios, Orlando, Florida. The author is grateful to the following former associates in the U.S. Dept. of Agriculture for permitting reproduction of a number of illustrations: Dr. H. A. Borthwick, Dr. G. H. Coons, Dr. W. C. Cooper, Dr. Frank M. Eaton, Dr. J. R. Furr, Dr. Frank E. Gardner, Dr. M. H. Haller, Dr. J. B. Kienholz, L. P. McColloch, W. T. Pentzer, Dr. Neil W. Stuart, Dr. John H. Weinberger, and J. R. Winston.

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The author has received helpful suggestions from Dr. Peter Gray, Dr. Ralph Buchsbaum, Dr. Richard H. McCoy and Dr. John R. Jablonski, all of the University of Pittsburgh; from Dr. James B. Lackey, University of Florida; and from William F. Keller and other members of the Blakiston staff.

The author should add to this imposing list the members of his own family who have also assisted with typing and with the preparation of illustrations for this book.

Pittsburgh, Pa.
July, 1953.

Erston V. Miller

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chapter 1

Plant Physiology in Everyday Life

Throughout the ages man has been dependent upon plants for many of his needs. The green plant is the initial source of food for man and other animals. In addition to food, plants supply us with wood, textile fibers, pulp (for paper), rubber, vegetable oils, gums, drugs, and other materials.

Because of their importance, plants have been the subject of study by both scientist and layman for a long time. The term *botany* is now applied to the general field of the study of plants. In this age of specialization a botanist usually concerns himself with one of the specialized fields. The study of plant structures, for instance, is called *plant anatomy;* the origin of these structures is called *morphology;* the classification of plants, *taxonomy;* their distribution and native habitats, *ecology. Plant pathology* embraces the diseases of plants, *genetics* includes their heredity and breeding. *Plant physiology* is concerned with the functions of the living plant. The physiologist attempts to interpret the processes of the living plant by means of physics, chemistry, and mathematics.

The study of *plant physiology* should be of use to anyone who has an interest in plants, whether he be amateur or professional, or whether his interest be of only an esthetic nature. The physiologist, in his contributions to the fundamental science of plant life, has benefited not only the farmer but all mankind. A few examples may be cited as an illustration of this last statement. The agricultural industry may be divided roughly into three categories. These are production, handling (harvesting, transportation and storage) and utilization (canning, concentrating, freezing, dehydration, and production of by-products). Let us consider a few physiological problems in these three categories with a brief discussion of how the problems were attacked.

Instances of Application of Plant Physiology

A. Production of Agricultural Crops

Plant physiologists have taken their place among other plant scientists in assisting with the production of agricultural crops. The successful use of fertilizers, correction of deficiency diseases, soil management, and other practices can usually be traced to fundamental investigations by the phys-

iologist in his laboratory or experimental greenhouse. The roles of both major and minor elements in the physiology of plants have been exhaustively studied by means of nutrient solutions or soilless culture, perhaps long before the laymen were aware that plants might be grown without soil.

Fundamental physiology has played its part in permitting the grower to speed up the opening of buds in the spring or to hold them back, to regulate the time of blooming, to induce fruit setting, or to cause the removal of unwanted leaves, flowers, or excess fruits. Many more examples might be cited, but because of lack of space detailed discussion of these examples must be reserved for treatment in appropriate chapters of this book.

B. Transportation of Plants

Abrupt changes in agricultural methods often produce new problems for the physiologist. One example of this is to be found in the modern method of handling vegetable seedlings. Tomato plants for the canneries were formerly grown in greenhouses in the canning areas and then transplanted to fields in the spring after danger of frost had passed. Now most



Experimental shipment of tomato plants by air. Physiologists are taking temperatures en route. (Courtesy, Delta Air Lines.)

of the tomato plants required by canners are grown outdoors in the southern states and these, called *field-hardened plants*, are shipped to the northern states for transplanting. Instead of moving the plants from greenhouse to field, they may be transported a thousand miles by train, truck, or plane.

Shippers of tomato plants soon had many problems to present to the physiologist. Carloads of plants were lost at times because of improper conditions of storage or transportation. Physiologists were asked to determine optimum temperatures and relative humidities during transit. It was necessary to study the effect of the respired carbon dioxide on the plants during their journey. Questions were asked regarding the formation of new roots by the plants. Many years of study and observation were required for the solution of these problems and physiologists in this instance worked in close cooperation with growers, canners, and transportation officials.

C. Storage Scald of Apples

One of the most interesting problems in the field of handling horticultural crops concerns the storage of apples. Prior to 1918, cold storage



Metabolic processes are retarded by low temperatures. Fruits and vegetables are transported in refrigerator cars to insure delivery in fresh condition. (Courtesy, Fruit Growers Express Co.)

men sustained severe losses because of a physiological disorder of apples known as *scald*. This disorder is characterized by browning of the skin, which looks much as if the apple has been scalded with hot water. Physiologists found that the fruits themselves were the cause of the scald. To be more specific, volatile substances evolved by the apples in the confinement of storage rooms produced scald in certain varieties of apples. When these volatile emanations were absorbed on either shredded oiled paper or oiled wraps, practically no scald developed. Thus the attractive

blue paper one often sees in the baskets or boxes of apples has been placed there, not for ornament, but for the sole purpose of controlling scald.

It should be added that at the present time physiologists are not entirely agreed as to the specific substance which is responsible for producing apple scald. In 1942 several investigators claimed that the active agent in emanations of apples is ethylene, an odorless and colorless gas with great physiological activity. But in 1948 those who held this theory were inclined to reverse their stand and it was then claimed that the physiological agent which causes apple scald is "some unknown storage gas."

D. Utilization of Agricultural Products

The story of canned orange juice offers an example of some of the results of scientific investigations by physiologists and others in the field of utilization of agricultural products. Probably few of us remember the not too palatable commodity marketed as canned orange juice a number of years ago. The product of today, which is so superior to that of the past, is the result of a number of improvements in processing, each having been discovered and applied separately and successively. Time and temperature of processing were both reduced; oxidation was prevented by packing either in nitrogen or in a vacuum; the catalytic effect of certain metals on aging of the juice was eliminated by changing over to stainless steel in the equipment; and the deleterious effect of the rind oil was removed by special improvements in the processing machinery. Thus physiologists, chemists, horticulturists, working in teams or independently in various parts of the country or of the world, have all helped solve these problems which concern all of us.

E. Duck Food Problem

Thus far the discussion has been limited to commercial agriculture. Years ago a problem of very different aspect was presented to biologists and later to physiologists, and was subsequently solved by a team of many different scientists. There were so many phases of plant physiology involved that it seems worth while to review the story briefly.

The story has been referred to by Crocker as the "Duck Food Problem." In the inland waters of North Bay and Back Bay in Virginia and Currituck Sound in North Carolina, is to be found one of the most important winter feeding grounds for migratory wild fowl in the United States. This area is bounded on the north by Chesapeake Bay, on the east by the Atlantic Ocean, and on the south by Albermarle Sound. In these waters at the beginning of this story (1918) there thrived a great abundance of submerged aquatic plants, all of which served as valuable food plants for wild ducks, geese, and swan. Examples of these plants are

Potamogeton pectinatus (sago pondweed), *Vallisneria spiralis* (wild celery), *Ruppia maritima* (ditch grass), and *Ceratophyllum demersum* (hornwort).

As can be imagined, this region constituted a sportsman's paradise, with all of its fishing and hunting. More than $5,000,000 were invested in marshlands and properties by outside interests. In addition to this, 10,000 people earned their livelihood, either directly or indirectly, by hunting and fishing. All this was prior to the year 1918.

Currituck Sound is connected with the waters about Norfolk and Portsmouth, Virginia, by means of the North Landing River and the Chesapeake and Albermarle Canal. During the first World War this canal was widened and the sea-level locks were abandoned. Almost simultaneously with the enlargement of this canal the water plants began to die out. By the end of 1926 large areas were practically denuded of their aquatic seed plants. With the disappearance of the food plants the geese and ducks failed to return. In the waters only low-oxygen-requiring, herbivorous fish were present in abundance. What was once a sportsman's paradise and a source of livelihood for 10,000 people now became a barren waste.

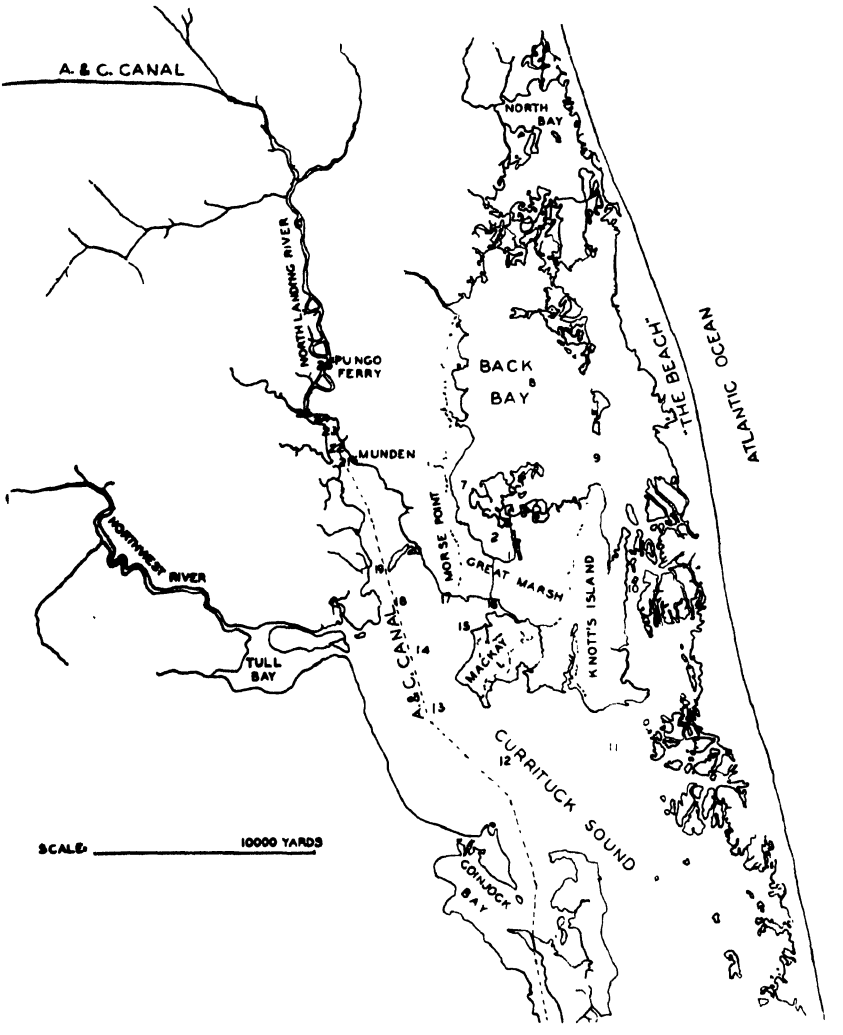
In 1925 the Boyce Thompson Institute for Plant Research, Yonkers, N.Y., was asked to study the problem. Early in 1926 Dr. W. S. Bourn began studying physical, chemical, and biological conditions of the water.

Army engineers in 1922 had claimed that the waters were made salty by washing of water from the ocean over the beach into Currituck Sound. Later they reversed this decision.

It is true that inland waters had become salty after widening of the A. and C. Canal. Dr. Bourn established the fact, however, that the salinity was introduced by the waters coming into the canal from Norfolk. For four years, monthly titrations for salt content were made at 26 stations distributed through this area. It was found that when a north wind blew, the water at station 26 (Pungo Ferry) reached values as high as 60% of sea water. When the south wind blew, the salt in Back Bay dropped to 3.2% of sea water.

But this was only circumstantial evidence. When scientists at the Boyce Thompson Institute tested various plants in the laboratory, it was found that some of the plants grew as well in diluted sea water as in fresh water and some even grew better. *Ruppia maritima* thrived in 0–80% sea water and stayed healthy in 150% sea water. So it was not the salt water that was doing the damage after all.

Brackish water which was swept down the canal from Norfolk with the north wind was polluted with sewage. Also a hydroid (*Cordylophora lacustris*) was carried along with the current. This hydroid attached itself to the leaves and stems of aquatic plants. Cordylophora was not a parasite. It fed on the plankton which abounded in the polluted waters. The hydroid forms a gelatinous sheath about the stems and leaves of plants



Map of the Back Bay, Virginia, and Currituck Sound, North Carolina, region, showing area in which scientists of the Boyce Thompson Institute for Plant Research worked on and solved the Duck Food Problem. (Courtesy, Crocker: *Growth of Plants*, New York, Reinhold Pub. Co., 1948, and the Boyce Thompson Institute.)

and the main injury occurs after the hydroid dies. This sheath constitutes a good medium for other organisms such as larvae, worms, diatoms, rotifers, fungi, and bacteria, which add to the injury. As a result of this smothering activity, the plants were partly killed, were broken from their moorings, and sank to the bottom, adding to the pollution and turbidity of the water.

About this time it was concluded that turbidity was the ultimate cause of denuding of these waters of plants. *That is, there was not enough light to permit the submerged plants to carry on photosynthesis.* There were two types of suspended particles in the water—inorganic and organic. The inorganic particles were mostly clay particles which were stirred up on the denuded bottom. This is what ecologists call *underwater erosion.* Organic matter consisted of particles of sewage in suspension.

During the growing season there was not enough light penetrating three feet below the surface to support plant growth. *Potamogeton pectinatus* thrives in 8 feet of water, and *Vallisneria spiralis* may grow in water up to a depth of 12 feet. Recent work indicates that these two plants may grow in two or three times this depth in clear water.

Let us pause now and review the significant chain of events which apparently caused the denuding of this area:

1. Removal of the sea-level locks in the A. and C. Canal permitted the north wind to blow brackish water from the Norfolk area southward.

2. Water from the Norfolk area contained sewage and the hydroid, *Cordylophora lacustris*, which found optimum conditions for growth in this water. This hydroid was not parasitic on the plants, but the gelatinous sheath left after its death, together with microorganisms which grew in this sheath, smothered the plants.

3. When the plants died and were uprooted there occurred underwater erosion. Particles of clay thus stirred up, produced turbidity in the water. In addition to this, organic matter (sewage particles) from Norfolk contributed to the turbidity.

4. With insufficient light, none of the plants could carry on photosynthesis and survive. Thus, with no plants there was no food for birds. With no photosynthesis, there was no oxygen for game fish. Without fish and fowl the erstwhile sportsman's paradise became a denuded waste land.

This story had the following ending: the sea-level locks were restored (about 1938) and life began anew. On June 25, 1943, Dr. W. S. Bourn, now in the United States Fish and Wildlife Service, wrote to the director of Boyce Thompson Institute as follows: "You undoubtedly will be delighted to know that the waters in question have returned to a maximum productivity of duck food plants. Last year the peak was reached and sago pondweed seed was washed ashore in windrows . . . "

Thus it will be seen that plant physiologists have helped solve problems originating in the production of fruits and vegetables, in their transport and storage, and in the utilization of these commodities. They have worked with land plants, with aquatic plants, with green plants, with chlorophyll-free plants, and with fruits, seeds, tubers, rhizomes. Furthermore, plant physiology, while it appears to be a specialized field of botany, may affect the everyday life of all of us at one time or another.

Many Unsolved Problems in Plant Physiology

The student will soon become aware that there are still many unsolved problems in plant physiology. In the chloroplast, for example, that microscopic, saucer-shaped body in the green cell, there lies the secret by which the plant converts water and carbon dioxide into precursors of sugar, starch, fats, and proteins. If the chemist could learn this secret and if he could synthesize foods in the laboratory, much could be done to correct the shortage of food in the world. Or even if test-tube cultures of unicellular plants could be made to produce large quantities of fats and proteins from carbohydrates, a great benefit to humanity would result.

In the matter of growth there is still much to be learned. Meristematic cells possess the ability to grow and divide. Growth-promoting substances (plant hormones) are present when growth starts, but we do not know why these substances induce growth in the young or meristematic cells and not in the mature ones. In the cancerlike growths of galls or plant tumors, there may reside the secret of abnormal growth of animal cells in malignant tumors.

To date only three plant hormones have been isolated and identified. There must be more than three of these substances present in plants. Ample circumstantial evidence exists to suggest the presence of a flowering hormone. Isolation and identification of such a substance would not only constitute a major contribution to fundamental plant physiology, but would also have considerable practical application.

The specific roles of all elements are not entirely understood. The list of ten elements essential for plant growth has now increased to 15 or 16. Perhaps additional elements will be added to the list of essential ones when salts are even more highly purified than at present, or when better techniques are developed for studying roles of the elements.

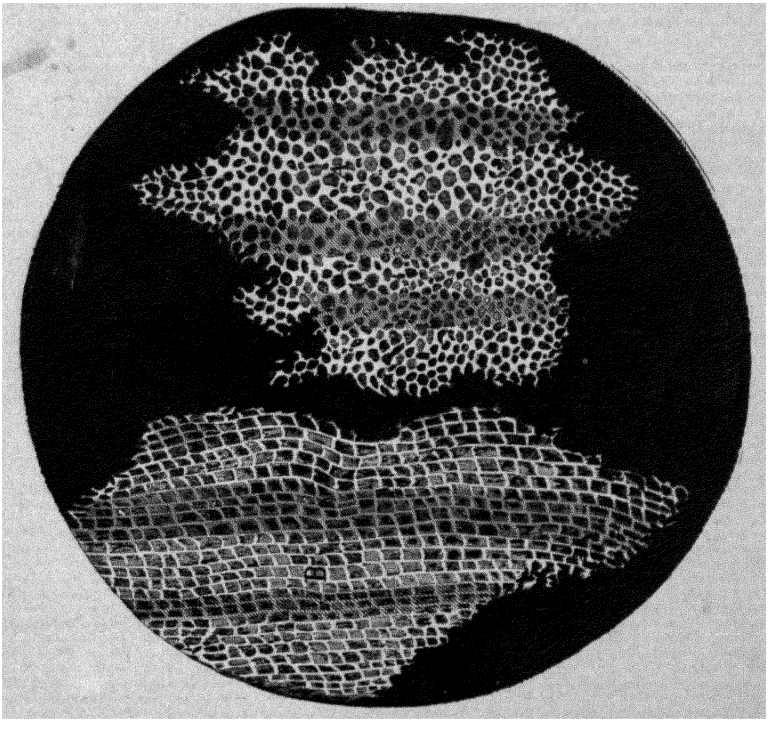
Several types of compounds are produced by plants and are useful to man, but we have not as yet discovered the function of these substances in the physiology of plants. A few examples are tannins, glycosides, alkaloids, and essential oils. A similar situation once existed with regard to vitamins in plants. At that time all evidence indicated that animals, and not plants, were dependent upon specialized food substances of this type. We now know differently.

Thus it will be seen that many problems still remain unsolved and that the field of plant physiology offers golden opportunities for pursuit of research. From time to time as we progress through the chapters of this book, we shall call the attention of the student to other problems which remain unsolved.

chapter 2

The Plant Cell

In 1667 Robert Hooke, an Englishman, looked through a microscope at a thin slice of cork and found it to consist of an enormous number of "empty boxes," fitted together somewhat like a honeycomb. Because



This is the first known drawing of cork cells and was published by Hooke in 1665. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

these boxes resembled the compartments of a monastery, Hooke called them *cells*. This name has remained in use for centuries. The cork cells in reality were like empty boxes because, being nonliving, they had lost their protoplasm and consisted of only a cell wall. As we shall see in a

moment, living cells contain varying amounts of protoplasm inclosed within the wall. It is interesting though to note that years after Hooke's discovery, the protoplasm in living cells was often overlooked largely because of its transparency. Today, with the aid of vital stains we are able to observe not only the protoplasm but many of the inclusions in addition.

A discussion of the cell seems to be the logical place to begin a course in physiology. We have just indicated that these microscopic compartments constitute the structural units of the plant or animal body. They are also the *functional* units. This means that the chemical reactions which accompany physiological processes are to be found within the living cell, and it is often possible to demonstrate these reactions in isolated tissues or even in *isolated single cells.*

Components of the Plant Cell

The components of the plant cell may be studied by placing a thin section of a leaf of the waterweed (*Anacharis canadensis*) under a compound microscope. It will thus be seen that all of the cells are surrounded by thin, translucent walls. Just within the cell wall is a thin layer of

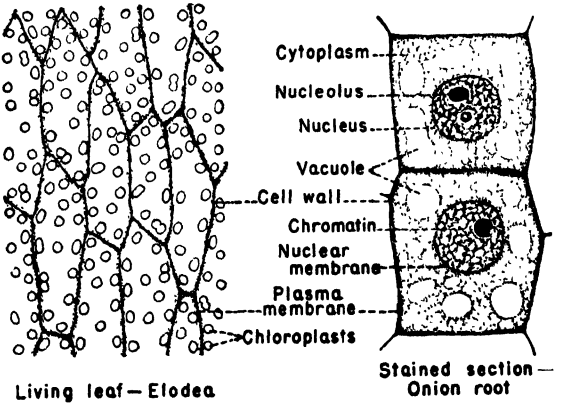


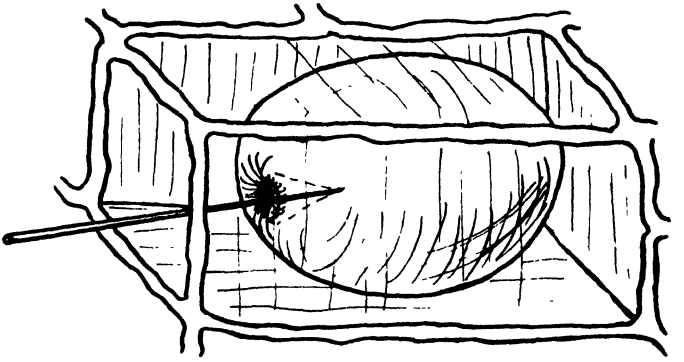
Diagram of plant cells showing principal parts. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

protoplasm containing disc-shaped, green bodies, the chloroplasts. In the center of the cell, and occupying most of the volume, is the vacuole. This contains the plant sap. Under favorable conditions protoplasm may be observed to stream or move around the border of the cell, carrying "inclusions" with it.

Protoplasm usually appears as a colorless fluid. As has been mentioned previously, the presence of protoplasm in the cell was overlooked for a long time because of the transparency of this substance. In mature cells the protoplasm consists of a narrow strip adjacent to the wall, and some-

times has crossing, interconnecting strands. The bulk of the volume within a mature cell is occupied by the vacuole. In immature cells, like those of the growing stem-tip or growing root-tip, most of the internal space is occupied by protoplasm. The vacuoles in the young cell are small and scattered. As the cell matures, these vacuoles tend to coalesce, with the formation of one large, central vacuole.

It must be borne in mind that a cell is three-dimensional. Actually, it is like a microscopic aquarium, with walls of cellulose, of course, and filled with sap, with the exception of the narrow layer of protoplasm around the walls.



The exposed living protoplast of a cell indented by a needle. (Redrawn by permission from *Protoplasm*, by William Seifriz. Copyright, 1936. McGraw-Hill Book Co., Inc.)

Protoplasm sometimes appears to be in the liquid state and at other times it appears as a gel. It is usually liquid in physiologically active cells. In dormant cells the protoplasm appears to have gelled.

It is customary to refer to the protoplasm itself, as distinct from its inclusions, as *cytoplasm*. A thin, delicate membrane separates the cytoplasm from the vacuole. There is apparently another membrane which separates the cytoplasm from the cell wall. It is believed that similar delicate membranes surround all of the inclusions in the cytoplasm.

The *nucleus* is of prime importance in the functioning of the cell. This is a spheroidal body containing cytoplasm of greater density than that surrounding it. Normally the nucleus is not visible unless the cell has been stained with a dye. It is believed that the nucleus controls the physiological activities of the entire cell. Experiments with a certain single-celled animal, like the ameba, have shown that when this organism is cut in half, only that part of the cell which contains the nucleus will survive and continue to grow. It has been known for a long time, too, that the nucleus plays an essential part in transmitting hereditary characters of the whole organism, whether plant or animal.

The nucleus consists of three distinct parts: *nuclear sap, nucleolus,* and *reticulum.* The nuclear sap occupies the greater portion of the volume of the nucleus and consists of a transparent sol or gel. The nucleolus is essentially the "nucleus of the nucleus." There may be many nucleoli. The reticulum consists of a system of delicate threads. Granular material on the reticulum is known as *chromatin.* During cell division chromatin is gathered together to form *chromosomes.* It is now known that chromosomes transmit hereditary characters of the cell.

Plastids are saucer-shaped bodies distributed in the cytoplasm. When green they are called *chloroplasts;* if red or yellow they are termed *chromoplasts.* Colorless plastids are *leucoplasts.* Chlorophyll pigments account for the color of the chloroplasts. Whenever chlorophylls are present there will also be found orange pigments, the *carotenoids.* In the latter group are included carotenes, xanthophylls, and derivatives of xanthophylls. Carotenoid pigments are not usually seen, while chlorophylls are present because of the masking action of the green pigments.

The importance of the chloroplast can be appreciated when it is realized that it is the center of photosynthetic activity, and that it is in this minute green disc that organic materials are synthesized from water and carbon dioxide.

Chondriosomes or *mitochondria* are small, rod-shaped, or granular bodies distributed throughout the cytoplasm. Various roles, mainly based on theory, have been assigned to mitochondria. Modern methods of experimental techniques, however, have served to indicate that chondriosomes are not merely inert inclusions. At least two functions are fairly well indicated. In animal cells mitochondria have been observed to be transformed into glycogen granules, yolk bodies, fat droplets, and other secretory bodies. In plants, or more specifically in the tomato, mitochondria have been observed to change into leucoplasts, chloroplasts, and finally into chromoplasts. Thus it appears that one function consists of acting as a precursor of more specialized inclusions.

In addition to the above, it is now apparent that mitochondria are seats of activity of hydrolytic and respiratory enzymes. This is suggested by the fact that the following compounds have all been identified on mitochondria: amylase, succinoxidase, cytochrome oxidase, cytochrome C, adenosine-triphosphatase, 18 amino acids and vitamin A. (Most of these compounds will be discussed in subsequent chapters.)

Still other inclusions might be observed in the cytoplasm. Stored foods may occur as starch grains, protein particles (which are often called aleurone bodies), and elaioplasts, which are believed to be centers of oil production. Sometimes crystals, like salts of citric, oxalic, and other acids, are to be found.

An outline of the components of the cell is shown in Table 1.

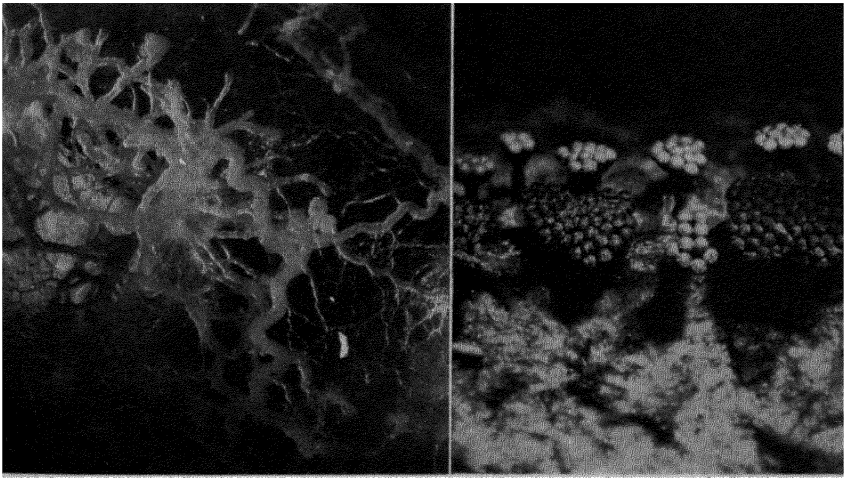
Table 1

COMPONENTS OF PLANT CELL

The Mature Plant Cell	Cell wall	Middle lamella	
		Primary wall	
		Secondary wall	
	Protoplasm	Cytoplasm	Plasmodesms
			Cytoplasmic membranes
			Undifferentiated cytoplasm
		Nucleus	Nuclear membrane
			Nuclear sap
			Reticulum
			Nucleolus
		Inclusions	Chloroplasts
			Chromoplasts
			Leucoplasts
			Elaioplasts
			Aleurone grains
			Chondriosomes or mitochondria
	Vacuole	Cell sap	Water containing compounds in solution or colloidal dispersion
		Crystals	

Chemical Constituents of the Cell

Most of the knowledge of the chemistry of protoplasm has been obtained from an analysis of myxomycetes. These are slime molds which, at certain stages in their life history, appear as amebalike masses of protoplasm. They are without cell walls in this stage and are found in moist places, usually on rotten logs. The constituents listed below have



A B

(A) Plasmodium and (B) clustered sporangia of a myxomycete. (Courtesy, Gibbs: *Botany*, New York, The Blakiston Co., Inc., 1950.)

been found in myxomycetes, and it is assumed that this is typical for protoplasm in metabolically active cells.

Protoplasm has been found to contain 90% or more water. (In storage organs, like seeds, the protoplasm may contain about 10% of moisture.) The bulk of the dry matter in protoplasm consists of nitrogenous compounds. Lipids (fats and fatlike substances) constitute a smaller proportion of protoplasm. Fats may occur in the cell either as emulsions or in the form of oil globules.

As would be expected, water-soluble substances are found in the vacuole. These consist of soluble carbohydrates, soluble proteins, amino acids, amides, organic acids, and water-soluble pigments. Mineral matter may be found in both the vacuole and cytoplasm. Salts of various organic acids may also occur in the vacuole.

The Cell Wall

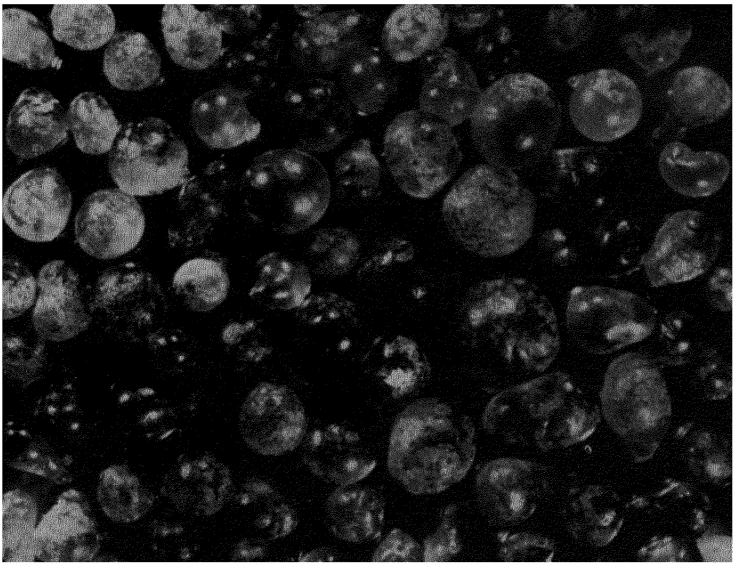
When the cell wall is first formed it consists mostly of cellulose. The first-formed wall is called the primary wall. Two primary walls of adjoining cells are held together by a layer of pectic substances, and the cementing layer is called the middle lamella. As cells become more mature a secondary wall is formed by addition of successive layers of cellulose on the inner surface. The primary wall may become strengthened by the formation of lignin, the substance that predominates in woody tissues. In some instances cell walls may become impregnated with suberin, thus becoming less permeable to water. The impregnating material is a waxlike substance. Cutin, a similar compound, functions as a water-proofing agent on the outside surface of cell walls of the epidermis of fruits, leaves, and stems. Connecting strands of cytoplasm have been found to extend through pores in the cell wall, thus forming a cytoplasmic link between cells. Strands of this type are called *plasmodesms*.

General Characteristics of Cells

Plant cells show great variety in regard to both shape and size. On the average, their diameters range from 10 to 100 microns. In certain varieties of cotton the fibers attain a length of 4 cm., while the phloem fibers of the ramie plant (*Bochmeria nivea*) may exceed 55 cm. in length.

Some of the lower plants are characterized by rather large cells and they therefore lend themselves admirably to investigation of cellular activity in plants. For example, cells of the green algae, *Cladophora* and *Nitella*, may reach a length of six inches. Likewise certain marine algae (*Valonia* and *Halicystis*) consist of large spheres, with a large, central vacuole which may contain as much as 5 ml of sap.

Mature cells, like those in parenchymatous tissues, retain a certain amount of protoplasm. Other types of cells lose their protoplasm as they mature. Fiber cells and water-conducting vessels of the xylem are exam-



Unicellular marine alga, Halicystis (natural size). Cells of this size contain sufficient sap to permit macrochemical analyses in the study of permeability. (Courtesy, General Biological Supply House, Inc., Chicago.)

ples of cells without protoplasm. In some fibers the cell walls may be thickened to such an extent that they occupy 50% of the cell volume. Both fiber cells and tracheae are distinguished by being elongated.

Short cells, which have thickened walls, and which have walls impregnated with lignin, are called *stone cells*. This type of cell is found in seed coats, the horny pericarp of the peach pit, and accounts for the grittiness in certain varieties of pears.

chapter 3

Solutions, Acids, pH

In the study of physiology it is essential to have a knowledge of standard solutions and methods for preparing them. Most everyone, at one time or another, has had occasion to use a solution in which the amount of dissolved substance (solute) is indicated by "percentage by weight." A 4% solution of iodine, 10% solution of sugar, and 1% salt solution, are typical examples.

It should be remembered that a 10% solution by weight consists of 10 Gm. of the solute plus 90 Gm. of the solvent. An error that is frequently made consists of dissolving 10 Gm. of a solute in 100 ml of the solvent and calling this a 10% solution. This, of course, is incorrect.

When working with two miscible liquids, it is customary to employ the term "percentage by volume." For example, 90% alcohol is customarily meant to imply that the solution consists of 90 ml of alcohol and 10 ml of water.

A very simple method may be employed for diluting a concentrated liquid to a lesser per cent by volume. This method is applicable only when a shrinkage of final volume does not occur as the result of combining two miscible liquids. Suppose, for example, one wished to prepare 17% alcohol from 95% alcohol. Seventeen ml of the 95% solution are measured out in a graduate and the volume is made up to 95 ml with water. The final mixture will then contain 17% alcohol. The mathematics involved are relatively simple. Thus 95% of 17 is equal to 17% of 95.

$$17 \times .95 = 95 \times .17$$
$$16.15 = 16.15$$

Among the most commonly used standard solutions in plant physiological work are *molar* or *volume molar* solutions. A molar solution consists of a gram molecular weight (or mole) dissolved in H_2O and made up to a liter at 20°C. This temperature is employed because most volumetric glassware is standardized at 20°C. which usually approximates room temperature.

A gram molecular weight of sucrose is 342.30, of dextrose, 186.16, of sodium chloride, 58.45.

It should be borne in mind that many salts contain water of hydra-

tion. A few examples are $CuSO_4 \cdot 5H_2O$, and $CaCl_2 \cdot 6H_2O$, and $MgSO_4 \cdot 7H_2O$. These molecules of water increase the weight of the crystals and must be considered in weighing out the required amount of the compound. Thus the gram molecular weight of $CuSO_4$ is 159.64, whereas that of $CuSO_4 \cdot 5H_2O$ is 249.64.

A gram molecular weight of the compound dissolved in 1000 grams of water, is called a *molal* solution. These solutions are often used in the investigation of osmotic phenomena.

It is more convenient to use volume molar than weight molar solutions in experimental work. For instance, if one has a *volume molar* solution, it is much simpler to dilute it because the original volume is always 1000. But in the case of a *molal* solution, the addition of a solute to a definite amount of water may cause a change in volume. For example, a mole of sucrose added to 1000 Gm. of water will increase the volume to 1207 ml. It will therefore be rather difficult to make up a half, a fifth, a tenth, or a 25th molal solution.

Normal Solutions

A knowledge of the molar basis of expressing concentrations is essential for an interpretation of results obtained in studies of osmosis, freezing-point depression, and in making up nutrient solutions for plants. Another type of standard solution is required where chemical reactions are involved. This is the normal solution, or fraction thereof. A liter of a normal solution contains an *equivalent weight* of an element or radical, by which is meant the weight which is equivalent in reactive power to one atomic weight of hydrogen. Thus if an acid, like H_2SO_4, contains two reacting hydrogens, the molecular weight dissolved in a liter of water constitutes a 2-normal solution. With H_3PO_4, a molar solution is 3-normal, whereas a normal solution of HCl is also a molar solution because the molecule contains but one reacting hydrogen.

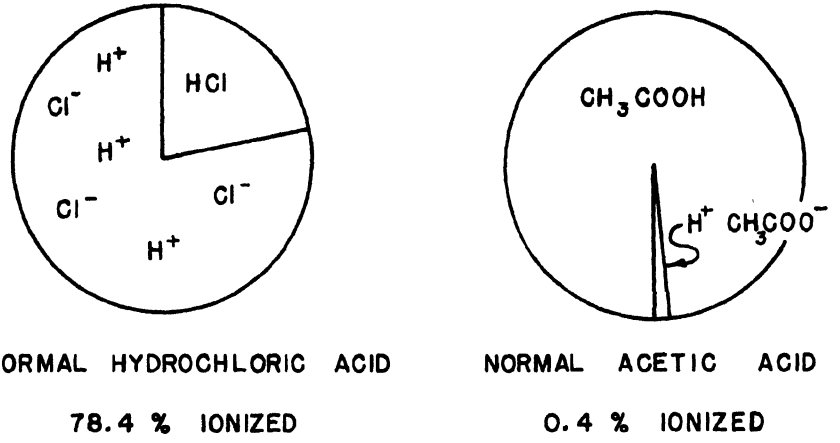
This must be taken into consideration in preparing normal solutions of organic acids. Acetic acid (CH_3COOH), although containing four hydrogen atoms, has only one reacting hydrogen and a molar solution is therefore a normal solution. A normal solution of oxalic acid $(COOH)_2$ contains one half of the gram molecular weight per liter.

Hydrogen Ion Concentration

The symbol *pH* has become almost a household term among many groups of people and no longer appears to be limited to the vocabulary of the scientist. It is very probable, however, that many persons speak of pH or of hydrogen ion concentration without completely understanding either term. Hydrogen ions are important, not only to the physiologist, but to every living individual. These ions play a role in every biological reaction in both plant and animal cells, and they are important to the

farmer and to the manufacturing chemist. It is, therefore, desirable for the student of physiology to learn something of the fundamentals of hydrogen ion concentration.

Vinegar contains about 4% acetic acid or, speaking in terms of the chemist, it is roughly 0.6 normal. Many of us have seasoned certain foods with vinegar and we enjoy the sour taste which is thus imparted to them. But if one were to substitute hydrochloric acid of equal normality in seasoning, the results might be disastrous. The effect of such a solution on the teeth and the lining of the mouth would be very unpleasant. Total acidity, as determined by titration with NaOH, would be the same for



Diagrammatic representation of difference in ionization of normal hydrochloric and normal acetic acids.

both solutions. However, hydrochloric is a strong acid and acetic is a weak one.

The main difference between this weak and this strong acid is to be found in the degree of *dissociation*. In 1887 Arrhenius proposed a theory which has since been found to be a fact. *Acidity* may be resolved into two components—the hydrogen ions and the acid which supplies this ionized hydrogen. Thus, a strong acid supplies more hydrogen ions than a weak acid. A normal solution of acetic acid contains 1.008 Gm. of *total hydrogen* per liter, as does a normal solution of hydrochloric acid. But, only 0.4% of the reacting hydrogen in acetic acid is ionized, whereas 78% is ionized in normal hydrochloric acid. That is, 78% of the hydrogen in normal hydrochloric acid is "mobilized," and 12% is retained in the "reserves." In normal acetic acid, 99.6% of the hydrogen is held in reserve.

This may be expressed mathematically. A normal solution of HCl contains 0.78×1.008 or 0.790272 Gm. of ionized hydrogen per liter. Normal acetic acid contains 0.004×1.008 or 0.004032 Gm. of ionized hydrogen per liter.

As a general rule hydrogen ion concentration of the solutions in the living cell is not high. In fact, it is often so low that it must be expressed as a decimal fraction which in turn may become cumbersome. To overcome this difficulty, we may call upon logarithms. If the quantity of hydrogen ion present in normal acetic acid is approximately 0.004 Gm., this may be expressed as $4/1000$, $4/10^3$ or 4×10^{-3}. Before multiplying 4×10^{-3} we must first convert 4 to its logarithm. This is $10^{0.6021}$. A time-saving manipulation in multiplication consists of adding logarithms of the numbers to be multiplied.

$$\log 4 + \log 10^{-3}$$
$$10^{0.6021} + 10^{-3} = 10^{-2.4}$$

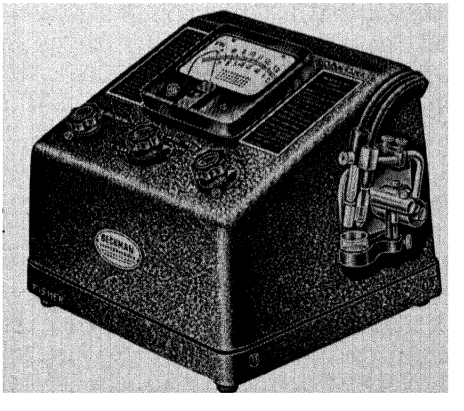
The concentration of hydrogen ions in normal acetic acid, which is 0.004032 Gm. per liter, may also be expressed as $10^{-2.4}$ Gm. per liter. S. P. L. Sørensen suggested simplifying the expression still further by dropping all but the exponent, changing the sign, and calling it pH. The pH of normal acetic acid, according to this calculation, is 2.4. Why Sørensen selected the symbol pH is not known, but it is assumed to signify *potential of hydrogen.*

The pH of normal hydrochloric acid may be computed as follows:

Normal HCl is 78% ionized

$$0.78 \times 1.008 = 0.790272 \text{ Gm. per liter}$$
$$0.790 = \frac{790}{1000} = \frac{790}{10^3} = 790 \times 10^{-3}$$
$$790 \times 10^{-3} = \log 790 + 10^{-3}$$
$$\log 790 = 2.8976$$
$$10^{2.8976} + 10^{-3} = 10^{-0.1024}$$

The pH of normal hydrochloric acid, according to this calculation, is 0.1024.



Beckman pH meter. (Courtesy, Fisher Scientific Company, Pittsburgh.)

The pH Scale

In titrating an acid with alkali, hydrogen ions of the acid are neutralized by the hydroxyl ions (OH^-) of the alkali to form water. It will thus be seen that a state of neutrality can occur only when the number of hydrogen ions is equaled by the number of hydroxyl ions present. Thus, in water at 25°C., it has been found that approximately one molecule in 10,000,000 is dissociated and that there are thus formed an equal number of both hydrogen and hydroxyl ions. One ten-millionth may be expressed as $1/10^7$. The pH of neutral water, therefore, is 7, and this figure is taken as the neutrality point in hydrogen ion determinations. We might say that in the above water the pH is 7 and the pOH is 7 also. It is customary, however, to express both alkalinity and acidity in terms of pH so that the scale extends from pH 0 to pH 14. It is apparent then, that the *lower* the pH reading the *higher* the hydrogen ion concentration. In Table 2 will be found pH values of a few common laboratory solutions.

Table 2

APPROXIMATE pH VALUES OF SEVERAL SOLUTIONS*

Solution	*pH*
0.1 N HCl	1.1
0.1 N H_2SO_4	1.2
0.1 N Oxalic acid	1.6
0.1 N Citric acid	2.2
0.1 N NaOH	13.0
0.1 N Na_2CO_3	11.6
0.1 N Borax	9.2
0.1 N $NaHCO_3$	8.4

* *Handbook of Chemistry and Physics*, 30th ed. Cleveland, Chemical Rubber Co., 1948.

Buffers

A small nation strategically located between two nations with opposing political ideologies is spoken of as a *buffer state*. It tends toward political neutrality. In the theory of hydrogen ion concentrations certain compounds are said to act as buffers. These compounds resist changes in hydrogen ion concentration. That is, they too, tend toward neutrality, but chemical neutrality, in this case.

The combination of a weak acid and a salt of the acid constitutes one type of buffer. Buffer action can be illustrated by considering a mixture of citric acid and sodium citrate. Citric acid is only slightly ionized so that when hydrogen ions are introduced into the solution containing this buffer, they are tied up by the citrate ions. Adding HCl to a citric acid-sodium citrate buffer lowers the pH only slightly, because hydrogen ions are taken up by citrate ions to form citric acid, which is slightly ionized. If NaOH is added to the solution, sodium ions combine with the citrate

ions, and the hydroxyl ions form water with the hydrogen of the weakly dissociated citric acid. In this manner the buffer solution resists changes toward either the acid end or the alkaline end of the pH scale. Other compounds which are useful in making buffer solutions are phthalate salts, boric acid, carbonates, Na_2HPO_4, and NaH_2PO_4 in correct combinations with HCl or NaOH. The amino acid, glycine, may also be employed in making up buffer solutions.

Buffer solutions are useful to the physiologist in many ways. Optimum pH for growing various organisms like bacteria or fungi may be maintained by adding the proper buffer to the mixture. Likewise, the necessary pH of substrates may be controlled in enzyme studies.

In nature, living cells contain organic acids and their salts, which serve to buffer the cell sap. This is still more important in animal cells, as is shown in the discussion of the pH of the blood.

Some Practical Considerations

It is doubtful if many people are aware of the important role played by hydrogen ion concentration in the physiology of plants and animals and in the everyday life of man. Natural bodies of water which contain large masses of submerged aquatic plants may show marked changes in hydrogen ion concentration between day and night. Respiration of aquatic plants and animals, as well as the decaying of organic matter, will result in an accumulation of CO_2 during the night. Carbon dioxide dissolves in the water, producing H_2CO_3 (carbonic acid). This lowers the pH of the water. In the daytime, that is in sunlight, aquatic plants carry on photosynthesis, thus removing dissolved CO_2. This in turn raises the pH. Diurnal changes may range as much as from pH 5 to pH 7. Variations of this type may on occasions be so pronounced as to limit the biological population of lakes or ponds.

The swelling of colloids is greatly influenced by the pH of the medium. This can be demonstrated with gelatin, and it applies to all natural proteins. This will be discussed in detail under the subject of *imbibition*.

Caldwell has shown how hydrogen ion concentration affects the development of fleshy fruits. According to this investigator, during the growing period of fruits, hydrogen ion concentration may increase from 10- to 80-fold. Concurrently with the beginning of the rise in acidity or shortly thereafter, fruits begin to absorb water and to enlarge at a rapid rate. It was concluded from these observations that alteration of the hydrogen ion concentration increased the capacity of the colloids for imbibing water. This applies to the colloidal material in both the protoplasm and in the cell wall.

There is some evidence that the absorption and accumulation of salts in the cell is affected by the pH of the medium, although definite relationships have not been completely established. The pH of the medium is a

highly important factor in its influence on the rate of enzymatic action. Different types of enzymes have definite optimal pH ranges. Hydrolytic enzymes generally require an acid medium, whereas oxidizing enzymes often function more efficiently at a pH near neutrality. This, of course, applies to the isolated enzyme under experimental conditions. There exists some evidence that enzymatic reactions in plants may depend upon the pH of the medium, that is, whether a compound will be hydrolyzed or synthesized. It will be shown later that the mechanism governing the opening and closing of the guard cells of the stomates in the leaves is dependent upon changes in the hydrogen ion concentration.

The pH of the soil may be of great concern to both farmers and home gardeners. Iron deficiency in plants has been observed in both acid and alkaline soils. In alkaline soils iron is precipitated or rendered insoluble. In acid soils it is possible that the excess acid serves to dissolve substances, like manganese, which interfere with the absorption of iron. It has been suggested that chlorosis (a deficiency symptom characterized by loss of chlorophyll) may be the result of altered pH in the leaves rather than a mineral deficiency, because the presence of iron can be demonstrated in chlorotic leaves. The explanation, according to this theory, is that the iron is of little use to the leaf because it remains insoluble.

The pH of the soil may determine the type of crop that can be successfully grown on it. Although plants as a whole grow in a wide range of soil reactions, some of them are very selective. Spurway has recorded pH tolerances of a large number of plants, both native and introduced. A few examples, showing a wide range, are cited in Table 3.

Table 3

SOIL REACTION (pH) PREFERENCES OF A FEW PLANTS*

Plant	*Optimum pH Range*
European blueberry (*Vaccinium myrtillus*)	3.5–5.0
Azalea	4.5–6.0
Sweet Corn	5.5–7.5
Bean, beet, cabbage	6.0–7.5
Box elder (*Acer negundo*)	6.0–8.0
Stonewort (*Chara vulgaris*)	7.5–8.5

* Spurway, C. H.: *Soil Reaction (pH) Preferences of Plants.* (Michigan State College Agricultural Experiment Station Special Bulletin 306.) East Lansing, 1941.

In some countries farmers are wont to plant the crop that suits the soil, whereas in America it is customary to change the pH to suit the crop if possible. It is often necessary, for example, to add lime to the soil, as in the growing of legumes. There may be occasions, however, when liming is overdone. In the eastern United States it seems to be common practice to add lime periodically to bluegrass lawns. What many gardeners do not appear to know is that bluegrass will tolerate a more acid condi-

tion than will some of the common weeds, like dandelion. Excessive liming on one's lawn may, therefore, encourage the growth of dandelions and other weeds at the expense of the bluegrass.

Table 4

pH VALUES OF SEVERAL FRUITS AND VEGETABLES*

Food	*pH*
Gooseberries	2.8-3.0
Apples	2.9-3.3
Peaches	3.4-3.6
Tomatoes	4.0-4.4
4.5	4.5
Pumpkins	4.8-5.2
Squash	5.0-5.4
Peas	5.8-6.4
Corn	6.0-6.5

* pH values of some foods which are frequently canned. A pH of 4.5 has been selected as the dividing line between acid and low-acid foods. Acid foods may be sterilized by heating for 10 minutes at 75°C. Low-acid foods must be heated for three hours at 100°C. pH values from *Handbook of Chemistry and Physics*, 30th ed. Cleveland, Chemical Rubber Co., 1948.

One way in which pH may enter everyday life is in the canning of fresh fruits and vegetables. In Table 4 is presented a list of foods with their pH values. It will be noted that a line is drawn at pH 4.5, thus separating the foods into two groups. Those having a pH above 4.5 are called *low-acid foods*. The others are termed *acid foods*. This division has been established by organizations connected with the canning industry. Acid foods may be satisfactorily sterilized by heating for only 10 minutes at 75°C. Low-acid foods must be heated for three hours at 100°C. to be absolutely certain that they have been completely sterilized.

If the student has found no point of contact between himself and the subject of pH thus far, perhaps he will do so in the consideration of the following. In the human body the pH of the blood is normally about 7.4. A slight change in either direction, that is, to more acid or more alkaline reaction, is accompanied by illness. Whether the change in pH is the cause or the effect of the illness is not known. Fortunately, natural buffers in the blood serve to resist changes during the reaction. Slight alkalinity (pH 7.4) of the blood facilitates the absorption of CO_2. When the blood's CO_2-absorbing power is reduced by the shift to a lower pH, a very serious condition may arise.

Sometimes individuals attempt to increase the body's alkaline reserve by the ingestion of fresh fruits, especially citrus fruits. Although oranges, lemons, and grapefruit are ordinarily classed as acid fruits, they do not necessarily produce an acid reaction in the blood when they are consumed. Acids in these fruits are organic in nature and during the process of digestion they are converted to water and carbon dioxide. Furthermore, fruit

juices of this type contain minerals like salts of potassium, magnesium, which may function as buffers in the blood.

Blatherwick and Long have conducted some interesting experiments which illustrate the effect of drinking orange juice on body fluids. (See Table 5.) The subjects consumed, in addition to a basal diet, from 600 to

Table 5

EFFECT OF INGESTION OF ORANGE JUICE ON pH AND CO_2 CAPACITY OF THE URINE*

Subject 0			
Date	*pH*	*CO_2 Capacity*[1]	
Nov. 4	6.8		Basal diet
5	6 8		" "
6	6 6		" "
7	7 1		" "
8	7.1	63 3	Basal diet + 600 ml orange juice
9	7 1		" " + 1200 ml " "
10	7.2		" " + 1800 ml " "
11	7 4		" " + 2400 ml " "
12	.	62.4	

* Blatherwick, N. R. and M. L. Long: "Studies in Urinary Acidity. I. Some effects of drinking large amounts of orange juice and sour milk." *Journal of Biological Chemistry* **53**:103, 1922.

[1] Ml of CO_2 per 100 ml of blood plasma.

2400 ml of orange juice daily. The pH of the urine of the control subjects ranged from 6.8 to 7.1. The pH of the urine of those who ingested orange juice ranged from 7.1 to 7.4. According to the report, it was impossible to overreach the organism's ability to oxidize the contained citric acid even though the amounts consumed in one day were equivalent to 48 Gm. of acids.

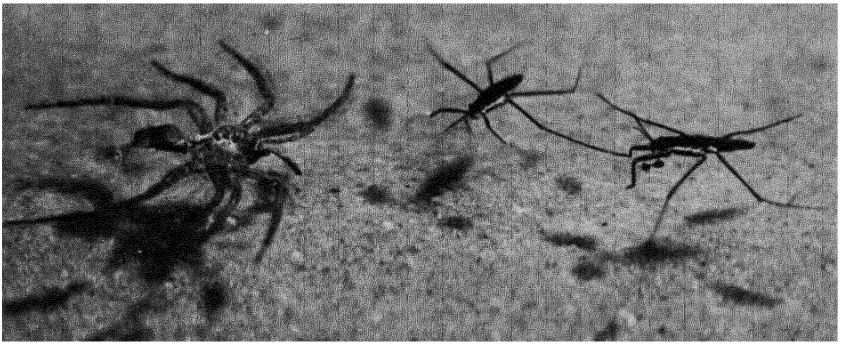
Fruits like prunes, cranberries, blueberries will tend to produce an acid urine if ingested in large quantities. These fruits contain relatively large quantities of benzoic acid or its derivatives. Benzoic acids are not as easily oxidized as are the aliphatic acids.

chapter 4

Interfacial Phenomena and Colloids

Surface Tension

Most everyone is aware that when water stands in a lake, a pond, or even a bowl, the surface layer possesses properties which are different from the rest of the liquid. Sometimes it appears as if there is a thin film over the surface of the water. Under proper conditions a steel needle may be made to float on the water. In nature certain insects are able to *walk on water.*



The ability of certain insects to walk on water is excellent evidence of a film caused by surface tension. Here are two water striders and the cast skin of a Dolomedes spider. (Courtesy, Lorus J. and Margery J. Milne.)

Experiments have shown that there *is* a film on the surface of water when exposed to the atmosphere. This film, however, consists of nothing but a layer of neatly oriented water molecules. Within the body of the liquid the molecules are attracted by other molecules and the force of attraction is equal in all directions. But that layer of molecules on the surface *feels* the attraction only from below. The gas phase (the atmosphere) above does not attract the molecules of water with the same force with which the water molecules attract each other. In addition to this, the molecules in the surface layer are oriented, that is, stacked in the same direction. This gives rigidity to the film. The strained condition of the surface layer of liquid molecules at liquid-gas boundaries is called *surface tension.*

The term *surface tension* is applied only to liquid-gas interfaces. *Interfacial phenomenon* is a more general term and applies to a condition existing between any two phases when there is tension. These might be liquid-liquid, solid-liquid, and solid-gas, as well as liquid-gas phases. No tension exists between gas-gas phases because there is little cohesive attraction among gas molecules.

Tension on the surface of a liquid tends to decrease surface area. It is for this reason that rain drops are spherical. A sphere is a geometrical figure which possesses maximum volume with a minimum surface area.

Expressed in another manner, surface tension is the work that must be done to increase the surface area by one unit. This is expressed by the formula:

$$S = \frac{\text{ergs}}{(\text{cm.})^2}$$

It is customary to express surface tension in dynes per centimeter. The shift to a linear unit rather than to one of area is purely accidental. Since ergs are equal to dynes times centimeters, the formula is simplified by canceling. All steps are shown below.

$$S = \frac{\text{ergs}}{(\text{cm.})^2} = \frac{\text{dynes} \times \text{cm.}}{(\text{cm.})^2} = \frac{\text{dynes}}{\text{cm.}}$$

Measuring Surface Tension

In 1884 Ludwig Traube, a German physician, developed an instrument for measuring surface tension. The instrument is called a *stalagmometer* (drop meter). It consists of a reservoir and a capillary tube with a flattened mouth on the lower end. The method is based upon the principle that the number of drops formed while delivering a definite volume of liquid is inversely proportional to surface tension. The number of drops formed by an unknown liquid is compared to the number formed by water.

$$\frac{S}{t} = \frac{n}{N}$$

Where S = surface tension of unknown
N = no. of drops of unknown
t = surface tension of water
n = no. of drops of water

$$SN = nt$$

$$S = \frac{nt}{N}$$

If the density of the unknown liquid differs from that of water then another factor (D = density of unknown) must be added to the equation.

$$S = \frac{nDt}{N}$$

Traube's invention of the stalagmometer gave impetus to additional studies on surface tension and biological phenomena, with the result that a better understanding of cellular physiology was obtained. Although many discussions of the role of interfacial phenomena in biological reactions are theory mixed with fact, it is evident that these phenomena play an important part in the living cell. A number of interfaces, for example, occur within the confines of the cell, such as between vacuole and cytoplasm, between nucleus and cytoplasm, and between the cytoplasm and the cell wall. The spherical shapes of the vacuole, nucleus, and nucleolus suggest the action of interfacial phenomena. This could also be true of plasmolyzed protoplasm with its spherical shape. Bacteria, spores of various kinds, and other free living cells assume either spherical or beautifully contoured shapes. These shapes all suggest the effect of surface phenomena in attaining a minimal surface.

It has even been claimed that the particular shapes of the isodiametric cells of the parenchyma in plant tissues may be attributed to interfacial phenomena. An analogy may be found in the appearance of a layer of soap bubbles when placed on a flat surface like a pane of glass. In the plane of contact the bubbles assume a hexagonal pattern and resemble very closely the cells in a cross-section of parenchymatous tissue.

If a solution of egg albumin is stirred vigorously or beaten, a stable froth is formed such as occurs in the making of frappés. It will be found that the stability is the result of the albumin's having concentrated at the interface between the air and the solution. This can be proved in the following manner. By successively removing the froth and whipping up more of it, practically all of the albumin may eventually be removed from solution. A similar experiment may be performed by adding a dye like methylene blue to a soap solution. Aerating the solution will produce colored soap bubbles. As in the case of the albumin, the dye may be removed from the solution by continuously removing the bubbles as fast as they are formed.

Adsorption

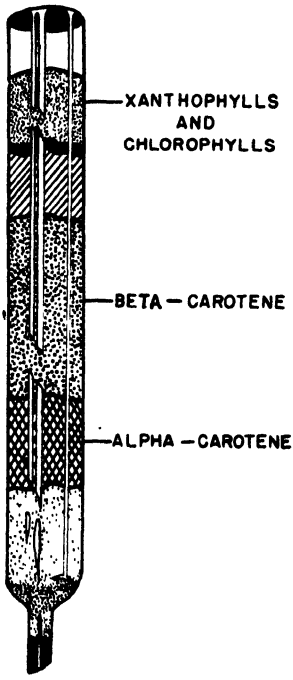
In 1864 C. Musculus, an apothecary, found that while salts had little effect on the rise of water in capillary tubes, such substances as acetic acid, alcohol, bile, etc., markedly reduced the capillary rise.

In 1878 J. Willard Gibbs proved by thermodynamic calculations that if a dissolved substance lowers the surface tension of a solvent, the dissolved substance tends to concentrate at the boundary layers or interfaces. These mathematical and physical deductions remained unnoticed for a number of years.

Interest in *surface activity* was revived by Traube's work in 1884 (which has already been mentioned). In fact, Traube originated the expression *surface active agents*. Since that time Gibbs' deductions have been

experimentally verified many times, and surface activity and the study of surface-active agents have opened new vistas in the biological sciences.

The concentration of solute molecules in the surface or interface of a liquid is called *adsorption*. When a compound like ether is added to water, the surface tension is lowered and, as predicted, the ether will be found to concentrate at the surface. The reason for this is that the water molecules have a greater attraction for each other than for the molecules of ether. Thus organic compounds, particularly nonpolar compounds, which are insoluble in water, tend to lower surface tension.



Separation of plastid pigments of leaves by means of chromatography. (Redrawn after Strain: "Leaf Xanthophylls," *Carnegie Inst. Publ.* No. 490, 1938.)

Adsorption is not confined to gas-liquid phases. Examples may be cited also in gas-solid and liquid-solid phases. In the gas mask, finely divided particles of *activated* charcoal serve to adsorb poisonous gases, as, for example, the small pieces of charcoal were once added to mixtures of chicken feed in order to adsorb undesirable gases in the fowl's digestive tract.

Charcoal is a very useful reagent in both the laboratory and in industry. Colored or cloudy solutions may be clarified by passing them over charcoal. This principle is employed in the commercial refining of sugar. The *chromatographic* separation of closely related compounds relies upon what has been termed *selective adsorption*. In this method a glass tube is carefully packed with an adsorbent which consists of finely divided and activated compounds like magnesium, or aluminum oxides, or others. When a solution of substances like leaf pigments is filtered through this column, each individual pigment is adsorbed at different rates, so that eventually a series of variously colored rings appears upon the column.

In *paper chromatography* (see Chapter 13) a large sheet of especially treated filter paper serves as the adsorbent. Compounds like amino acids or others, which are selectively adsorbed, appear as very definite spots on the paper and can be identified by their relative positions.

Adsorption in Plant Physiology

As fundamental principles such as hydrogen ion concentration, surface tension, adsorption, imbibition, diffusion, and osmosis are discussed in

this book, it may appear to the student that one may be considered more important than the other. It is not intended to create this impression. It is true that, in the past, following each classical discovery, physiologists were wont to concentrate upon a certain fundamental principle and to attempt to apply the new discovery to an interpretation of many, if not all, of the physiological processes in the cell. Modern physiologists are inclined to believe that all of these fundamental principles are involved in the cellular activities, possibly at the same time, and that each is integrated with the other.

In the plant cell, protoplasm, being colloidal in nature, certainly acts as an adsorbing agent. Mention has been made of the interfaces within the cell and the tendency of certain substances to accumulate in these interfaces. It is believed that enzymes function so efficiently because they adsorb reacting compounds, concentrating them on their surfaces. Thus large quantities of reactants are brought together into intimate relationship. In like manner chondriosomes in the protoplasm of the cell have been found to adsorb certain enzymes.

Adsorption in Life

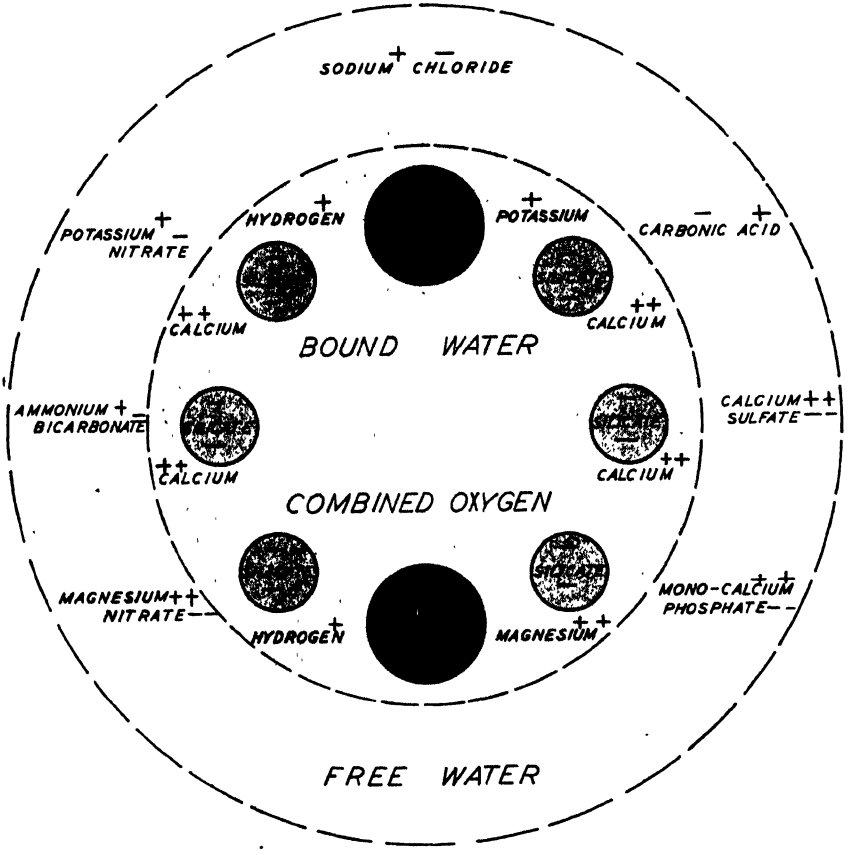
It is impossible to say how many living processes are concerned with adsorption. Many of the examples listed under plant physiology would also apply to animal physiology because essentially identical processes occur in both types of cells.

H. Freundlich went so far as to state that poisoning may be due to adsorption. Otto Warburg conducted an experiment which tended to prove this theory. This was accomplished by first adsorbing oxalic acid on blood charcoal. This acid is a stable one and is not readily oxidized by atmospheric oxygen. When adsorbed on blood charcoal, however, oxalic acid is readily oxidized. But when narcotics, like alcohols, were added, the rate of oxidation was retarded. It appeared that the alcohol was adsorbed by the charcoal, reducing the amount of surface available to the oxalic acid. Warburg suggested that this is what happens in narcosis or anesthesia of animals. According to this theory, in normal respiration of the animal, sugar is adsorbed on small blood particles and oxidized there. When narcotics or anesthetics are administered to the subject, the surfaces on the blood particles are occupied by the poison and the rate of respiration is retarded. Adequate dosage of either substance will produce death.

Adsorption: Physical, Chemical, or Electrical?

Adsorption is usually considered to be a purely physical phenomenon because the efficiency of the adsorbent often depends more upon its physical structure than upon its chemical constitution. Charcoal for gas masks is more efficient if made from compact woods like coconut shells and

peach pits. Also, for adsorbing alcohol it has been found that charcoal is more efficient than talcum, and talcum is more efficient than sulfur. If a series of alcohols are adsorbed, the same order of adsorbability is retained, indicating that it is a physical rather than chemical reaction.



Imaginary clay particle (micelle) many times enlarged. The clay particle is made up of aluminum silicate immersed in bound and free water. (Courtesy, Hester: *Soil Fertility in Tomato Production*, Dept. of Agricultural Research, Campbell Soup Co., 1941.)

However, evidence can also be cited which indicates that adsorption is chemical, and in some instances, electrical. Often it is stated that there are three types: physical, chemical, and electrical. The process might include three. With what we know of the structure of the atom, it is not always possible to distinguish between chemical and electrical phenomena. Examples of physical adsorption have already been presented. Let us consider a few of the other types.

Some solids when placed in water assume negative charges. Common

examples of this are sand, clay, and cellulose. If a piece of filter paper, which is mainly cellulose, is placed in solutions of dyes, the degree of adsorption will depend upon the electrical charge of the dye. Suppose the paper is dipped in a mixture of methylene blue and of eosin. Eosin will rise much higher in the filter paper than methylene blue. The reason for this is that eosin is negatively charged and is repelled by the cellulose. The molecules are free to move upward in the capillary pores of the paper. Methylene blue, being positively charged, is attracted to the cellulose and is adsorbed.

The adsorption of minerals by the clay colloids in the soil constitutes another type of electrical adsorption. Clay particles are negatively charged. The ions which are adsorbed are positively charged ones (cations) like K, Mg, NH_4, etc. This is certainly an electrical phenomenon.

Let us now consider examples of chemical adsorption. When iodine is added to a solution of starch, a dark blue compound is formed. This is employed in the well-known starch-iodide test. It has been shown that a definite chemical compound is formed between the iodine and the starch.

Another example of chemical adsorption is to be found in the selective dyeing of fabrics. A dye bath containing amacid milling scarlet, amanil sky blue, and S.R.A. golden yellow will dye silk scarlet, viscose rayon blue, and Celanese yellow. If we are attempting to interpret adsorption as being an electrical phenomenon, then the selective dyeing with two colors is explainable. But three different colors, as just cited, points strongly to chemical adsorption. By this is meant that the chemical constitution of the fabric is the determining factor.

Colloids

A discussion of interfacial or surface phenomena leads logically to a consideration of colloids because, as will soon become apparent, it is not always possible to distinguish between interfacial and colloidal phenomena. Colloids are also important in physiology because all living organisms are fabricated on a colloidal pattern. Few physiological processes proceed in the absence of colloids of one type or another.

The word *colloid* was proposed in 1861 by Thomas Graham, an English chemist. This term means *gluelike* and was assigned by Graham to those substances which are slow to diffuse. Common examples, in addition to glue, are gum arabic, agar, gelatin, and protoplasm. Graham gave the name *crystalloids* to rapidly diffusing compounds like sugar, salts, etc., which normally form crystals. We now know that no substances are inherently colloidal or crystalloidal. A colloid represents merely a state of matter, and many compounds may occur in either the colloidal or crystalloidal state.

A colloidal particle is distinguished from others mainly by its degree of dispersion or particle size. In fact we have set up arbitrary classes for

suspensions, colloids, and true or molecular solutions. The characteristics of these types of solutions are presented in Table 6.

In a true solution the particles (molecules and ions) have a diameter smaller than 0.001 micron. (A micron is $\frac{1}{1000}$ of a millimeter.) They are not visible under the ordinary microscope. The diameter of a colloidal particle lies between 0.001 and 0.1 micron. Usually, though not always, a colloid consists of an aggregate of molecules. The qualifying portion of the foregoing statement is necessary because there are certain molecules which are large enough to be called colloids. Examples are molecules of proteins, starches, pectins, and certain dyes. From a physical standpoint however, these molecules possess the properties of colloids, so that the classification by particle size is still valid.

Table 6

PHYSICAL CHARACTERISTICS OF SUSPENSIONS, COLLOIDS, AND TRUE SOLUTIONS

	Suspensions	*Colloidal Dispersions*	*True Solutions*
Particle Size (Diam.)	Larger than 0.1 micron	0.1 to 0.001 micron	Smaller than 0.001 micron
State	Aggregates of molecules	Aggregates of molecules	Molecular
Stability	Unstable	Stable	Stable
Filters	Separated by ordinary filters	Separated by ultrafilters (like cellophane bags)	Not separated by ultrafilters
Other Characteristics		Show Tyndall effect and Brownian movement (especially hydrophobic colloids)	

Suspended particles are those which are larger than 0.1 micron in diameter. They are visible under the ordinary microscope. Suspensions differ from the other two types of solution, not only in particle size, but also in regard to stability. A suspension sooner or later settles out of solution. Colloidal and molecular solutions, on the other hand, are permanent. To obtain an example of a suspension, a colloid, and a true solution all together, we might consider the following. During a storm along the ocean giant waves frequently wash away portions of cliffs along the shore line. In the seething mass of turbid water will be found *suspended* grains of sand, *colloidally* dispersed particles of clay, and true *solutions* of the salts that characterize sea water.

Ultrafilters

Graham observed another important difference between colloidal and crystalloidal solutions. It has been mentioned that colloids are slow to diffuse. Graham found that colloids would diffuse very slowly if at all through a differentially permeable membrane, like parchment paper or an animal bladder. Thus if a mixture of starch (colloid) and sodium chlo-

ride (crystalloid) are placed in an animal bladder and immersed in water, the sodium chloride will diffuse into the water and the starch will remain within the bag. In this manner one type of solution may be separated from the other. The process is called *dialysis*. It has many applications in biology and industry. It is customary, for example, to prepare pure proteins by *salting out* or precipitating with sodium chloride, ammonium sulfate, etc. The mixture of precipitated protein and salt solution is then placed in a bag of parchment paper or other similar membrane and the salts will separate from the colloid by *dialyzing* out into the water. This process is also called *ultrafiltration* and the differentially permeable sack is termed *ultrafilter*.

The Ultramicroscope

We have said that colloidal particles, although larger than most molecules, are still invisible under the ordinary microscope. One of the characteristics of colloidal solutions is that they are translucent and not opaque like suspensions. Beautiful red and purple solutions of gold were produced by the alchemists of the 17th century even before the word *colloid* was ever proposed. Michael Faraday suggested that gold was present in such solutions as extremely minute particles. He reported that this could be illustrated by collecting rays of the sun into a cone by means of a lens and sending the part of the cone near the focus into the solution. This then was the origin of the ultramicroscope. This principle, whereby submicroscopic particles become apparent in a cone of focused light, is called the *Tyndall effect*. It was named after John Tyndall (1820–1893) who employed this method of illumination rather extensively. A slit of sunlight admitted to a dark room will reveal colloidal dust particles or the colloids in smoke. We see, not the particles, but rather the results of their diffraction of light. In other words, these minute particles produce *halos* of light under these conditions.

The ultramicroscope then, consists of an ordinary microscope so constructed that a colloidal solution can be placed under the lenses and a beam of light projected into the solution. Colloidal bodies are seen to glisten like myriads of stars in the sky. They differ from stars, however, because they appear to be dancing about.

Brownian Movement

This movement of colloidal particles, which is observable under the ultramicroscope, is quite pronounced. Particles quiver and describe irregular paths, sometimes traversing distances which are several times their diameter. This motion is caused by the impact of the surrounding molecules on the particle. It is known that molecules are in constant motion. As they collide with the larger, colloid particles, the latter are seen to quiver and to move about.

This movement is not confined to colloidal particles. It was first observed in pollen grains in 1827. The observer was Robert Brown, a botanist, and the motion which he first observed is now called the *Brownian movement.*

Terminology

Before continuing the discussion of colloids it seems advisable to explain a few of the terms which have been developed in this branch of science. First, it should be recalled that all three phases of matter (solid, liquid, and gaseous) may enter into colloidal combination. The different types, with at least one example, are indicated in Table 7.

Table 7

EXAMPLES OF COLLOID TYPES

Type	*Example*
Gas-in-liquid	Foams
Gas-in-solid	Some minerals
Liquid-in-gas	Clouds, fogs
Liquid-in-liquid	Oil in water, etc.
Liquid-in-solid	Certain gems, like pearls
Solid-in-gas	Smoke
Solid-in-liquid	Solutions of gelatin, colloidal metals
Solid-in-solid	Some alloys

If a solid is dispersed in a liquid, as for example, an aqueous solution of starch, then starch is the *dispersed* or *discontinuous phase*, and water is the *disperse medium* or *continuous phase.*

A colloidal solution in which a solid is dissolved in a liquid is termed a *suspensoid.* (The word *suspension* is more properly applied to dispersion of particles which are larger than colloids.) Likewise, if a liquid in a liquid involves globules of colloidal size, the combination is called *emulsoid* to distinguish it from an *emulsion.* Suspension and emulsion are similar in regard to size of the dispersed particles. *Micelle* is perhaps a more appropriate term than *particle* in referring to colloids because it is equally applicable to gases, liquids, or solids.

Colloids are also classified in regard to solubility of the dispersed phase in the disperse medium. Inasmuch as water is the most common solution encountered in plant physiology, this discussion will be limited to solubility in water. Colloidal solutions containing water-soluble micelles are termed *hydrophilic* (water-loving). Many examples of these have already been mentioned, such as solutions of starch, gums, pectins, and proteins. Colloidal solutions of insoluble substances like metals are termed *hydrophobic* (water-hating).

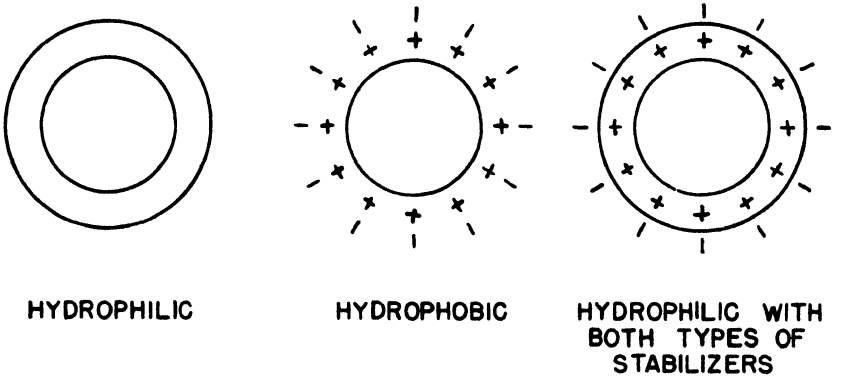
The gold solutions of the ancients are examples of hydrophobic colloids. A metal may be dispersed in a colloidal solution either by discharging an electric current in a solution of a salt of the metal or by adding a reducing agent. The addition of tannin to a solution of silver nitrate will

cause the silver ions to aggregate into colloidal micelles. The addition of plant extracts may accomplish the same results.

Graham, who contributed so much to the field of colloid chemistry, originated the terms *sol* and *gel*. When a colloidal dispersion is in the fluid state it is called a *sol*. When it congeals or becomes solid, we say the solution has *gelled*. The solid phase is called a *gel*. When one prepares a solution of agar by heating, the *sol* becomes a *gel* upon cooling. Changing from a solid to liquid state is called *solation;* the reverse process is called *gelation*.

Stabilizing Factors in Colloids

The question now arises as to what keeps colloids dispersed. The micelle is too large to constitute a molecular solution but too small to



Diagrammatic representation of the theory of the stabilization of colloid particles. Outer circle represents shell of water molecules.

settle out. Among the many theories which have been offered, the following seems most tenable. It is supposed that a hydrophilic colloid is stabilized by a shell of water molecules which prevent the micelle from coalescing with other micelles and precipitating. The shell consists of successive layers of oriented molecules. One reason for believing this is that the addition of a dehydrating agent like alcohol to a hydrophilic colloid brings about coagulation. Colloidal substances like pectin and proteins may be precipitated from plant extracts in this manner.

Stabilization of hydrophobic colloids, according to this theory, depends upon a shell of electrical charges. First, there is a shell of oriented charges all of one kind. Surrounding this oriented layer are layers of opposite charges, each layer becoming farther from the other. Flocculation of a hydrophobic colloid is accomplished by neutralizing the charges in the particle itself. This may be done by adding to the solution a colloid of opposite charge or an electrolyte, in which case neutralization of the colloid is brought about by the action of oppositely charged ions.

Sometimes a colloid may be stabilized by both water of hydration and by a shell of electric charges. Examples of this type are proteins, agar, etc. Flocculation requires both dehydration and neutralization. One of the commonest methods is *salting out* by the use of salts like Na_2SO_4, or $MgSO_4$, or $(NH_4)_2SO_4$. These salts are capable of taking up large quantities of water. (Their crystals contain molecules of H_2O.) Salting out consists of dehydrating the colloid and neutralizing it with the ions of the same salt. Salting out of proteins is a very common practice in preparation of these substances.

chapter 5

Diffusion and Osmosis

Diffusion

Ample evidence exists that materials move *into, out of, and all through the living plant.* Perhaps a gardener has observed the beginning of wilting in potted plants and has added water which soon finds its way up through the plant and into topmost leaves. A beaker placed over the branches eventually becomes fogged with moisture, indicating that the water has moved out of the plant too. Between the time that the moisture left the leaves and condensed on the sides of the beaker it was invisible. It was water vapor—a gas. As we shall learn later, other gases are continually passing into and out of the leaves. During photosynthesis CO_2 is being absorbed and O_2 is released. As the result of respiration (in the absence of photosynthesis) O_2 is absorbed and CO_2 is released. Other examples of the movement of material in plants might be cited. Carbohydrates, which are manufactured in the leaves, may be subsequently transported to other parts of the plant such as the seeds, stem, roots, and tubers.

Once again we shall draw upon a physical principle as we attempt to explain how materials move through plants. The principle involved is that of *diffusion.* It must be added, however, that although the movement of gases may be satisfactorily explained by the principles of diffusion, explaining the means by which liquids and solutes (dissolved substances) are transported in plants may involve other principles in addition to those of diffusion. These are discussed under *osmosis, permeability, imbibition,* and *translocation.*

Diffusion of Gases

If a bottle of ammonium hydroxide were opened in a classroom, the penetrating odor of this compound would gradually become noticeable farther and farther from the mouth of the bottle. A visual demonstration of the same phenomenon may be produced by standing a bottle of concentrated hydrochloric acid beside a bottle of ammonium hydroxide. After the stoppers of the two bottles have been carefully removed, a white "smoke" will appear above the bottles. Fumes of HCl have left the bottle of acid and have met fumes of ammonia which have also escaped. As the two gases meet they form a white, solid, ammonium

chloride (NH_4Cl), which accounts for the white smoke. Thus the molecules of each gas have moved from a region of greater to one of lesser concentration. This migration of the molecules is initiated as the result of kinetic activity. We have said previously that molecules are in constant motion. (Brownian movement was shown to be the result of constant bombardment of larger particles by surrounding molecules.) The gradual movement from areas of greater to lesser concentrations is explained as follows. In the areas of highest concentration the molecules are continuously colliding with each other so that the direction of the motion is constantly changing. At the border of the area collisions are less frequent so that these molecules move farther and farther "away from the crowd." To be specific, *diffusion is the net movement of the molecules of a given substance in a given direction.* And to be absolutely correct one should say that molecules of water vapor *move out* of the leaf as the result of diffusion rather than that they *diffuse out.* However, usage, rather than derivation, ultimately serves to define the words of a language. Furthermore, it is felt that it will appear less complicated to the student if we use the term *diffusion,* rather than *net diffusion,* to indicate molecular movement in a given direction. It must be borne in mind too that diffusion must not be confused with mass movement of a gas. Diffusion of gases occurs in a stagnant atmosphere. The mixing of two gases may be accelerated by stirring or fanning the mixture but this introduces mass movements. Likewise convection currents produced by heating a mixture represent mass movements and not diffusion.

Different gases have different rates of diffusion when all other conditions are constant. Thomas Graham determined experimentally that the rate is inversely proportional to the square root of the relative density. Taking the density of hydrogen as 1, the density of oxygen is 16, and that of carbon dioxide is 22. The rate of diffusion of hydrogen is proportional to $1/\sqrt{1}$, of oxygen it is $1/\sqrt{16}$, and of CO_2 it is $1/\sqrt{22}$. From this it is apparent that hydrogen will diffuse four times as fast as oxygen and nearly five times as fast as carbon dioxide.

The relationship may be expressed mathematically with the aid of *the law of equal partition of energy.*

$$\tfrac{1}{2}MV^2 = K$$

In this equation M is the mass of a particle in a diffusing system, V is its velocity, and K is a constant. The velocity is inversely proportional to the square root of the mass ($V \propto 1/\sqrt{M}$). This is called *Graham's law of diffusion.*

Dissolved Gases

The beginning student of physiology may become confused in trying to apply Graham's law to the absorption of CO_2 by plants. In this in-

stance we must consider other factors. After gaseous CO_2 diffuses through the stomates and into the air spaces of the spongy tissue it comes into contact with the cell walls, which are impregnated with water. Carbon dioxide dissolves in this water. The velocity of the movement of dissolved gases is directly proportional to the coefficient of solubility of the gas in the solvent. Carbon dioxide is more soluble in water than are the other atmospheric gases (O_2, N_2) and is therefore absorbed in relatively large quantities.

Diffusion of Solutes

The diffusion of solutes may be demonstrated by simple experiments. If a crystal of a colored salt, like copper sulfate or potassium dichromate, be placed at the bottom of a beaker of water, the diffusion of the salt to all parts of the solution may be observed as the solution eventually becomes colored throughout. As with gas molecules, a number of factors affect the rate of diffusion of solutes. Furthermore, we may be dealing with both ions and molecules in a solution. Among the factors which affect the rate of diffusion of ions or molecules in solution are size, mass, and degree of hydration of the ion or molecule, and the temperature and viscosity of the medium.

Acids and alkalies are characterized by a relatively high rate of diffusion. This is attributable to the speed with which both hydrogen and hydroxyl ions diffuse. In Table 8 will be found the absolute velocities of

Table 8

ABSOLUTE VELOCITIES OF SEVERAL IONS*

Ion	*Absolute Velocity Cm. per Sec. at 18°C.*
H	32.50×10^{-4}
K	6.70×10^{-4}
Na	4.51×10^{-4}
Li	3.47×10^{-4}
Ag	5.70×10^{-4}
OH	17.80×10^{-4}
Cl	6.78×10^{-4}
NO_3	6.40×10^{-4}
CH_3COO	3.20×10^{-4}

* Clark, W. Mansfield: *The Determination of Hydrogen Ions*, 2nd Ed. Baltimore, The Williams and Wilkins Co., 1927.

several ions. It will be observed that whereas both H^+ and OH^- diffuse more rapidly than the other ions which are listed, hydrogen ions possess the most rapid rate of all. That mass is an important factor in determining rate of diffusion may be illustrated with potassium and chlorine ions. The atomic weight of potassium is 39.096 and the absolute velocity of the potassium ion is 6.70×10^{-4} cm. per second. Chlorine, with an atomic weight of 35.457, has a velocity of 6.78×10^{-4} cm. per second in the ionic form. It is for this reason that potassium chloride is suitable for use in

hydrogen ion determination. The so-called KCl bridge is often called a neutral bridge because there is not enough difference in the rate of diffusion of the two ions to develop a potential difference. Effect of size on the rate of diffusion may be illustrated by citing diffusion constants for several different particles. For example, the diffusion constant for hydrogen is roughly seven times greater than that of the sodium ion, and 50 times greater than the value of the diffusion constant of egg albumin. As particle size increases from small to larger ions, to that of a relatively small organic molecule, and finally to a colloidal micelle, the rate of diffusion progressively decreases with increase of particle size.

Other Factors Affecting Diffusion

At least two factors exert a direct affect on the rate of diffusion of both gases and solutes. One of these factors is temperature. An increase in temperature causes an increase in the rate of diffusion. Since raising the temperature of a gas or liquid serves to speed up the kinetic activity of the component molecules, it is obvious that an increase in diffusion rate will follow.

Of equal importance to the physiologist is the *diffusion gradient*. We have already said that molecules move from the region of greater concentration to one of lesser concentration. The speed with which they move is determined by the difference in the concentrations of the two regions. It is also governed by the distance through which the molecules must move. The speed of the molecules is directly proportional to the difference in concentration, and inversely proportional to the distance between the two regions. The diffusion gradient is therefore determined by dividing the difference in concentrations by the distance between regions of two different concentrations.

Osmosis

Thus far the discussion of diffusion has been limited to conditions in which the molecules in question are free to move in any direction. We shall now consider a special type of diffusion. This is a system in which two solutions, or a solution and the solvent, are separated by a membrane which is more permeable to molecules of the solvent than to those of the solute. The principle involved is *osmosis* and the membrane just described is said to be *differentially* permeable.

The principle of osmosis may be demonstrated as follows. A membrane, like an animal bladder, is tied over the end of a glass tube. Molasses, syrup, or a solution of sugar is placed in the lower part of the tube which is then lowered into water. In a short time the solution will begin rising in the tube. This is an indication that molecules of water are diffusing through the membrane into the tube more rapidly than they are coming out and that molecules of sugar are not passing out into the water. This is osmosis and the apparatus is called an *osmometer* or an *osmoscope*.

Osmosis was discovered by Abbé Jean Antoine Nollet in 1748 and promptly forgotten for nearly a hundred years. Interest in it revived in 1837 by René Joachim Henri Dutrochet, a Frenchman. The type of apparatus employed by both men was identical or similar to that described above. In 1867 Traube developed a membrane which was a little more selective than Dutrochet's animal bladder. Traube's membrane was made by dropping a crystal of copper sulfate ($CuSO_4$) into a solution of potassium ferrocyanide [$K_4Fe(CN)_6$]. A precipitate of copper ferrocyanide [$Cu_2Fe(CN)_6$] is immediately formed at the area of contact between crystal and solution. This precipitate forms an "enveloping membrane." It acts as a differentially permeable membrane. Water diffuses through the membrane but the copper ions cannot escape. Soon a pressure is built up within the membrane and it bursts. As soon as a new surface of copper is exposed to the ferrocyanide solution another "precipitation" membrane forms. The process is repeated indefinitely until the system appears to "grow" like a "submarine garden."

Traube's type of osmometer was improved later by supporting the copper ferrocyanide membrane within the walls of a porous clay cup. A porous cup is filled with a solution of copper sulfate. A long glass tube is fitted to the open end at the top. The cup is then immersed in a solution of $K_4Fe(CN)_6$ as was previously done with the crystal of $CuSO_4$. A membrane is now precipitated where the two solutions meet within the walls of the cup. An osmometer of this type is less fragile because of the supporting framework of the porous cup.

In 1877 Hugo de Vries and Wilhelm Pfeffer applied the principles of osmosis to plant cells. This simple explanation of the movement of water into the cell was well received by the botanists of the 19th century because, up to this time, only vague, vitalistic theories had been offered in explanation of the phenomenon. The trend at the time was away from vitalism and toward mechanism. The studies of de Vries and Pfeffer were extended by other investigators who made many valuable contributions to plant physiology. As is quite often the case, this newly developed principle was employed to explain many different phenomena in plants. It is known today that not all water relations in plants are susceptible to explanation based on the principles of osmosis. However, osmosis plays a very important part in many of the living processes in the plant although other principles are also involved.

Osmosis in the Plant

The cell wall does not enter into this discussion because it is usually permeable to most solutions. We must look for permeable membranes within the confines of the wall. Two of them enclose the protoplasm. These are so delicate that they have never been seen. As mentioned previously, the membrane which separates the vacuole from cytoplasm is called the

tonoplast, and that which separates cytoplasm from the cell wall is the *plasmalemma.* These are differentially permeable membranes *par excellence.* It is probable that other membranes exist within the cell. There is evidence that differentially permeable membranes surround the nucleus, the plastids, and many other of the particulate bodies.

Actually these intracellular membranes are films. If they are formed as the result of interfacial tensions, they may be *monomolecular* in thickness. At any rate, they differ from the types of membranes which are customarily employed for demonstrating osmosis. Animal bladders, the membrane lining the shell of the egg, seed coats, and onion skins are examples of the last-mentioned. They are sometimes called multicellular membranes. Still another type of membrane may be useful for demonstrations. This type is represented by certain synthetic membranes, such as *pliofilm* (rubber) or *cellophane* (cellulose film). A type of experiment which more nearly approaches the natural phenomenon of osmosis consists of using a raw carrot for the osmometer. This is accomplished by boring a small hole in the top of the carrot root, cementing a glass tube in the hole and immersing the carrot in water. The tube will gradually fill with water, as was true when an animal membrane was used. In this manner osmosis may be demonstrated in the living plant.

The delicate membranes enclosing protoplasm are of vast physiological importance. They control the movement of water between the vacuole and cytoplasm or between cytoplasm and the exterior of the cell. Membranes of this kind are of extreme importance to the living cell. It has been aptly said that free diffusion between protoplasm and its environment will cause instant death, and that the limiting membrane of protoplasm is not a dead partition, but acts as the guardian of life.

In the plant cell the vacuole is regarded as an osmotic mechanism. As was mentioned in the chapter on the plant cell, most of the water-soluble substances occur within the vacuole. Both organic and inorganic solutes are present in relatively large concentrations and this in turn accounts for the osmotic pressure. Prior to the discovery of osmosis, botanists were at a loss to explain the mechanism by which plants maintain *turgor.* And still more puzzling was the phenomenon of wilting, which resulted from loss of turgor. The principle of osmosis serves to explain the development and maintenance of turgor in the cell.

Let us look at the plant as a whole. Osmosis accounts for the entrance of water into the roots, the movement from cell to cell. The passage of water up through the xylem involves other principles which will be discussed under the topic of transpiration. The movement of water from the xylem elements into adjacent living cells, however, may in most instances be attributable to osmosis. Thus, after the water is absorbed and has entered the transpiration stream, then its distribution to all cells depends upon osmosis.

The force by which roots and the first shoots of plants push themselves through the soil during growth is the result of turgor pressure. It is inconceivable that a plant could grow, even above the soil, without having first developed sufficient turgor to keep all parts erect or extended.

We have stated that turgidity in plants is produced by osmotic pressure of the cells. Let us look at the dynamics of this principle. In the ordinary osmometer which is employed for laboratory demonstration, the solution within the bag rises in the glass tube. If the tube is removed and the bag is closed at its neck then a certain amount of pressure will develop. Likewise osmotic systems in the plant cell have no outlet. Pressure is built up causing the membranes to distend. Turgor develops as the cell contents are pushed against the relatively inflexible cell wall.

To return to the cell, we might think of the plasmalemma surrounding protoplasm as being somewhat like the rubber bladder of a football. The leather covering of the ball corresponds to the cell wall. The cell wall, like the leather cover of the football, possesses a limited amount of flexibility. As turgor pressure is built up, wall pressure acts in the opposite direction. When fully turgid, the turgor pressure and wall pressure will be equal.

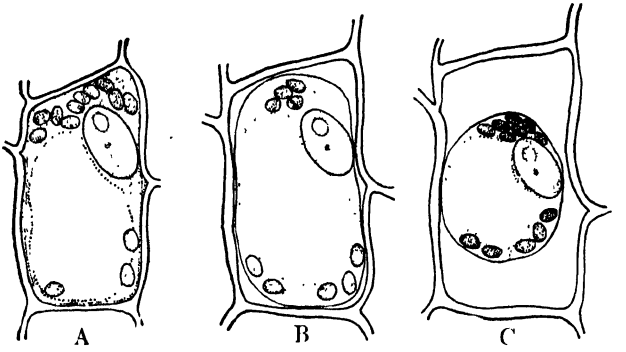
It should be recalled that in the osmotic system the solvent moves more rapidly from the region of less solute concentration to one of greater concentration. That is, a 1-molar solution of sucrose enclosed in a differentially permeable membrane will build up turgor pressure not only in water, but in any concentration of sugar between 0- and 1-molar.

Measuring Osmosis

Direct Method. Early workers measured osmotic pressure by direct methods. Pfeffer's osmometer consisted of a porous cup and a precipitated membrane which has been previously described. A one-hole stopper was placed in the top of the cup and a mercury manometer was attached by inserting one end in the hole of the stopper. By this means Pfeffer was able to measure osmotic pressure in millimeters of mercury which in turn could be converted to pressures in atmospheres.

Plasmolytic Method. It has been said that in an osmotic system, water molecules move from a less concentrated solution through the membrane to a solution of higher concentration. (We are speaking of the concentration of the solute molecules and not of the water molecules.) If a plant cell were placed in a solution of higher concentration than that in the vacuole, then the process of osmosis would be reversed. That is, water will diffuse from the plant cell into the surrounding solution. This process is called *exosmosis*, to distinguish it from *endosmosis*, which is applied to the movement of water *into* the cell. Exosmosis may proceed to such an extent that protoplasm within the cell shrinks away from the wall, eventually assuming a spherical form. This shrinking of the protoplasm as a result of withdrawal of the water is called *plasmolysis*.

The technique of the plasmolytic method of determining osmotic pressure is as follows. Thin slices of plant tissue are placed in solutions of various concentrations of a nontoxic substance like sugar. Each successive solution is gradually more concentrated than the previous one. The cells are observed under the microscope to ascertain the concentration of sugar which first causes plasmolysis. When this has been observed, an average is struck between the last concentration that did not cause plasmolysis and the first one that did cause it. This average of the two readings is called the *critical plasmolytic concentration*. It is the concentration which just does not produce plasmolysis.



(A) Diagram of normal cell. (B) and (C) progressive stages of plasmolysis. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

When the concentration of the sugar solution bathing the cell is equal to that in the vacuole, it is said to be *isotonic* with the cell sap. That is, it is *in tune*, or has the same tone. A solution having a higher osmotic concentration is said to be *hypertonic* with reference to the lower one. When the concentration is lower, the solution is *hypotonic*.

Cryoscopic Method. It is known that adding a solute to water serves to raise the boiling point or to lower the freezing point, and that the degree of change in these values is directly proportional to the amount of dissolved substance in the solvent. This principle is utilized in the cryoscopic method of determining osmotic pressure. It is more convenient to determine the depression of the freezing point than to measure the raising of the boiling point when working with plant extracts. An essential part of the equipment for this determination is a Beckmann thermometer. This instrument does not cover the range of temperatures covered by the ordinary thermometer but is so graduated as to indicate differences in temperature as small as 0.01°C. The thermometer is first standardized with pure water. Next the freezing point of the unknown is determined and the results are expressed as the depression of the freezing point of water. Osmotic pressure of the unknown solution can be computed from the following equation:

$$\frac{\text{O.P.}}{22.4} = \frac{\Delta}{1.86}$$

$$\text{O.P.} = \frac{22.4}{1.86}\Delta$$

Δ = observed freezing point depression of unknown
1.86 = the freezing point depression of a one molal solution of a nonelectrolyte
22.4 = osmotic pressure of a one molal solution of a nonelectrolyte

Relationship between Osmotic Pressure and the Gas Laws

It has been customary to speak of osmotic pressure when actually *osmotic concentration* is implied. Pressure is not developed unless the solution is set up in an osmometer. Again, when pressure is produced in the plant cell it is actually turgor pressure and not osmotic pressure. On the other hand, the term *osmotic pressure* occurs so frequently in textbooks and in the literature that it seems advisable to employ this term although actually we mean *potential pressure*.

The discovery of osmosis represented an important milestone, not only in the development of the science of plant physiology, but in its contribution to the field of physical chemistry as well. If real advances in science are made by the development of new concepts, then the discovery of osmosis represents the beginning of some very significant advances. In the discussion which follows it will be noted that in certain instances one investigator developed his theory from the data of one of his predecessors.

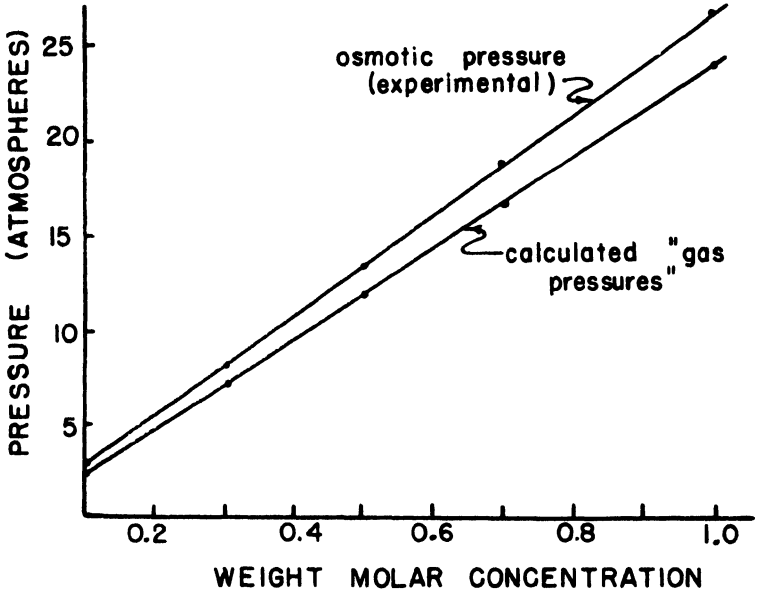
In 1887 van't Hoff showed that in dilute solutions osmotic pressures obey the gas laws. Some of the results of Pfeffer's investigations were cited to prove this point. According to Boyle's law, the volume of a gas varies inversely with pressure if the temperature remains constant. Charles' law states that volume varies directly with temperature if the pressure is constant. From these two laws it can be deduced that if the volume is held constant, the pressure of a gas varies directly with temperature.

Returning to the subject of solutions, it has been shown experimentally that osmotic pressure increases with temperature. Raising the temperature increases the velocity of the molecules and this in turn increases the rate of diffusion. Van't Hoff showed by calculation that a quantitative relationship exists between temperature and osmotic pressure. The calculations were made as follows. Pfeffer worked with a solution which contained 4 Gm. of sucrose per 100 ml. Such a solution would contain 40 Gm. per liter and this is $^{4}0/_{342}$ molar. According to Avogadro's hypothesis, equal volumes of gases at the same temperature and pressure contain the same number of molecules. At 0°C. and one atmosphere pressure (76 cm. of Hg) a gram molecular weight or mole of a

gas occupies 22.4 liters. If this gas is compressed to 1 liter then the pressure will equal 22.4 atmospheres. If solutions resemble gases then the osmotic pressure of a mole of sucrose will be equal to 22.4. The osmotic pressure of the above solution of 40 Gm. per liter would be equal to $^{40}/_{342} \times 22.4$ atmospheres.

$$^{40}/_{342} \times 22.4 \times 76 = 199.1 \text{ cm. of mercury}$$

The interesting thing about this calculation is that it agrees rather closely with Pfeffer's experimental results, his value being 197.4 cm. of



Comparison of calculated "gas pressures" with experimentally determined osmotic pressures of various concentrations of sucrose. (Plotted from data of Morse: "The Osmotic Pressure of Aqueous Solutions." *Carnegie Inst. Publ.* No. 198, 1914.)

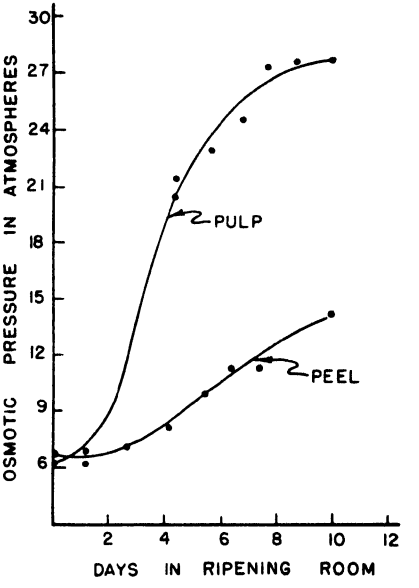
mercury at 0°C. This shows a remarkably close agreement between theoretical and experimental values, considering the equipment with which Pfeffer must have worked.

De Vries' data (published in 1884) afforded material for the development of another new concept. This was the theory of ionization. De Vries found that osmotic pressure of electrolytes was higher than that of nonelectrolytes. This went unexplained until 1887 when Arrhenius was proposing his theory of dissociation of electrolytes. De Vries' data were cited as evidence in support of the theory. According to Arrhenius, an electrolyte dissociates into its component ions, each of these ions acting as a molecule in so far as contributing to osmotic pressure. Thus if a compound

were 75% ionized then in every 100 molecules there would be 25 undissociated molecules and 150 ions (2 × 75). The osmotic pressure of a mole of this compound would be 1.75 times that of a molar solution of a nonelectrolyte.

Significance of Osmotic Pressure

Many interesting examples might be cited in which osmotic pressures play significant roles in the physiology of fresh fruits, where the conditions may be both normal and abnormal. Banana fruits are picked in the "mature green" stage and are ripened in ripening rooms. During the process of ripening, the peel changes from green to yellow, the flesh softens, tannins are rendered insoluble, and starch is converted to sugar. In addition to these changes, a definite increase in osmotic pressure occurs in both peel and pulp. However, the changes are much more pronounced in the pulp than in the peel. Stratton and von Loesecke have reported that during 10 days of ripening, osmotic pressure of the peel increases from 6.57 atmospheres to 14.68, whereas in the pulp the pressure increases from 6.19 atmospheres to 27.88 in the same time. These changes in osmotic pressure during ripening are sufficient to bring about a transfer of water from peel to pulp, and from the stalk through crown and neck to both peel and pulp. Thus in a ripe banana the pulp contains more sugar and water than the peel.

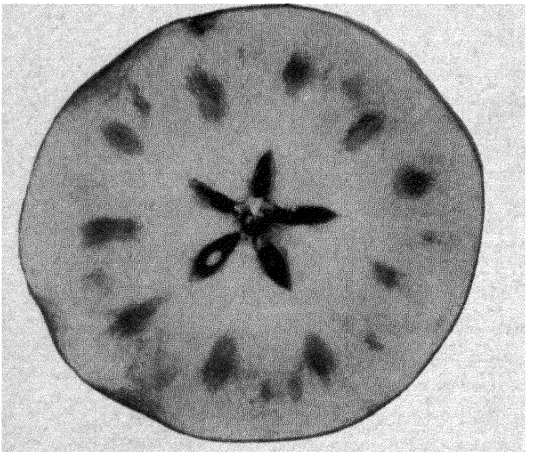


Changes in osmotic pressure of pulp and peel of the banana during ripening. (Redrawn from data of Stratton and von Loesecke: "Changes in Osmotic Pressure of Bananas During Ripening," *Plant Physiol.*, **6**:361–365, 1931.)

Another interesting example may be found in the sweet-cherry industry of the United States. Growers in the eastern part of the country often suffered financial losses because large numbers of fruits developed cracks in their flesh as they matured. The following sequence of events caused this abnormality in sweet cherries. Occasionally, the weather remained hot and dry for a period of ten days or more. Osmotic pressure in the ripening cherries was increased as the leaves manufactured sugar in the strong sunlight, and transpiration (loss of water) tended to make the solution of sugar in the sap more concentrated. When the hot-dry period was

broken by rain, the cherries swelled up and cracked as the result of movement of the water into the cells. This sudden increase in turgor caused the epidermis of the fruits to rupture. In the arid regions of the West, where irrigation is necessary for the production of crops, water relations of the trees may be kept more uniform and damage of the above kind is more easily avoided.

Water-core of apple fruits affords another example in which osmotic pressure may produce an abnormal condition in tissues. This disorder is characterized by watery or glassy areas in the flesh of the apple. At the



Apple showing water core. The watery or glassy spots in the cortex are produced by exudation of sap from cells into intercellular spaces. Exudation is the result of excessive osmotic concentration. (Courtesy, Bureau of Plant Industry, U.S. Dept. of Agriculture.)

beginning of the disorder, affected areas may appear merely as small dots or streaks. In extreme cases the whole fruit may be involved. Ordinarily the glassy spots are not much larger than 0.5 cm. in diameter. It has been found that this glassy appearance results from a filling of the intercellular spaces with water or sap instead of air.

Early workers suggested that water exuded from the cells under pressure. In 1926 Brooks and Fisher reported that there was a close correlation between sap concentration and water-core in apples. These investigators showed experimentally that osmotic concentration (expressed in atmospheres pressure) in water-cored apples averaged 16% higher than in apples which were free from water-core. Osmotic concentration in water-cored tissue averaged 14% higher than in normal tissue from the same apple. Increased osmotic concentration was found to be associated with certain environmental conditions such as exposure of the fruits to intense sunlight and reduction of water supply to the trees.

Many problems in this field remain unsolved and it is highly probable that knowledge of the principles of osmosis may be useful in solving these problems. For example, what causes the blossom half of an orange to contain more sugar than the stem half? This is undoubtedly the reason that the stem half and not the blossom half shows frost damage first. But why does granulation appear almost exclusively in the stem half of certain oranges as they approach senescence? This particular disorder gives the orange the appearance of having dried out at the stem end. However,



Mangroves send their roots into brackish water in tributaries adjoining the seacoast. The cells in plants of this type have relatively high osmotic concentrations.

analyses of affected tissue have shown that granulated cells are not lower in moisture than normal cells. Actually the water has been "bound" by hydrophilic colloids. Fundamental principles of physiology are involved here and this question stands as a challenge to physiologists of the future.

Osmotic pressure enters into the daily life of animals, including man. The freezing point depression of human blood is 0.56 and it fluctuates between 0.51 and 0.62. Greater variation than this indicates disorder, usually traceable to the improper functioning of the kidneys.

A number of experiments involving principles of osmosis have been conducted with the eggs of the sea urchin. The unfertilized eggs are usable because they are spherical in shape and changes in volume can easily be computed. J. Loeb showed that parthenogenesis could be produced by

adding a nonelectrolyte like sugar to sea water, thus producing a solution which was hypertonic to that in the egg.

A marine invertebrate, the spider crab (*Maia*) cannot regulate the salt content of the blood and cannot adjust itself to living in fresh water. The shore crab (*Carcinus*) on the other hand is able to live in a medium of high or low osmotic concentration, as shown by the fact that it may move from the open sea into estuaries or even considerable distances up the rivers.

Osmotic pressures of aquatic plants are usually much lower than those of land plants. In the water weed (*Anacharis canadensis*) osmotic pressure may be as low as 5 atmospheres. Osmotic concentration of land plants has been reported to range from 3 to 50 atmospheres.

Plants whose roots tolerate large quantities of salt can be expected to contain sap with high osmotic concentration. Mangrove trees have been reported to have an osmotic concentration of 25 and 34 atmospheres. The highest osmotic concentration reported for plants is 153 atmospheres. This was found in plants in the region of the Great Salt Lake.

Other Types of Osmosis

Under certain conditions it is possible to demonstrate osmosis between solutions of the same concentration. The two solutions are separated by a differentially permeable membrane as in all previous cases. Next the temperature of the one solution is raised slightly above that of the solution on the other side of the membrane. It will be found that molecules of water will pass from the region of higher temperature through the membrane to the region of lower temperature. We have said that osmosis represents diffusion of water through a differentially permeable membrane. Diffusion is the result of the kinetic activity of molecules. Raising the temperature increases the rate of diffusion and in this instance is responsible for osmosis between two solutions of the same concentration. This is called *thermal osmosis*.

Electro-osmosis represents still another type of osmosis. As indicated by the title, electric potentials are involved in this type. Instead of maintaining a difference in temperature between the solutions separated by the membrane, a difference of electric potential is introduced. Under these conditions hydrogen ions will move through the membrane toward the negative electrode or cathode. If the solution is in a glass tube, the hydroxyl ions will be adsorbed on the walls of the tube. Since electric potentials have been observed in plants it is highly possible that electro-osmosis occurs in living cells at times. Cellulose, in contact with water, acquires a negative charge. This is doubtless the result of adsorbing hydroxyl ions of the water, as was described in the instance of the walls of a glass tube. A discussion of electric potentials in plants appears in a subsequent chapter.

chapter 6

Imbibition and Permeability

Imbibition

Imbibition is the power possessed by a body of taking up relatively large volumes of a liquid without actually forming a liquid solution. The imbibant undergoes swelling during the process.

The swelling of starch grains, gelatin, or agar when placed in water illustrates the common effects of imbibition. Blocks of wood may be observed to swell as they soak up water, and the sticking of doors in a house during the rainy season is an example of the same principle at work. The swelling of seeds when placed in water is another very common example of imbibition. The greatest degree of swelling is displayed by protein substances; starch swells less, and cellulose swells the least of these three types of substances. This is the reason that peas and beans swell to a greater extent than seeds like wheat and corn. Legumes (peas and beans) are rich in proteins, whereas cereals (corn, wheat, etc.) are rich in starch. Since both proteins and carbohydrates swell to a greater extent than cellulose, this accounts for the fact that seeds usually burst their coats in the early stages of germination. The seed coat is largely cellulose and therefore does not swell proportionally with the imbibing seed.

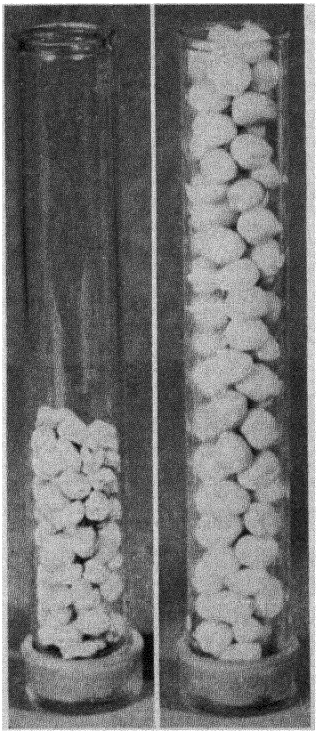


Illustration of increase in volume of garden pea seeds following imbibition of water. Each cylinder contains the same number of seeds.

Water is not the only liquid that may be imbibed. Organic solvents, like alcohol and ether, are imbibed by rubber. If a rubber stopper is left

too long in a flask containing an organic solvent, the lower part of the stopper will swell to such an extent that it becomes wedged in the neck. This is true even though only the fumes of the liquid may have come in contact with the imbibant in this instance.

A substance which imbibes alcohol or ether will not imbibe water. Likewise, substances which imbibe water will not take up organic solvents.

Albumen will not imbibe alcohol, benzene, or ether. Rubber will not imbibe water. In this way imbibition is distinguished from adsorption. Hydrophilic colloids will adsorb organic solvents although the quantity may be slight.

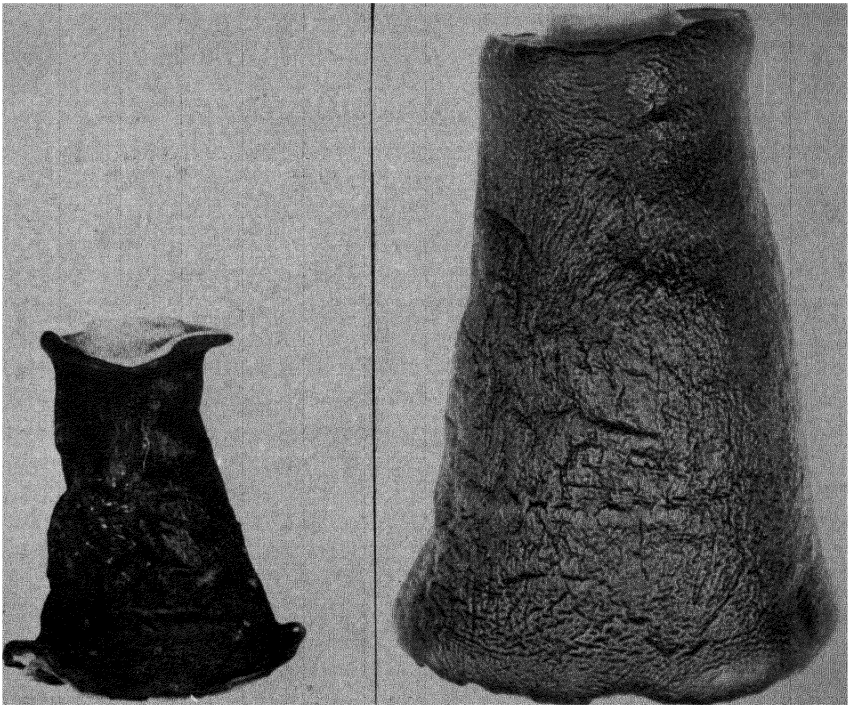
In the living plant only water is imbibed and we need not concern ourselves with other liquids in this discussion. It will be observed then, that imbibition in the plant is a characteristic of hydrophilic colloids. Gums and mucilages may be employed for demonstrating imbibition. Dry cherry gum, for example, when permitted to imbibe water freely, increases in volume but does not go into solution. The magnitude of the water imbibed by certain substances is apparent from the following examples. Under favorable circumstances gluten from wheat will imbibe 300% of its weight. A piece of dried stipe of the giant kelp (*Pelagophycus porra*) can absorb fifteen times its own weight of water.

The amount of water imbibed, when no other factor is involved, depends upon the cohesive force of the molecules of the particular colloid. Thus, molecules of wood exert greater attraction for each other than do molecules of gelatin. Because of this difference in cohesive force of molecules, a block of wood will not imbibe as much water as an equal weight of gelatin. The volume of the imbibant increases during imbibition. However, the volume of the whole system (imbibant plus solution) *decreases*. This may be demonstrated as follows. A volumetric flask is loosely filled with shreds of gelatin. Water is added up to the calibration mark on the neck. As the gelatin imbibes water it will be noted that the level of the water in the neck of the flask gradually falls. The reason for this is that molecules of water are oriented on the surface of the imbibant. They occupy less space because they are more efficiently "stacked."

During imbibition a certain amount of energy is released in the form of heat. A simple experiment will illustrate this principle. Dry starch or nonliving wheat seeds are placed in a thermos bottle. Water is added and a stopper with a thermometer is inserted in the neck of the thermos bottle. In a few minutes the temperature of the system will be observed to rise. The source of the heat is the kinetic energy of the molecules of water. As these molecules are adsorbed on the colloid in an oriented pattern, they lose some of their kinetic energy.

Alexander, in his textbook on colloid chemistry, relates an interesting story in which the force of imbibition was used to advantage. When the great obelisk was being erected in the Piazza of St. Peter's Cathedral it

Rome, Pope Sextus V ordered complete silence under penalty of death so that engineers would not be disturbed. They used blocks and tackles and raised the stone as far as they could but the monolith was not settled on its base. Suddenly a sailor in the crowd of spectators called out: "Wet the ropes." This was done and as the fibers of the ropes imbibed water and swelled, the ropes shortened. This was just enough to raise the obelisk to the required height and thus it was seated on its base.



Stipe of giant kelp before and after imbibing water. Weight increased from 6.0 to 54.9 Gm.

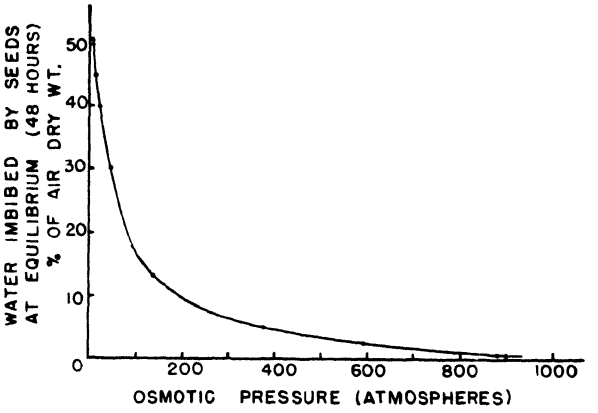
A number of factors may influence the rate of imbibition. Primarily, however, it is the diffusion pressure of the imbibed molecules which governs the rate of degree of imbibition. It will be recalled that this statement holds for other processes which have been considered, such as diffusion and osmosis.

Temperature and Imbibition. Since a rise in temperature will automatically increase the kinetic activity of molecules or ions, it follows that imbibition will be affected in the same way. Thus the rate at which the liquid is taken up increases with the rise of temperature.

Osmotic Concentration and Imbibition. Another factor which has been shown to affect diffusion pressure is the presence of organic

solutes. During the discussion of osmosis we showed how water molecules moved from the region of lower to a region of higher concentration of a solute. It was indicated that in a concentrated solution of sugar, water molecules are not as free to diffuse as in pure water. The molecules of water of a sugar solution enclosed in a membrane do not diffuse outwardly as rapidly as molecules of water from the external phase diffuse inwardly. Let us now suppose that the osmometer is replaced with a block of gelatin. In distilled water gelatin will imbibe a maximum quantity of the liquid. In a dilute solution of sugar less water will be imbibed. The concentration may be gradually increased until no water is imbibed at all.

Charles A. Shull demonstrated this principle, using seeds of the cocklebur (*Xanthium pennsylvanicum*) as the imbibant. Instead of sugar,



Relation between osmotic concentration and of amount of water imbibed by seeds. (Plotted from data of Shull: "Measurement of Surface Forces in Soils," *Botan. Gaz.* **62**:1-31, 1916.)

Shull used varying concentrations of sodium chloride and a saturated solution of lithium chloride. When cocklebur seeds were soaked in water they imbibed water equal to more than 50% of the dry weight. In tenth molar NaCl, the water imbibed by the seeds equaled 46.33% of the dry weight. When the seeds were soaked in saturated NaCl only 6.35% of water was imbibed. In a saturated solution of lithium chloride the seeds imbibed no water. (Osmotic pressure of sat. LiCl was 965.0 atmospheres.) It must be borne in mind that these experiments were possible only because seed coats of cocklebur are permeable to water but not to chlorides of sodium and lithium. These rather remarkable data are significant for two reasons. In the first place they suggested to Shull a technique for determining the diffusion-pressure deficit of soil moisture, that is, the difference between diffusion pressure of soil water and that of pure

water. Shull placed cocklebur seeds in silt loam soil containing varying amounts of water. At the lower range the soil was air-dry. At the upper range the soil contained 18.8% moisture, which was the approximate wilting point. The amount of water taken up by the seeds from the soil was compared with that taken up from the salt solutions previously described. Thus, if the seeds imbibed about 38% of water, the diffusion-pressure deficit of the soil was assumed to be 19 atmospheres. In the air-dry soil the seeds imbibed no water at all. This indicated that the diffusion-pressure deficit of the soil moisture was equivalent to 965 atmospheres or, in other words, like that of a saturated solution of lithium chloride, in which the seeds also failed to imbibe any water at all.

The second important point apparent from Shull's results is the magnitude of imbibitional pressures which may be developed by hydrophilic colloids. Examples have already been cited in which men have utilized imbibitional forces in order to accomplish work. Yet it is difficult to appreciate the magnitude of these forces even when expressed in atmospheres. Perhaps it will help if we think in terms of water pressure. The highest value reported by Shull was 965 atmospheres. Hydrostatic pressure of 965 atmospheres would be found six miles below the surface of the sea.

Concentration of Ions. In discussing the effects of osmotic pressures on imbibition, we have restricted our discussion in general to various concentrations of the same compound. Under the present heading we are concerned with identical concentrations of different ions. It will be found that the degree of imbibition is dependent upon the type of ion. For instance, the order in which the following ions are arranged is definitely related to the order of their effect on imbibition:

$$\begin{matrix} \text{Li} - \text{Na} - \text{NH}_4 - \text{K} - \text{Rb} - \text{Cs} \\ \text{Mg} - \text{Ca} - \text{Sr} - \text{Ba} \end{matrix}$$

If a block of agar were placed in equimolar solutions of the chlorides of each of these elements or radicals, the amount of imbibition would decrease as we progressed from left to right in the series. This is true of both monovalent and divalent ions. A similar effect is produced by acid radicals. A series of this type is called the *lyotropic series*. Many other physical processes are affected by these ions in the same order.

Hydrogen and hydroxyl ions exert varying effects upon imbibition of colloids, depending on the electric charge of the imbibant. If a negatively charged colloid is placed in a solution in which hydrogen ions predominate, the degree of swelling will be lower than that in pure water. If hydroxyl ions predominate, the negatively charged colloid will show increased swelling. The response of a positively charged colloid is similar to the negatively charged one with respect to the two ions; that is, swelling of a positively charged colloid increases in solutions of hydrogen ions and diminishes in one of hydroxyl ions.

The effect of hydrogen and hydroxyl ions on amphoteric colloids is a little more complex. Amphoteric colloids possess both negative and positive charges. Proteins are examples of amphoteric compounds. The positive charge results from the hydrogen in the acid group ($COOH^+$) and the negative charge from the amino group (NH_2^-). Tests have been made in this connection with gelatin, which is a "processed" protein. A minimum amount of imbibition is shown at the isoelectric point, which is pH 4.7. The isoelectric point indicates the hydrogen ion concentration in which the colloid is neutralized, that is, possesses no electric charge. Swelling of the gelatin gradually increases as the pH is changed in either direction, that is, a maximum is shown both in the acid range and also in the alkaline range. The swelling then falls off after the maximum is reached, when the medium is made more acid, or when it is made more alkaline.

Perhaps the preceding discussion has suggested other examples of a possible relationship between hydrogen ion concentration and imbibition, or has raised questions regarding certain phenomena in animal cells. What, for example, is the cause of local swelling in the human body, such as that produced by insect stings? Is this the result of the action of organic acids on imbibition of colloids? A sting may be simulated if a needle is dipped into acid and then inserted into a plate of gelatin. In many degenerative diseases of the body the blood becomes less alkaline and the patient loses weight. Is this an indication that body colloids have been forced nearer their isoelectric point and that their water-holding capacity diminishes?

Permeability

It has been said that problems in science have their day, but there is one problem in biology which has held a foremost position since its inception for approximately a hundred years. That problem is *permeability*.

Living cells possess the unique capacity of controlling the entrance and exit of various substances. This selective or differential permeability of protoplasm is dependent upon membranes. As a matter of fact, permeability is a characteristic of the membrane rather than of the substance which diffuses through it.

We have already mentioned some of the membranes which are believed to be present in the living cell. Between the cell wall and the protoplasm is found the *plasmalemma*. Surrounding the vacuole there is the *tonoplast*. A nuclear membrane surrounds the nucleus. It is believed too that membrane surrounds each and every plastid.

What is a membrane? Generally speaking it is a thin, pliable sheet. Plant physiologists often use the term rather loosely. They may have in mind intracellular membranes which are so delicate that they may be more properly called *films*. This may take us into the realm of "mono-

molecular" dimensions. This type of membrane is also found at the surface of most animal cells and on naked masses of plant protoplasm, like the plasmodium of slime molds. On the other hand we might mention the tough pellicles which surround blood cells or the ameba under certain circumstances. These are *multimolecular membranes*. All of the membranes thus far mentioned might be called *unicellular membranes*. Certain seed coats, onion skin, animal bladders represent still another type of membrane. These are multicellular. All may be differentially permeable.

Examples of Selective Permeability

Let us consider several examples of the selective permeability in living cells. The study of relative rates of absorption of potassium and sodium serves to illustrate the principle. These two elements occur in the plasma and corpuscles of rabbit blood in the following concentrations.*

	Plasma	*Corpuscles*
Potassium	0.026%	0.523%
Sodium	0.111%	0.008%

It can thus be seen that nearly all of the sodium of the blood is to be found in the plasma. Potassium, on the other hand, is present in both plasma and corpuscles, but the concentration in the corpuscles is 20 times that in the plasma.

A considerable amount of information on selective permeability has been obtained from studies of algae. Some algal cells are six inches in length and they contain several milliliters of sap. The fresh-water alga, *Nitella*, is found in ponds in which the potassium content is exceedingly low. Analyses of the sap of this organism have indicated that the quantity of potassium in the sap is a thousand times that of pond water.

Somewhat similar results have been reported with marine algae, like *Valonia* and *Halicystis*. Cells of these plants may be as much as two or three centimeters in diameter.

Table 9

ANALYSIS OF SEA WATER AND VALONIA SAP*

	Molar Concentration	
Constituents	*Bermuda Sea Water*	*Valonia Sap*
Cl + Br	0.58	0.60
Na	0.5	0.09
K	0.012	0.5

In Table 9 will be found an analysis of the sap of Valonia compared with sea water in which this alga was found growing. In this instance, there is 40 times as much potassium in Valonia sap as in sea water,

* Mitchell, Philip H.: *A Textbook of General Physiology*, 4th ed., New York, McGraw-Hill Book Co., 1948.

whereas there is only one-fifth as much sodium in the sap as in the sea. These phenomena cannot be explained entirely on the basis of difference in mobility of the ions.

It is true that potassium ions diffuse faster than sodium ions but the rate is only six times as great, whereas actually potassium may enter the Valonia cell 200 times as rapidly as sodium. Still better proof that it is not the speed of the ions, but rather a characteristic of the membrane, which accounts for unequal absorption, may be obtained from a comparison of the constituents of two different species of Valonia. It has been reported that *Valonia utricularis*, growing at Naples, shows little preference for potassium over sodium. This is in considerable contrast to *Valonia machrophysa*, which shows considerable preference for K over Na.

How Do We Know That Membranes Exist in the Cell?

As has been stated previously, the belief in the presence of membranes in the cell has been largely based upon circumstantial evidence. Selective permeability of living cells, and the fact that under certain circumstances the cell acts as an osmometer, are indications that membranes are present. In addition to these facts, a certain amount of information has been obtained by the use of micromanipulation or *micrurgy*. By means of a micropipet, dyes have been injected into the cytoplasm of living cells. The dye, consisting of molecules of colloidal size, did not move into the vacuole nor did it pass out into the surrounding medium. When the dye was injected into vacuolar sap there was no diffusion into cytoplasm from vacuole. A more convincing demonstration has been obtained with the vacuolar membrane or tonoplast. It is possible to withdraw plasmolyzed protoplasm from a cell by means of microneedles. After removal in this manner the protoplasm coagulates. The protoplasm may be stripped off, leaving the vacuole suspended like a sac which is filled with water. Whether the tonoplast is a membrane in the true sense of the word, or is a monomolecular film which forms at interfaces, may be open to question. But from the standpoint of selective absorption this is not important. Equally convincing evidence of the existence of a nuclear membrane has been obtained by micromanipulation. A part of an ameba containing the nucleus may be pinched off. The nucleus coagulates. By means of two microneedles it can be shown that the nucleus is surrounded by a very delicate membrane. It can be lifted off by the needles.

Mechanics of Differential Permeability

Several theories have been offered to explain the mechanics of differential permeability of living cells. Traube (1867) has been given credit for developing what has since been called the *sieve theory*. According to this theory, plasma membranes serve as molecular sieves, permitting

smaller molecules to pass more readily than larger ones. For example, water, with a molecular weight of 18, is perhaps the smallest molecule which commonly passes in and out of the cell, and it diffuses through the membrane more rapidly than glucose with a molecular weight of 180. Sucrose, which represents a still larger molecule, diffuses through cell membranes less readily than glucose.

This theory has been given practical application recently in an attempt to solve a problem originating in the utilization of potatoes. A relatively high concentration of glucose in the tubers is undesirable in the manufacture of potato chips, because the resulting chips assume a dark color during processing. Improvement is made in the final product by first soaking raw potato slices in water, permitting excess quantities of glucose to diffuse out. Sometimes a chemical or heat treatment is first given the slices prior to soaking in water, but the operator is always cautioned against any practice which will cause the loss of larger molecules, such as sucrose or dextrin.

The sieve theory of selective permeability has certain weaknesses. It has been found to apply in experiments in which artificial membranes have been employed, but it is not universally applicable in regard to living cells. It can be shown, for example, that with certain organic compounds, the degree of permeability increases with increase in the size of the molecule. This is illustrated in the discussion which follows.

Other explanations for selective permeability fall into the category of *solution theories*. It is held, for example, that those substances which are soluble in the cell membrane are capable of passing through it. A leading advocate of this theory was E. Overton (1895) who expressed the view that cell membranes are permeable to substances which dissolve readily in lipoids. This is sometimes referred to as the *lipoid solution theory*. Overton drew his conclusions from the following evidence. Fat solvents, like alcohol, ether, glycerol, benzene, etc., readily penetrate the cell. J. Willard Gibbs had already stated that compounds which decrease surface tension tend to accumulate at the surface or interface. According to Overton then, fatlike substances (lipoids) accumulate in the membranes of the cell because a membrane actually constitutes an interface. Fat solvents therefore penetrate the cell by dissolving in the lipoidal layer at the surface. A better understanding of the theory may be obtained by consideration of the difference between *polar* and *nonpolar* compounds. Polar compounds are those which have structures tending to form electrically charged ions or molecules. Water ($\overset{+}{H}O\overset{-}{H}$) is made up of two polar groups. This compound ionizes (although slightly) into hydrogen and hydroxyl ions. Other polar radicals are COOH, SH, CSN, NH_2, etc. Nonpolar compounds are represented mainly by hydrocarbon compounds, such as CH_3, C_2H_5, C_6H_5, and others. Many organic compounds, such

as the alcohols (CH_3OH, C_2H_5OH, C_6H_5OH), possess both polar and nonpolar radicals.

Overton's theory holds in instances when Traube's sieve theory fails. This is illustrated by the following results. Loeb studied the rate of penetration of a series of fatty acids into sea urchin eggs. In these tests the compound with the largest molecular weight penetrated the eggs most rapidly. This was nonylic acid, $CH_3(CH_2)_7COOH$. The rate of penetration decreased with decrease in size of the molecule in the series. Formic acid, H·COOH, penetrated sea urchin eggs the least rapidly. These experiments tend to refute the theory that the smaller the molecule, the more rapidly the compound will penetrate a differentially permeable membrane. These data do fit into Overton's theory, in that permeability appears to be directly associated with the number of nonpolar radicals in the molecule. In nonylic acid, with its eight hydrocarbon radicals, 69% of the molecular weight is attributable to nonpolar radicals. Acetic acid, which was next to last on the list, contains only one nonpolar radical. This represents 25% of its molecular weight.

On the other hand, Overton's theory fails to account for the ease with which water, a polar compound, is able to penetrate the living cell. Several attempts have been made to explain this apparent paradox. One explanation is that differentially permeable membranes of the cytoplasm are living and are constantly undergoing changes in composition. The surface layer may at one time be predominantly lipoidal material and therefore be permeable to fat solvents. At another time the surface layer may consist mainly of proteinaceous materials. Under this condition, the membrane would be permeable to water.

The following elaboration may help clarify the issue. The term lipoid does not necessarily mean a fat. It may indicate a fatlike substance, such as a phosphatide. Lecithin is a commonly occurring phosphatide which is often employed as an emulsifying agent. Because of the presence of both polar and nonpolar groups in its molecule, lecithin serves to keep oils dispersed in water. It is possible that lecithin may function as an emulsifying agent in cell membranes, permitting the passage of both polar and nonpolar compounds.

An expansion of this theory consists of the assumption that the surface membrane is composed of two layers of fats separated by one layer of proteins or an other water-soluble compound. Support for the theory has been obtained from a study of blood corpuscles. If a mass of blood corpuscles is separated from blood, centrifuged, and stirred with water, the corpuscles will burst. The contents dissolve in the water and the outside envelope or *stroma* remains undissolved. These empty envelopes have been called "ghosts of corpuscles" and they may be collected by centrifuging. It has been found that *lecithin*, *cephalin*, and *cholesterol* compose some 40% of stroma solids. It will be recalled that all of these are lipoids.

Protein and inorganic materials constitute the remainder of the composition of stroma solids. The actual weight of protein was found to exceed that of the lipid by 40% to 100%. However, assuming the molecular weight of the protein to be 67000, there would be 60 or more lipid molecules to every protein molecule. The proteins would therefore occupy a third of the volume or one layer of the three.

A Few Salient Facts

In conclusion, a few salient facts regarding permeability can be summed up as follows:

1. Any inert membrane, if at all permeable, would eventually permit equilibrium to be attained.
2. The living cell is different from the inert membrane. Solutes may diffuse into it against a concentration gradient (potassium in blood corpuscles, and potassium in Valonia cells).
3. Sap of injured Valonia cells becomes more and more like sea water until, at death, it is identical with it. *Even colloids diffuse into dead cells.*
4. Accumulation of solvents in a cell against a concentration gradient might be explained by assuming that the solutes are adsorbed on the surface of colloids, thus becoming an integral part of the protoplasm.
5. Energy is required to accumulate a solute against a concentration gradient. It is possible that this energy is supplied by the process of respiration.

chapter 7

Loss of Water by Plants

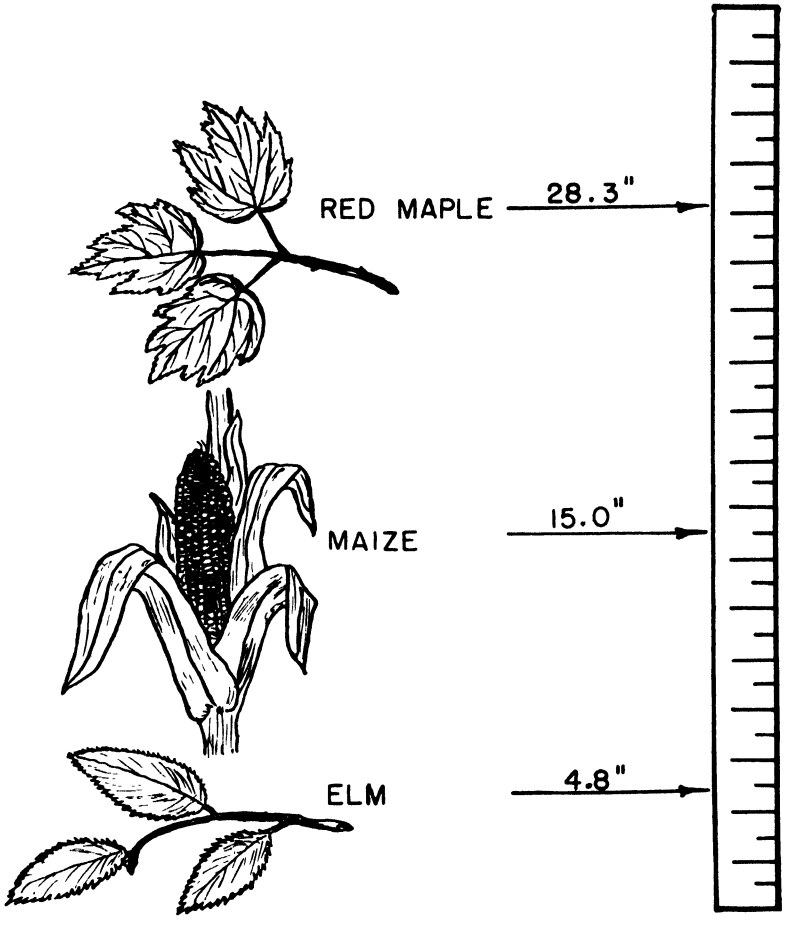
Transpiration

If the terminal of a leafy branch of a tree were enclosed in a jar during the daylight hours, a film of moisture would be seen to collect on the inside walls of the jar. This is evidence that the plant is losing moisture through its leaves. Since the water is lost in the gaseous form, it is not visible until it has condensed in the form of small drops. This loss of water vapor by living plants is called *transpiration*.

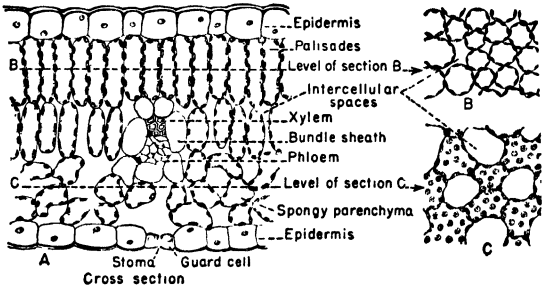
Although the actual passage of water from the plant to the air is not visible, the end results of severe water loss are readily apparent. Wilting of plants under conditions of severe drought occurs because water is lost more rapidly than it is taken up. The magnitude of this invisible process of transpiration is much greater than is generally realized. It has been estimated that a single corn plant may transpire 188 kilograms (over 400 pounds) of water per season. These figures are still more impressive if expressed in another way. Suppose that the water of transpiration from a whole acre of corn were to condense and fall on the ground instead of being dissipated into the atmosphere; and suppose that this water does not sink into the soil but accumulates on the ground as in a rain gauge. Assuming all of these conditions, we would find that the season's transpired water from an acre of corn would stand 15 inches high around the bases of the corn stalks. Under similar conditions an acre of red maple trees would transpire the equivalent of 28.3 inches of water during the course of a year. An acre covered with grass or clover might evaporate from 500 to 750 tons of water during a single season. An acre of 30-year-old apple trees may transpire 600 tons of water in a season.

The Mechanics of Transpiration

By what means is water transpired from a plant? And how does the plant exercise control over the rate of water loss? To answer these questions we must first familiarize ourselves with the structure of the leaf. Students of botany are no doubt acquainted with parts of the leaf. The fleshy portion is covered by the epidermis. This consists of a layer of colorless cells somewhat similar to the skin of animals. Over the epidermis

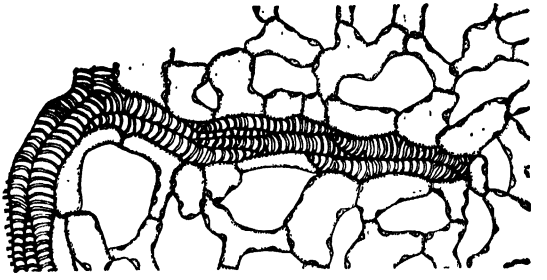


Acre inches of water transpired by red maple, maize, and elm during a normal season.

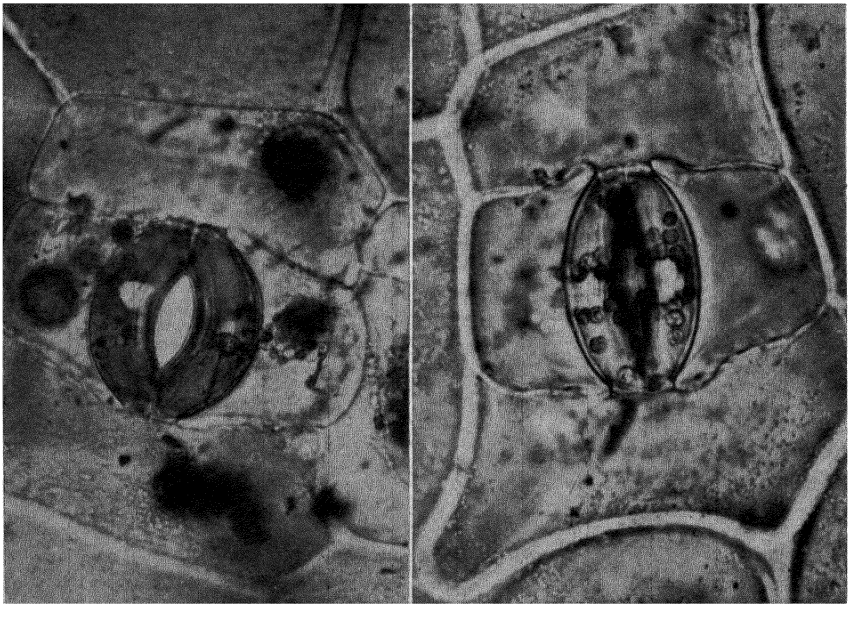


Diagrammatic cross-section of a leaf. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

is a structureless layer of cutin. This is a waxlike substance of complex composition. It is insoluble in ethyl ether. On top of the cutin is a layer of wax laid down in scales. This is soluble in ethyl ether and may be removed by dipping the leaf in this solvent. The combined layers of cutin



Drawing of xylem elements terminating among spongy parenchyma of a leaf. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)



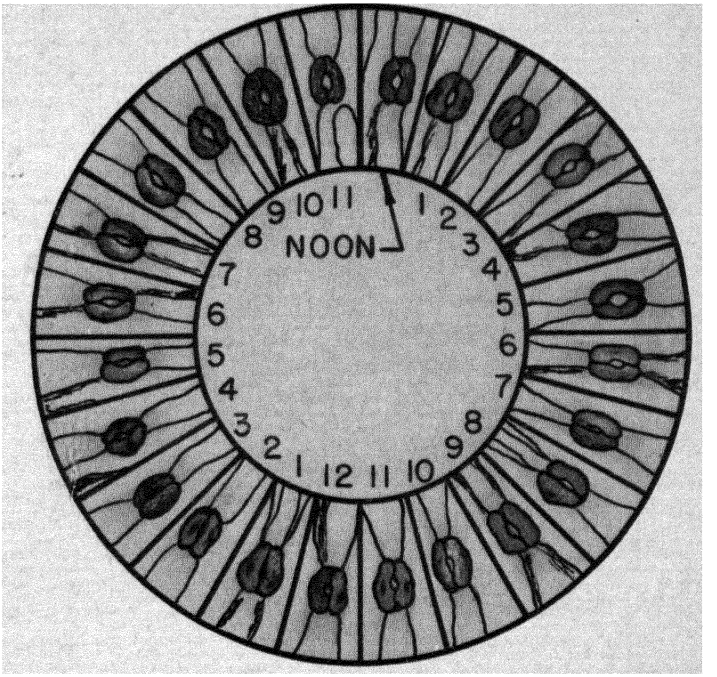
Stomates from underside of leaf of Zebrina pendula. Left: guard cells open; right: guard cells closed. (Courtesy, Gibbs: *Botany*, New York, The Blakiston Co., Inc., 1949.)

and wax comprise the cuticle, which in turn serves as a water-proofing layer for the leaf. It will be observed that the leaf possesses both an upper and lower epidermis, and that each is covered by a cuticle.

Just below the upper epidermis will be found the palisade cells. These are elongated and contain the green plant pigment, chlorophyll. Below

the palisade cells is found *spongy tissue*. Cells in this layer are usually isodiametric, and they too are green. Spongy tissue gets its name from the fact that many of the cells are separated by air spaces.

The cross-section of a leaf will also reveal a portion of one or more vascular bundles. These bundles contain the conducting tubes of the plant. The rather dense cells are the *sieve tubes*. These serve to conduct food. The large, open cells are the tracheae or water-conducting cells.

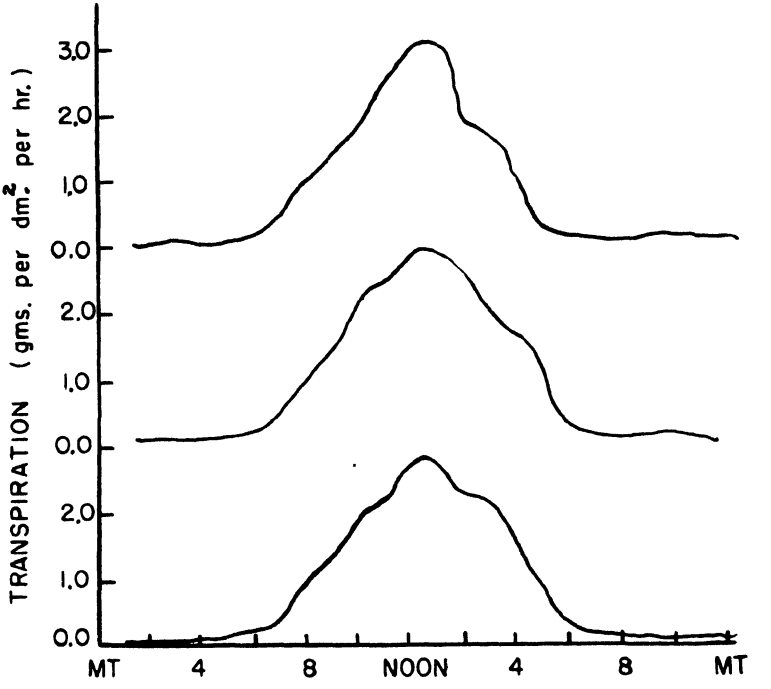


Hourly variation in opening and closing of stomates. (Courtesy, Loftfield: "The Behavior of Stomata," *Carnegie Inst. Pub.* No. 314, 1921. Redrawn after Maximov.)

Sieve tubes, with their surrounding cells, constitute the *phloem*. The tracheae, with their accompanying cells, make up the *xylem*. These two tissues will receive detailed treatment later. We are concerned now with the means by which water is lost by the plant. Tracheae bring the water to the surrounding cells. The vacuoles, protoplasm, and walls of the cells in the mesophyll of the leaf all become saturated. Finally the surface of the cells which border the air spaces become saturated too. Water then diffuses in gaseous form into the air spaces of the spongy tissue.

We must now look more closely at the epidermis in order to understand the means by which water vapor passes from the leaf to the atmosphere. Inspection of the lower epidermis of a leaf under considerable magnification will show stomates (singular, *stoma* or *stomate;* plural,

stomata or *stomates*) or pores through which water vapor escapes. Stomates may or may not be present on the upper epidermis too, although when present they are usually not as abundant as on the under side of the leaf. The stomate is bordered by two elliptical cells, called *guard cells*, which differ in shape from other cells of the epidermis. These two crescent-shaped cells derive their name from the fact that they guard the aperture to the spongy tissue of the leaf. During the daylight hours, if the plant is adequately supplied with water, the stomates will be wide open. Should a water deficit develop, the stomates will be closed to a mere slit, like the pupils of a cat's eye at noon. Normally stomates are closed at night also. Before discussing the mechanism by which the guard cells open and close, let us consider the hourly course of transpiration.



Hourly variation in transpiration rate of three mullein (*Verbascum thapsus*) plants. (Redrawn after Sayre: "The Relation of Hairy Leaf Coverings to Resistance of Leaves to Transpiration," *Ohio J. Sci.* **20**:55–86, 1920.)

Daily Variation in Transpiration Rate

During the course of a normal day in the growing season the transpiration rate of a plant will show considerable variation. This can be illustrated nicely if the hourly rates are plotted against time. The rate remains near zero during the hours of darkness. At sunrise the transpiration rate

begins rising and it reaches a peak around noon. After this it begins falling off until, at sunset, the rate is again near zero. It should be borne in mind, however, that results of this type would be obtained only if an adequate supply of moisture were available to the plant and if other conditions for growth were favorable.

Stomatal Mechanism

Stomates of a living leaf open when exposed to light. The speed with which they open is rather remarkable. In some instances the stomates open within sixty seconds after light impinges upon the surface of the leaf. For many years physiologists have been interested in the delicate mechanism by which the stomates are opened and closed. J. D. Sayre (1926) and G. W. Scarth (1932) were among the early workers to contribute toward a better understanding of the functioning of the guard cells, but the final answer was not obtained until 1951. Let us consider in detail some of the physiological changes that occur in guard cells during opening and closing of the stomates.

1. Observations made on the guard cells of Wandering Jew (*Zebrina pendula*) showed that in the dark the pH of the sap was 5 or less. In the light the pH increased to the range of from 6.0 to 7.4.

2. It was observed that starch grains are present in the guard cells and that the number of these grains was larger in the dark than in the light.

3. Amylase is an enzyme which converts starch to sugar or sugar to starch. The direction of the reaction depends upon the pH of the medium. At a low pH, like that found in guard cells in the dark, sugar is converted to starch. At a higher pH (from 6.0 to 7.4) starch is converted to sugar.

Thus it appears that when light strikes the leaf, starch in the guard cells is converted to sugar. This would account for the reduction in the number of starch grains during the day. The accompanying increase in sugar would increase the osmotic concentration of the sap.

4. Studies showed that osmotic concentration of the sap in the guard cells does increase when leaves are exposed to light. Furthermore, the osmotic concentration of other epidermal cells is not affected by exposure to light. Water then moves into the guard cells from adjacent epidermal cells and the turgor of the guard cells is increased. In a common type of guard cell the wall which borders the stomatal aperture is thicker than the opposite wall. Because of this, an increase in turgidity will cause guard cells to become less elliptical and more circular; that is, as these cells bulge away from the stomates, the opening becomes larger.

It is now well-established that alterations in osmotic concentration and the consequent changes in turgor are responsible for the opening and closing of the stomates of a leaf. But why should light cause a change in pH of the cell sap? Some physiologists claimed that light exerted a photo-

chemical effect. Others claimed that light inaugurated photosynthesis, which in turn accounted for the subsequent physiological reactions. The second theory could not be substantiated because no one had thus far proved the pigment in the guard cells to be chlorophyll. In fact doubt was expressed, at least up to 1950, that the guard cells contained chlorophyll.

In 1950 Freeland demonstrated that the guard cells contained chlorophyll *a*. This investigator carefully stripped off the epidermal cells of the leaves, examining them under the microscope to make certain that none of the palisade cells adhered. This tissue was extracted in acetone to remove the pigment. When the green pigment was purified and analyzed it was found that the absorption spectra coincided with those of chlorophyll *a*.

Additional contributions were made by Scarth and Shaw in 1951. These workers demonstrated that photosynthesis does occur in the guard cells. During the night, as a result of respiration, CO_2 is released in the cell sap. This becomes H_2CO_3 when dissolved, thus lowering the pH. During photosynthesis, CO_2 is absorbed by the chloroplasts, and the pH of the sap is raised. Thus a more complete picture can now be presented. The whole explanation of the opening and closing of the guard cells has been crystallized and presented diagrammatically in Table 10.

Table 10

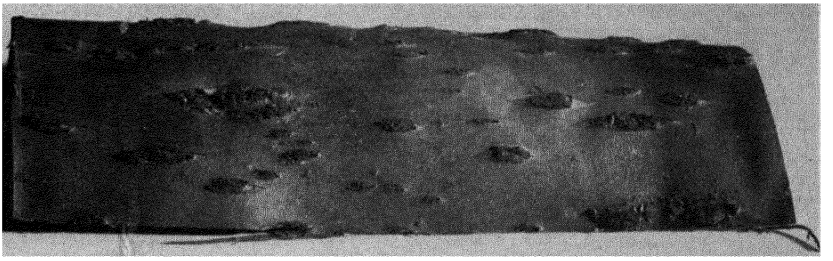
DIAGRAMMATIC REPRESENTATION OF STOMATAL ACTIVITY OF ZEBRINA PENDULA IN LIGHT AND DARKNESS

	Darkness	*Light*
Guard Cells	pH 5 or less	pH 6.0 7.4
	Starch	Sugar
	Low osmotic pressure	High osmotic pressure
	Flaccid	Turgid
Stomates	Closed.	Open

Different Types of Transpiration

Up to the present time we have discussed only loss of water through stomates, called *stomatal transpiration*. Plants may also lose water through lenticels, a process known as *lenticular transpiration*, and through the cuticle, called *cuticular transpiration*. After stomates have ceased to function they may be replaced by lenticels which since they consist of loose tissue, facilitate the exchange of gases. Lenticels are found on woody stems and may be seen also on certain fruits like apples, pears, and plums, and on potato tubers under certain conditions. Lenticels on fruits usually appear as mere specks to the naked eye, but under a hand-lens will be recognized as small openings surrounded by a raised layer of cork. These openings in the epidermis are particularly noticeable on the bark of cherry trees and on the bark of paper birch. The lenticels are elongated in these instances.

Cuticular transpiration refers to loss of water directly through the cuticle. Since this wax-like coating constitutes one means of conserving moisture in succulent plants, it does not seem logical that very much moisture can be lost by cuticular transpiration. As a matter of fact, succulent plants lose most of their water through the stomates. Investigators have covered the stomates with a petroleum jelly (when stomates were present only on the lower epidermis) and have recorded a loss of water through the cuticle of leaves. This of course represents an artificial condition. It is possible that fruits, flowers, and herbaceous stems lose a certain amount of moisture as a result of cuticular transpiration. However, lenticels are usually present on the surface of fruits and stems, so that it is difficult to distinguish between cuticular and lenticular transpiration in examples of this type. It is a known fact that many succulent fruits and vegetables



Bark of cherry tree showing lenticels.

lose water after harvest and consequently become shriveled. In some instances the loss of water may be by way of the cuticle, but this may be traceable to the fact that much of the waxy coating has been removed as a result of processing. This therefore is an artificial case, as was true of the one in which the stomates were covered with a petroleum jelly in order to demonstrate loss of water through the cuticle of the leaf.

Other Means of Losing Water

Not all water is lost in the gaseous state by plants. One of the commonest examples of loss of water in the liquid form is that of *guttation*. If both soil and air are saturated with moisture, plants may then lose water by guttation. Water appears to ooze out of the leaves at the tips. This may be demonstrated by covering potted wheat or oat seedlings with a glass jar. Soon after excess water has been added to the soil, drops of moisture will appear on the leaves. This water has not come from stomates but rather from structures beneath the epidermis which are called *hydathodes*. It is possible that many of the "heavy dews" in the tropics are not the result of formation of dew, but are rather attributable to guttation.

Several other examples might be cited of the loss of water in liquid form. One of these is the *secretion* from glands such as the nectaries of flowers, and another is *bleeding*, such as is found in grape vines, sugar maple trees, etc.

Methods of Measuring Transpiration

Frequently physiologists find it important to determine the rate of transpiration of a plant. A number of methods have been employed for measuring water losses. Several of these will be described.

1. Hygrometric Paper. Filter paper may be made *hygrometric* by soaking it in 3% cobalt chloride and then drying it. This paper is blue when dry and pink when wet. If cobalt thiocyanate [$Co(CNS)_2$] is substituted for cobalt chloride ($CoCl_2$), the colors become a little more intense. The rate of transpiration of a leaf may be measured by clamping a piece of blue (dry) cobalt paper to the leaf and recording the time required for the paper to become pink. This method is customarily employed either for qualitative demonstrations of transpiration or for indicating relative rates. It can be used for quantitative determinations by recording the time required for the paper to attain a definite shade of pink. The end point is determined by matching the color with one in a standard color chart.

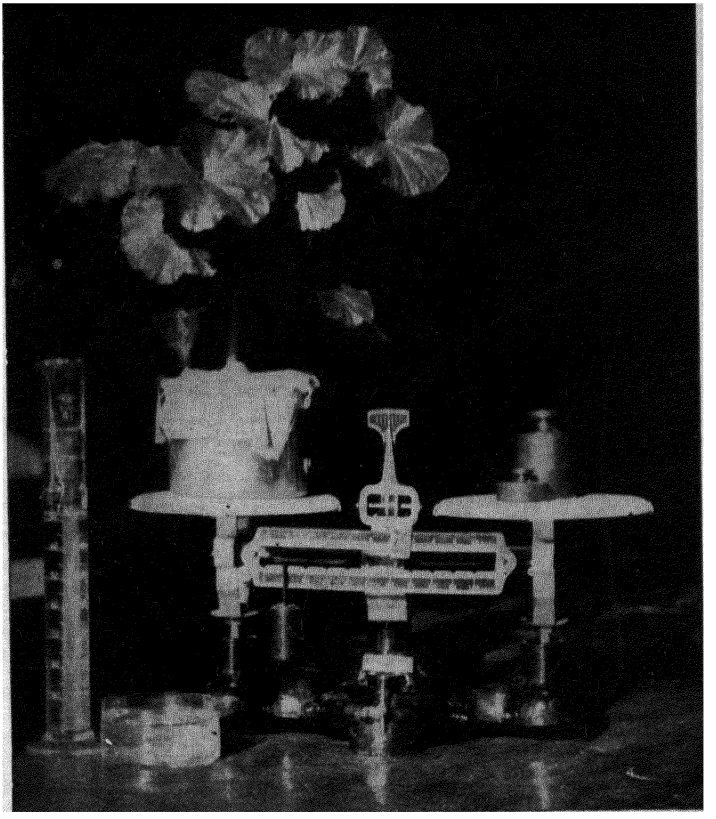
2. Weighing Potted Plants. This method is adaptable to classroom demonstration. The plant is transplanted to a tin can to prevent loss of moisture from the walls of the container. The surface of the soil is covered with paraffin. The stem of the plant is protected from the melted paraffin with a wad of cotton. It will facilitate computation of leaf area if a plant with leaves which are nearly circular is selected. The common geranium is suitable for this reason. The plant and container are weighed at regular intervals, such as every two hours. The rate of transpiration is indicated by the gradual loss of weight.

3. Collecting Transpired Water Vapor. In employing this method the plant must be enclosed in a transparent chamber. A stream of air is swept through the chamber, and the moisture is collected over an absorbent like calcium chloride. Corrections must be made for the amount of moisture present in the introduced air. After this correction has been made, the rate of transpiration is computed from the increase in weight of the calcium chloride tube.

The methods just described will be found suitable for determining the rate of transpiration on relatively small plants, but they are not practicable for studying transpiration in mature trees. Several methods have been developed, however, which may be useful in studying transpiration of large shade or fruit trees.

4. Potometer. This instrument, which means literally "drink meter," may be employed for measuring uptake of water. Since water absorbed is

usually proportional to water transpired, the procedure serves as an indirect method for measuring transpiration. The potometer consists of a tube with side-arms, one of which is graduated. A leafy shoot or even a small plant is placed in the main cylinder which is filled with water. The stem of the plant is inserted in a one-holed rubber stopper. As the plant transpires, the water moves into the main cylinder from the graduated



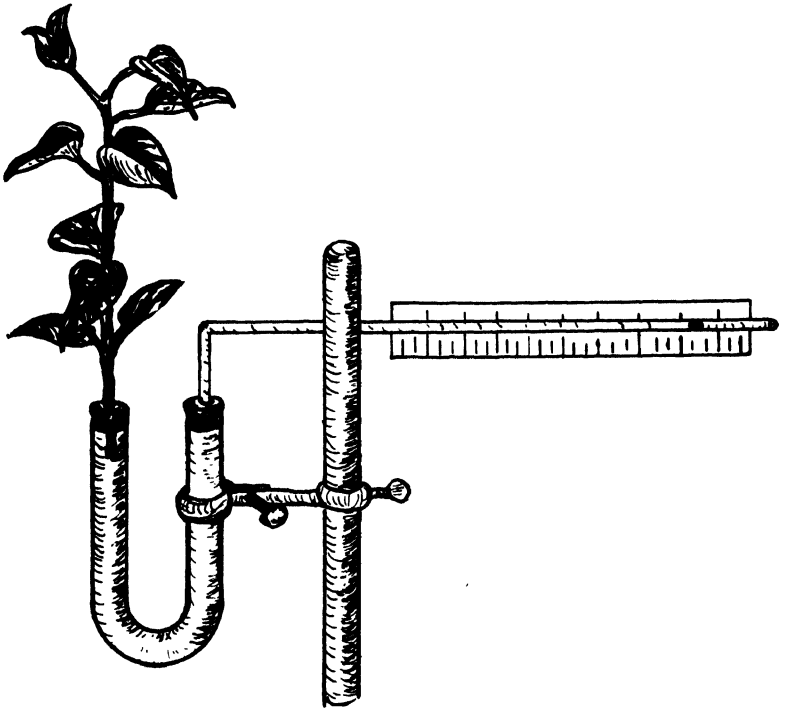
Illustrating a method of measuring transpiration. Periodic loss of water is evidenced by loss of weight of the plant. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

side-arm. Observations made at regular intervals yield information on the rate of transpiration.

5. Weighing of Detached Leaves. A convenient method for measuring transpiration in large trees consists of studying individual, detached leaves. In this method the leaves are weighed at short intervals after removal from the tree. Progressive losses in weight are interpreted as losses in moisture by transpiration. One disadvantage of this method is that an abnormal condition has been created because the leaf has been removed

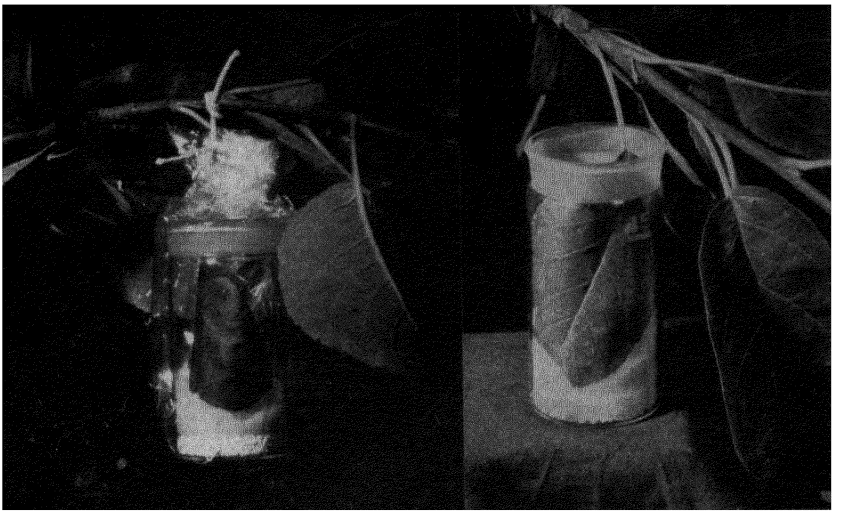
from its source of water. In fact it has been reported that accuracy demands that all weighings be made within a period of 60 seconds after the leaf has been removed from the tree.

6. Use of Attached Leaves. It is possible to determine the transpiration rate of leaves of a large tree without removing them. A recently developed method consists of clamping a section of a leaf between the mouth of a weighing bottle and its top. The weighing bottle contains a small amount of calcium chloride, which is separated from the leaf by a



Transpiration rate may be measured by a potometer, which indicates the quantity of water absorbed. (Redrawn after Molisch.)

small wad of cotton. A satisfactory modification of this method consists of lowering the whole leaf into the weighing bottle and then enclosing the system in a Pliofilm (rubber hydrochloride) bag. The rate of transpiration may be determined by weighing the bottle before and after the leaf has been exposed to the calcium chloride. One apparent objection to this method is that there are many leaves on a large tree, and a representative sample might require a very large number of individual leaves. Nevertheless, it represents a method which may be employed without destroying either leaf or tree, and without removing the plant from its normal environment.



Method for determining transpiration rate without removing leaves from tree. (Left) Leaf is enclosed in a weighing bottle containing calcium chloride and a sheet of Pliofilm is tied around bottle and leaf. (Right) Pliofilm has been removed in the photograph.

Factors Affecting Transpiration

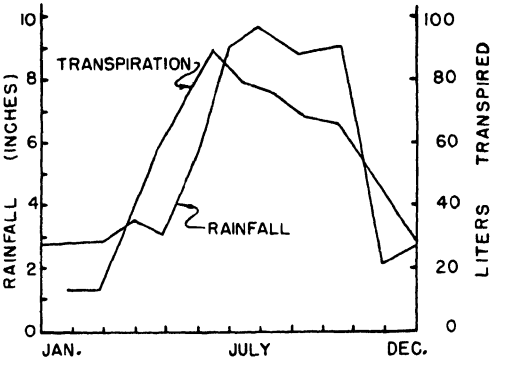
Any number of factors, both external and internal, may affect the rate of transpiration of plants. Perhaps light can be considered one of the most important environmental factors, because of its role in causing the stomates to open. But other factors may be almost as important. The condition of the atmosphere with regard to moisture is of considerable importance, because the rate of water loss from plants is greatly affected by the capacity of the atmosphere to take up moisture. This condition is usually referred to as *relative humidity*, which refers to the per cent saturation of the atmosphere at a given temperature. Since warm air has a greater capacity for holding water than cold air, relative humidity is not a true index of the amount of moisture that can be evaporated. The term *vapor-pressure deficit* is preferred by many physiologists. By this is meant the difference between actual vapor pressure and the vapor pressure of the atmosphere when saturated at the same temperature.

The effect of atmospheric humidity on transpiration has many practical applications. Truck gardeners have long made a practice of setting out vegetable seedlings on foggy or rainy days. Under these conditions the atmosphere is saturated or nearly so, and the transpiration rate of the plants is practically zero. The home gardener, if he finds it necessary to transplant seedlings on a sunny day, will cover the plants until their roots become established in their new environment. The atmosphere of

the modern home, with its centralized heating, is often too dry for growing plants. The air in a commercial greenhouse, on the other hand, is much more humid, and one becomes aware of this as soon as he enters.

Temperature may affect the rate of transpiration in several different ways: first, as has been shown, a rise in the temperature of the atmosphere increases the capacity of the air for holding moisture; second, leaves exposed to direct sunlight may be warmer than the air by several degrees. The kinetic energy of the water molecules in the spongy tissue is increased, and this in turn increases the diffusive capacity of the water.

Roy and Gardner studied monthly transpiration rates of orange trees by growing them in culture solutions in stone jars. The work was done in Florida where the rainy season coincides with the growing season, that is,



Transpiration rate (liters per 28 days) of orange trees grown in sand culture, and mean monthly rainfall in Orlando, Fla. area. U.S. Weather Bureau data. (Redrawn after Roy and Gardner: "Seasonal Absorption of Nutrient Ions by Orange Trees in Sand Culture," *Proc. Fla. State Hort. Soc.*, 1945.)

during the summer months. A glance at the results, as illustrated graphically, might suggest that the transpiration rate was correlated with the monthly rainfall. However, the trees were grown in jars and were adequately supplied with water at all times. A closer scrutiny of the results indicated that the monthly rates of transpiration more or less paralleled the monthly changes in temperature.

A certain amount of acceleration in transpiration results from exposing plants to wind. Unprotected seedlings, when transplanted on a windy day, are very likely to wilt. The same effect has been observed in the transportation of plants in open trucks. As a rule, however, the rate of transpiration increases in winds having velocities of up to two or three miles an hour, and then becomes constant. Winds of high velocity probably bring about closure of stomates.

A number of investigators have reported that the rate of transpiration is proportional to the quantity of moisture in the soil. This means not the

total moisture but the available moisture. As the available moisture is gradually reduced, the rate of transpiration falls off, largely because of the closing of the stomates. Water may be present in the soil and still not available to plants. Examples of such conditions will be cited under the discussion of water absorption.

Cultural Practices Affecting Transpiration

It has been known for many years that fungicidal sprays which contain copper may increase the transpiration rate of plants. There exists considerable difference of opinion as to the exact cause of this increase in transpiration, but it is generally agreed that the presence of free copper is to some extent responsible for the effect. Whatever the actual cause, the results have stimulated a search for fungicidal materials which produce less drastic effects than some of the older copper sprays. Conversely, it is now possible to employ a spray for reducing the transpiration rate of plants. An oil emulsion or a wax-oil emulsion, when applied to leafy plants, has been found to reduce transpiration. Such a spray is used commercially by nurserymen when transplanting evergreen shrubs and trees. The spray reduces the loss of water in the plants until the roots become established in their new environment.

Absorption of Water and Its Movement through the Plant

Our discussion thus far has been restricted to the plant's loss of water. Little attention has been given to its absorption of water. It might appear to some readers that we have reversed the order in which these two topics should be developed. This was done only because it is believed to be an advantage to the student to have a clear picture of transpiration before considering the entry of water into the plant.

Plants absorb water (and mineral nutrients) by means of their root systems. Inspection of the tip of a root reveals a white cap called the *rootcap*. The terminal portion of the root (other than the cap) consists of meristematic tissue. This is followed by a zone of cell elongation. Above this zone is found the region of root hairs. These are filamentous outgrowths of epidermal cells. It is apparent that the absorbing surfaces of roots are greatly enhanced by the formation of root hairs. A large part of the water taken from the soil is absorbed by root hairs when they are present. In the absence of root hairs, water is absorbed by nonsuberized portions of the roots. Experiments have shown that roots themselves take up some of the water even in the presence of root hairs.

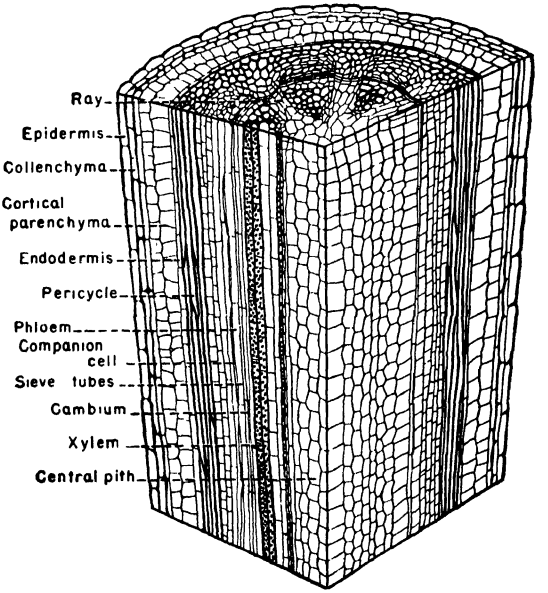
Evaporation of water from the leaves during transpiration produces a diffusion-pressure deficit or tension in the cells of the spongy tissue. This tension extends through the vascular system down to the root tips. The cohesive force of water molecules for each other enables the column of water in the tracheae to withstand the tension which develops. When a

greater diffusion-pressure deficit exists in the root cells than in the soil, the roots absorb water. It is probable that osmotic and imbibitional forces also play a part in the absorption of water by the roots.

From the root hairs and simple epidermal cells, water moves across the cortex of the root, through the endodermis and pericycle, and into the tracheae or vessels. As mentioned previously, the tracheae constitute continuous columns which terminate in the spongy tissues of the leaves.

Factors Affecting Absorption of Water

Although roots may slowly absorb water when plants are not transpiring, it has now been well established that the rate of absorption of



Semidiagrammatic drawing of young stem. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

water is greatly influenced by the rate of transpiration. In other words, active absorption of water by roots is not important in supplying the plant with water when the transpiration rate is high.

When other conditions are optimal, plants transpire more rapidly when the available moisture content of the soil is high. As the percentage of moisture in the soil decreases, the water is held by the soil with greater force, so that when the soil approaches complete dryness, a force of about 10,000 atmospheres is required to remove more water.

In some instances uptake of water by the roots may be prevented by higher osmotic concentration of the soil solutions. Such a condition may occur in the so-called alkaline soils of the semi-arid regions; it is rarely

found in humid regions except where salts may have accumulated in the soil as a result of heavy application of fertilizers. Retention of moisture in the soil as a result of high concentration of salts is sometimes termed *physiological drought.*

Lowering of soil temperature has been shown to limit absorption of moisture by plants. Kramer cites a number of examples in which plants have wilted in an abundant supply of soil moisture, only because the temperature of the soil was so low that uptake of moisture was retarded. Plants adapted to warmer climates (cotton, cucumber, watermelon) have been reported to show a greatly reduced uptake of water when soil temperature is lowered.

How Does Water Reach the Top of the Tallest Trees?

We have attempted to describe the paths by which water moves from the soil into the roots, up through the axis of the plant, into the leaves, and out into the atmosphere through the stomates of the leaves. The next question is, "How does water reach the top of the highest tree?" For many years physiologists have been searching for the physical principle which would account for the force which is necessary to carry water through plants in the transpiration stream. In looking for an answer we must bear in mind that the tallest trees in the world, like the giant redwoods of California, are nearly 400 feet high. Certain physical principles which we have elaborated thus far might be employed to explain the rise of water in succulent shrubs and small trees, but the principles are not valid for trees 400 feet tall. For instance, one first thinks of capillarity and osmosis in discussing the rise of liquids. However, the capillary rise of water in tubes 10 microns in diameter would amount to only about 9 or 10 feet (3 meters). Likewise, osmotic pressures of sufficient magnitude are not often reported in plants. Atmospheric pressure is not sufficient to account for the rise in water in the tallest trees. One atmosphere will lift water only about 30 feet. It has been estimated that approximately 50 atmospheres pressure would be required to lift water 400 feet in a tree. It takes 12 atmospheres to lift the water and the remaining force to overcome friction.

Three theories have been advanced to explain the rise of water in plants. These are: the vital theory, the root-pressure theory, and the cohesion theory.

The *vital theory* was proposed by Godlewski in 1884. He claimed that living cells bordering on the xylem developed a higher osmotic concentration than surrounding cells, and that caused water to move from the xylem into the cells with higher osmotic concentration. This in turn caused water to move up in the xylem to replace that which was lost to adjacent cells. In 1893 E. Strasburger performed experiments which tended to refute the vital theory. He killed a 75-year-old oak tree by

sawing it off at the base, and immersing the cut end in picric acid. The tree was 22 meters high. Three days after the picric-acid treatment, the cut end was placed in a solution of dye. The dye moved up the entire trunk of the tree. Similar stems were killed by exposing them to a temperature of 90°C. These dead stems were also able to conduct water to the top of the tree. Strasburger concluded from these results that living cells are not necessary for the movement of water through the vessels of the xylem.

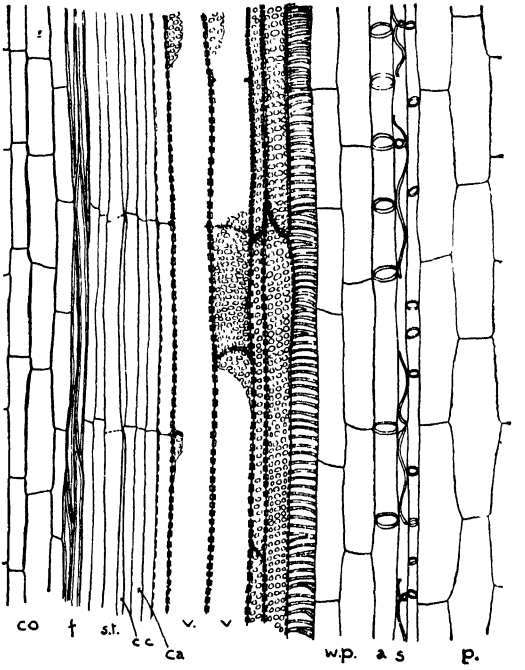
The *root-pressure theory* is based upon the observation that plants have been known to develop internal hydrostatic pressures. In certain woody plants, like grape vines and maple trees, there occurs a type of pressure which apparently causes a flow of sap. If grape vines are cut or maple trees are tapped, the sap flows in considerable quantities. This is often referred to as *bleeding*. Internal pressure in plants may be demonstrated by a very simple experiment. The stem of a potted plant is cut and a mercury manometer is fitted over the cut end by means of a rubber tube. The mercury column will rise, indicating hydrostatic pressure. The range of pressure which has been demonstrated is from 2 to 6 atmospheres. The force which causes the mercury to rise (and the sap to flow) is called *root pressure*.

Several objections have been raised against the root-pressure theory. The most important objection is that the magnitude of pressure (2 to 6 atmospheres) which has been demonstrated is far from sufficient to account for the water's rise to the top of the highest tree.

In 1897 E. Askenasy developed what is known as the *cohesion theory*. This theory has now received more support than any of the others. Askenasy bent a glass tube in the shape of a U, with one arm longer than the other. A porous cup was attached to one end. The tube was filled with water, and the lower or longer end was immersed in mercury. To remove all air, the end with the porous cup was lowered into water and the water was heated. When the cup was exposed to air, water evaporated from its surface and the column of the water in the tube rose, pulling up mercury with it.

Thus laboratory experiments of this type show there is a cohesive force present in a narrow column of water and that a pull on the upper end will cause the column to rise. These experiments served to demonstrate Askenasy's principle although the maximum force produced was never greater than 2 or 3 atmospheres. Years after the theory was proposed, H. H. Dixon, a leading advocate of the theory, performed a number of experiments which served to support the cohesion theory. Dixon, using the centrifuged sap of the holly plant (*Ilex aquifolium*), demonstrated a cohesive force of 207 atmospheres. This is a much greater force than has been demonstrated in any of the root-pressure experiments, and is far in excess of what is required in the largest plants.

Still greater pressures had been previously demonstrated by A. Ursprung in 1915. It has been observed that tremendous forces are set up by the sporangia of ferns in discharging spores. As the annulus turns inside out and stretches backward it acts as a coiled spring. It is the force of the cohesion of water molecules in the epidermal cells which maintain the tension. When suddenly the tension does break, the annulus springs back, discharging the spores in the atmosphere. The cohesive force of the water may be determined by balancing it against osmotic pressure.



Radial longitudinal section through part of a young dicotyledonous stem. (Courtesy, Gibbs: *Botany*, New York, The Blakiston Co., Inc., 1950.)

The annuli of the sporangia are permitted to come into equilibrium with known vapor pressures produced by various osmotic concentrations. In this manner the highest vapor pressure is determined at which rupturing of the water occurs. Results show that the cohesive force developed in the annuli of the fern sporangia is between 300 and 350 atmospheres. These experiments, and many others, have given strong support to the cohesion theory.

The Significance of Transpiration

Physiologists are divided in their opinions regarding the significance of transpiration in plants. One school of thought says the process is as important as photosynthesis, respiration, and other functions. Another

school claims that transpiration is a necessary evil. The latter group holds that transpiration is a habit carried over from the era when all plants were aquatic and when there was an abundance of water at all times. It is claimed too that the stomates open for the purpose of introducing gases required in photosynthesis, and that large quantities of water escape while these "doors" are open.

The following are the roles generally assumed to be played by the transpiration process:

1. Translocation of water to all parts of the plant.
2. Translocation of minerals.
3. Dissipation of radiant energy.

A few of the opposing views on the significance of transpiration will be presented here. It will give the student an opportunity to do his own thinking on the subject.

Is transpiration essential for the translocation of water?

Yes

Maximov. Transpiration is very probably of major importance in translocation of water to the leaves. A certain saturation of the plant is essential for functions like flowering and fruiting. Optimum degree of turgor is maintained by transpiration.

No

Meyer and Anderson. Although under conditions of high transpiration the movement of water in plants is more rapid than under conditions of low transpiration, translocation of water (to the extent that it is used in metabolic processes) continues even though the transpiration rate is negligible. For example, sufficient water for metabolic processes is supplied to the cells at night, after transpiration has ceased.

Is transpiration necessary for the translocation of minerals?

Yes

Raber. Higher mineral content is associated with increased transpiration, and analyses show that leaves (terminal organs in transpiration) contain more calcium than other parts of the plant. Years would be required for a molecule like NaCl to reach the top of a giant sequoia tree by diffusion alone.

Haas. Increased amounts of calcium have been found in citrus leaves when the transpiration rate was raised by exposing plants to wind.

No

Meyer and Anderson. Greater accumulation of salts, under conditions favoring high transpiration rates, occurs only under certain metabolic conditions, and there is no consistent correlation between the transpiration rate and the rate of absorption of minerals.

Hoagland. Movement of water and solutes are not governed by the same laws, and in a given tissue, during a given period, solute and solvent may move in opposite directions.

Does transpiration function in dissipating radiant energy?

Yes

Maximov. Overheating of leaves has been known to occur in greenhouses where the transpiration rate was reduced by high humidity. Temperatures of wilted leaves may be 4 to 6 degrees higher than that of turgid ones.

No

Meyer and Anderson. Assuming that only 50% of the radiant energy of the sunlight is absorbed, this would be enough to raise the temperature of a leaf 37°C. in a minute at noon on a hot summer day. In a few minutes the thermal death point of protoplasm would be reached. However, it would take the evaporation of a 6.6 Gm. of water per square decimeter per hour, to prevent this rise in temperature. This figure is rarely reached in the transpiration of plants on the desert, and leaves having their stomates plugged with a petroleum jelly rarely attain temperatures much higher than that of the surrounding atmosphere. It is highly possible that leaves lose heat by thermal emission, i.e., in a physical manner as do inanimate objects.

chapter 8

Soils

The surface of the earth on which we live was once a solid rock. Weathering processes have gradually reduced a relatively thin layer of this rock to smaller particles. As the earth continued to age, plants and animals lived and died on the surface, leaving their remains in the crust. Thus the soil, which is the outer portion of the earth's crust, is made up of both inorganic matter from rocks, and organic matter from the remains of plants and animals. During the evolutionary stages of the making of a soil, the per cent of organic matter becomes a fixed quantity unless disturbed by man through various cultural practices.

Organic matter may be estimated by incinerating oven-dried soil in a muffle furnace. The material which remains following incineration is called inorganic matter, mineral matter, or ash. This method is subject to a slight error in that some of the minerals may have been broken down and volatized during the process. Errors of this kind may be reduced by oxidizing organic matter by means of chromic acid. As a rule, however, the method of ashing soil samples as described above is sufficiently accurate for making classroom analyses, or for obtaining information required by amateur gardeners.

The Mineral Matter in the Soil

As the rock or parent material crumbles, it may undergo both physical and chemical changes. There may be fine particles which are actually minute forms of large rocks or boulders. On the other hand, material like clay differs from the original rock because it has undergone both physical and chemical changes. Inorganic particles are classified according to their size. This classification is purely arbitrary but it facilitates the study of soil types. Inorganic particles with a diameter between 2 and 0.02 mm. are called *sand;* those between 0.02 and 0.002 mm. in diameter are termed *silt;* and those whose diameter is less than 0.002 mm. fall into the category of *clay.* Sand grains are visible to the unaided eye and they feel gritty when held between thumb and fingers. Silt is barely visible to the naked eye. When held in the hand it feels like flour. Particles of clay are not distinguishable by the eye, and many of them are not visible under the

compound microscope. In other words, many of them are colloidal in size. It is the clay component that imparts stickiness to soil.

The suitability of a soil type for plant growth depends, among other things, upon its texture and structure. Texture is determined by the nature of the individual particles. Structure refers to the arrangement of the particles in the soil. Ordinarily soils are classified according to relative portions of sand, silt, or clay which account for individual textures. Listing soils in the order of decreasing proportions of sand and increasing proportions of clay, there is sand, loamy sand, sandy loam, loam, silt loam, clay loam, and clay. Contrary to popular opinion, the term *loam* does not necessarily refer to a soil which is relatively rich in organic matter. Instead, a loam is a soil the mechanical composition of which is about midway between sand and clay, and it contains such a mixture of sand, silt, and clay as to exhibit light and heavy properties in approximately equal proportions (Lyon and Buckman, 1952). Soils may be roughly classified as sand, silt, or clay by observing the feel to the fingers since they will feel respectively gritty, flourlike, or sticky. Results of the mechanical analysis of three types of soils are given in Table 11.

Table 11

CLASSIFICATION OF SOIL PARTICLES ACCORDING TO THE SYSTEM OF THE INTERNATIONAL SOCIETY OF SOIL SCIENCE, AND THE MECHANICAL ANALYSIS OF THESE SOILS*

Fraction	*Diameter (Millimeters)*	*Sandy Loam* %	*Loam* %	*Heavy Clay* %
Coarse sand . .	2 00-0.20	66 6	27.1	0 9
Fine sand	0 20-0.02	17 8	30.3	7 1
Silt	0 02-0 002	5 6	20 2	21 4
Clay .	Below 0 002	8 5	19 3	65 8

* Lyon, T. Lyttleton, and Harry O. Buckman: *The Nature and Properties of Soils*, 5th ed. New York, The Macmillan Co., 1952.

Organic Matter in the Soil

Total organic matter in the soil includes both inanimate materials and living organisms. We shall concern ourselves for the moment only with inanimate matter. As mentioned previously, organic matter in the soil is formed by plants and animals, the greater proportion being supplied by plants. Under natural conditions and in a mature soil most of the organic matter comes from decayed roots. This is the reason that soil under a grass sod contains more organic matter than that under a forest floor. The floor of the forest contains layers of leaves in various stages of decay but there is usually a sharp line of demarcation between the forest floor and the true soil. Both farmers and home gardeners are generally aware that productiveness of a soil is improved by the presence of organic matter. The higher the organic matter, the darker the color of the soil. Black muck soils in bogs and abandoned lake beds are examples of soils containing

large amounts of organic matter. In peat soils the plant remnants have been only partially decomposed so that much of the material is still recognizable as being of plant origin. In muck soils organic matter is in a later stage of decomposition.

Sandy soils and loams contain much less organic matter than peat or muck. Soils which are relatively low in organic matter are usually characterized by lighter color. Soils of coastal plains, dunes, and deserts afford examples of extremely light types, some of them containing less than one



Soils rich in organic matter are dark in color. This is a typical abandoned lake bed. (Courtesy, Bureau of Plant Industry, U.S. Dept. of Agriculture.)

per cent of organic matter. Despite this paucity of organic matter, one often sees a definite though limited amount of vegetation on many of the white sandy soils.

Decomposition of plant and animal residues in the soil continues endlessly. The ultimate products of the decomposition of these residues are carbon dioxide and water, with the mineral matters they contain being deposited in the soil. Decomposition of the organic residues is accomplished by soil microorganisms such as bacteria and fungi. It is obvious then that those conditions which are most favorable for growth of microorganisms are likewise most effective in promoting decomposition of organic matter. This means that in light soils in warm climates the organic

matter may be entirely oxidized to CO_2 and H_2O by lower organisms. Soils in tropical forests are normally not as rich in organic matter as are those in temperate zones. Organic matter accumulates in swamps and bogs because of insufficient oxygen for the growth of microorganisms which aid in the decomposition of plant and animal residues.



Soils which are low in organic matter are light in color. A few species of plants are able to maintain themselves even on sand dunes.

Soil *humus* represents a definite stage in the decomposition of soil organic matter. It has no specific chemical composition but consists of a homogeneous, amorphous, dark-colored, and practically odorless material.

Soil Profiles

The vertical cross-section of a soil in nature is spoken of as its *profile.* Opportunity to observe a soil profile may be obtained in instances where engineers have cut through a rural hillside for a railroad or a highway. At the very top will be seen the *topsoil.* It is also called the *A horizon.* It may be several inches or a foot in thickness. This soil is darker in color because of the presence of organic matter. Most of the roots of the plants growing in the soil are also found in the A horizon. Just below the topsoil is the *B horizon* or *subsoil.* It contains less organic matter and fewer roots. Topsoil and subsoil are considered the true soil. The third layer, *horizon C,* is usually referred to as the *parent material.* In this layer is found the disintegrated and disintegrating material which supplies mineral matter to topsoils and subsoils.

Down below the C horizon is found bedrock or other impervious

materials like certain types of clay. This layer constitutes the *hardpan*. Excess water from rain and snow percolates to this layer and stops. The surface of this "free" water is called the water table. The level rises and falls, depending upon climatic conditions. In good agricultural lands the

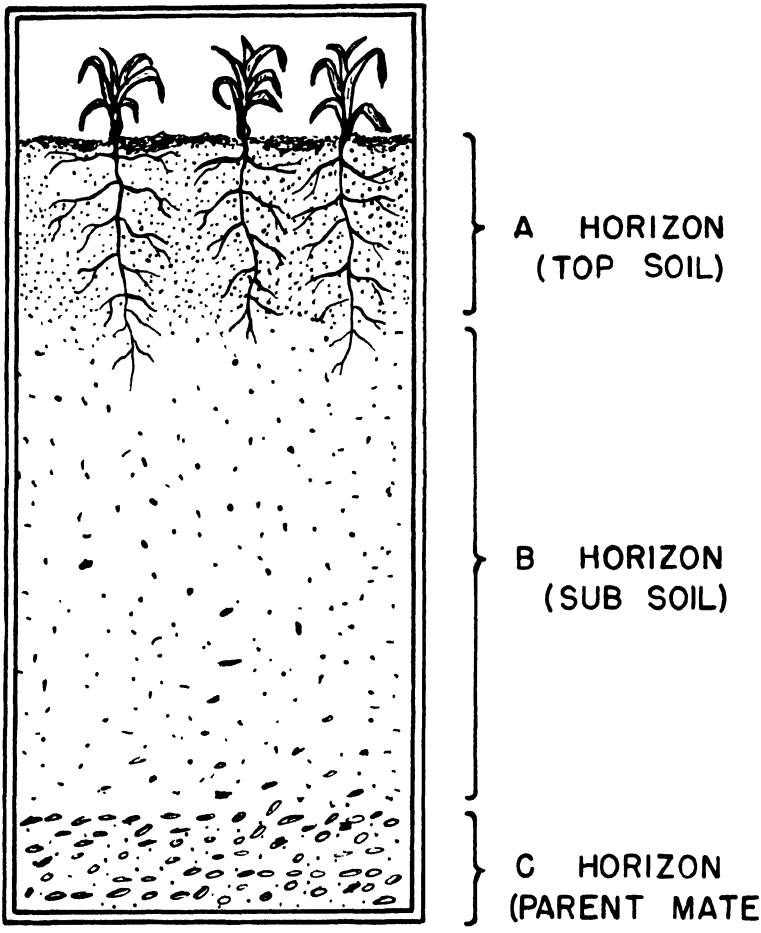


Diagram of a typical soil profile. (Redrawn, with permission, from Lyon and Buckman: *The Nature and Properties of Soils*, New York, the Macmillan Co., 1952.)

water table rarely comes near the surface of the soil. In regions where the water table is only 3 or 4 feet below the surface of the soil, it may rise during the rainy season and create a bog or swamp.

Soil Organisms

As one treads through pasture fields, woods, or garden, it is doubtful if he realizes the tremendous amount of biological activity which takes

place in the soil beneath him. Both plants (the *flora*) and animals (the *fauna*) abound there and play their own particular roles. The fauna include protozoa, nematodes, earthworms, insects, insect larvae, and burrowing species of higher animals. Among the flora are bacteria, fungi, and algae.

Animals contribute to the inanimate organic content as they die and leave their bodies. The burrowing types (earthworms, some insects, and larger animals) assist in aerating the soil. What the protozoans and nematodes contribute to the soil is not clear. The large number of protozoa present in the soil suggests that they must play an important role in underground biological activity, yet physiologists have generally ignored them. In the plant kingdom the lot of the algae is similar to that of the protozoans: ever present in the soil, but with function unknown.

The bacteria and the fungi in the soil have been the object of extensive investigations. Both types of organisms assist in the decomposition of organic matter and serve to make the soil better suited to plant life. They are very important in the production of humus. Many other types of bacteria occur in the soil, some of them with specific functions. Sulfofying bacteria serve to oxidize sulfur to sulfates. One group of nitrifying bacteria converts ammonia to nitrites, and another group oxidizes nitrites to nitrates. Nitrogen-fixing bacteria are able to utilize atmospheric nitrogen in the building up of proteins. When these organisms die, a certain percentage of the nitrogen is returned to the soil, thus enriching it. Some of these nitrogen-fixing bacteria are saprophytic, that is, they live upon dead material, obtaining their carbohydrates from plant residues in the soil. Others are parasitic, or perhaps it would be more accurate to say, symbiotic. This type enters the roots of legumes (plants bearing pods) and produces tubercles. Some of the nitrogen which is fixed by these organisms is used by their hosts and some by the organisms themselves. When the plants die or are plowed under, a certain amount of nitrogen is left to enrich the soil.

The number of bacteria in the soil is fantastic. A gram of soil may contain between 2,000,000 and 200,000,000 individual bacteria. It is now known that soil organisms, primarily bacteria, are concentrated in the immediate vicinity of the root hairs, rootlets, and roots. This area is called the *rhizosphere*. Plate counts indicate that from 5 to 50 times as many bacteria are present in the rhizosphere as are found outside this zone. This suggests that much is still to be learned regarding symbiotic relationships between soil organisms and higher plants. It also suggests that transplanting trees with "balled" earth around their roots may have other advantages besides keeping the roots intact, for it is possible that moving the soil population along with the plant may constitute an added advantage.

Soil Colloids

Colloidal matter in the soil is of considerable value to growing plants. Colloids in the soil may be either organic (humus) or inorganic (clay). Since colloids possess the capacity for imbibing water, it is apparent that they perform the important function of retaining soil moisture. This is illustrated by a comparison of farming practices in different regions. In light, sandy soils, (which are low in both humus and clay) farmers may be seen cultivating their fields immediately after, or even during, a light



Illustrating the water-holding capacity of three types of soil. Left to right: fine sand, silt loam, muck. Water retained by 100 Gm. of each soil was as follows, listed in same order: 31.4, 47.5, 99.5 Gm.

rain. In clay soils (such as are found in the limestone regions) farmers must wait until the soil crumbles before they plow. Soils of this type retain considerable quantities of moisture for several days after a rain, and they remain sticky for a long time in the early spring. Organic colloids have the capacity of retaining even greater quantities of water than the inorganic colloids. It has been found that a given amount of humus may hold nine times its weight of water.

This water-holding capacity of colloids may cause inconvenience to the farmer at times but it has its value too. It enables vegetation to survive during extended periods between rains. In the light sandy soils, farming is not practicable unless frequent rains occur during the growing season or unless the soil is irrigated.

Inorganic colloids perform another important function in soils. This consists of the retention and gradual release of minerals. (Organic matter or humus may also adsorb minerals, and may be the main source of colloidal material in some types of soils.) It was shown previously that hydrophobic colloids are stabilized by the presence of a shell of electrical charges. Clay colloids in the soil are negatively charged and therefore attract positively charged ions (cations). Thus the elements calcium, magnesium, potassium, sodium, and hydrogen are adsorbed by the colloidal micelles and are retained in the soil. The cations which are absorbed by the roots of the plants may have been those which were removed from the clay colloids. This process is referred to as *base exchange*. More will be said about this subject during the discussion of mineral nutrition. Suffice it to say that the clay colloids serve to prevent the minerals from being washed down with the rains. It explains why light, sandy soils require so much more fertilizer than the heavier soils.

Soil Reclamation

Truog (1951) has said that 1,000 years are necessary to produce an inch of average topsoil. It has been said by others that nations go through three stages in farming their lands: exploitation, conservation, and rebuilding what has been lost. In our own country we are in the midst of the second stage, although many individuals, including home gardeners, seem to be attempting to rebuild their soils. The need for conservation should be apparent when it is realized that continued cultivation of the land will gradually reduce organic content of the soil, and that plowing under of cover crops serves only to replace what has been lost through cultivation. It is important, of course, to add organic matter by means of approved methods of conservation, but it would require a life time or two to restore to topsoil the organic matter which has been squandered through years of careless cultivation. A plot of ground which has been cropped to the point of being capable only of marginal agriculture had best be planted in pasture and permitted to lie fallow for several years.

Air and Water in the Soil

Thus far we have discussed only the solid matter in the soil. We must now consider the liquid and gaseous phases of matter because air and water constitute major requirements for the roots of plants. These two phases may be discussed concurrently because the pores in the soil are filled with either air or water, depending upon the degree of saturation of the soil.

The soil moisture is classified in accordance with the manner in which it is held in the soil. The extent of its availability is also indicated by its type. (See Table 12.)

Table 12

INTERRELATIONSHIPS AMONG CERTAIN SOIL MOISTURE CONSTANTS (PERCENTAGES) OF A GROUP OF SOILS*

Moisture Constant	*Clay*	*Loam*	*Silt Loam*	*Sandy Loam*	*Fine Sand*
Moisture equivalent	28.4	21.7	16 1	9 5	3 2
Permanent wilting percentage	13 4	10 3	7.5	2 9	1 0
Growth-water storage capacity	15 0	11 4	8 6	6 6	2 2

* Daubenmire, R. F.: "Plants and Environment. Data of Veihmeyer, F. J. Evaporation from soils and transpiration." *Am. Geophys. Union Trans.*, **19**:612–619, 1938.

Important types of soil moisture are (1) hygroscopic, (2) capillary, and (3) gravitational moisture.

Hygroscopic Moisture. If a sample of soil were spread out on a table in the laboratory, this soil would dry down to a point at which the moisture content would be in equilibrium with that of the air. It would then be considered an *air-dry* sample of soil. That it would still contain a certain amount of water may be demonstrated by heating it for 24 hours in an oven at 105°C. It would still lose weight. The moisture which passes off during oven drying of an air-dry sample is called *hygroscopic* moisture. The layman's term for air-dry loam soil is *dust*. Hygroscopic moisture is held on the surfaces of colloidal particles. It is retained so tenaciously by the soil that it is unavailable to plants.

Capillary Moisture. As indicated by its name, this type of moisture is held in the capillary pores of the soil. When a soil contains its maximum quantity of capillary moisture it is said to be at *field capacity*. This condition exists when all of the capillary pores have been filled with water and the air in these pores has been displaced. It is the capillary water upon which plants draw for their continual supply of moisture.

Gravitational Moisture. A thoroughly saturated soil contains gravitational water in addition to capillary and hygroscopic moisture. This type of water takes its name from the fact that it moves through the soil by gravity; hence it moves only in a downward direction. Gravitational water occupies the noncapillary pores. Many a schoolboy, walking along the road following a heavy summer rain, must have wondered why the earthworms have all come out. Their dwellings have been flooded with water and they have come up to the surface to prevent becoming water-logged. The roots of plants are not as fortunate, and if exposed to gravitational water continuously, they will soon die. Fortunately, in well-drained soils, gravitational water moves downward in three or four days.

Gravitational water differs from the other two types in that it actually exerts pressure in the soil. Capillary and hygroscopic moisture, on the other hand, are present in the soil in a state of tension. Also, whereas gravitational water moves downward only, capillary water may move in any direction, and hygroscopic water rarely moves at all.

Quantitative Determinations of Soil Moisture

It is often desirable to determine the actual amount of moisture in a soil or more particularly to determine the quantity of available moisture present at a given time. In addition to the three kinds of soil moisture previously described, there might be added several expressions which relate to the "physiological significance" of the quantity of water present. Some of these terms, which are of primary interest to plant physiologists, are *maximum water-holding capacity*, *field capacity*, and *wilting coefficient*.

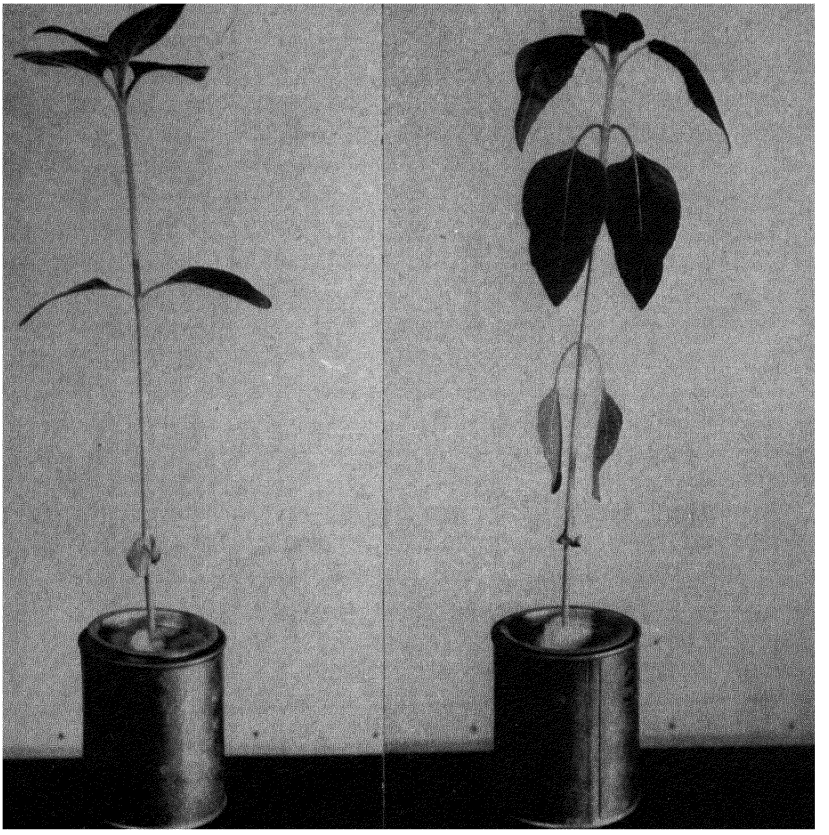
Total Moisture. In soil analysis it is customary to determine total moisture by drying the sample of soil for 24 hours at 105°C. The loss of weight represents the total amount of moisture which was present when the sample was collected. If the soil were saturated then all three types of water would be removed. This determination is of value only in determining the dry weight of soil. This is important because all percentages are based on dry weight, and not on wet weight.

Field Capacity. This is the quantity of water held by the soil after all of the gravitational water has drained away. It represents the maximum amount of capillary water which a soil is capable of holding. The standard method of determining field capacity consists of collecting a sample two or three days after a rain and drying it down to equilibrium at room temperature.

Maximum Water-holding Capacity. This value is obtained entirely by a laboratory determination. A sample is placed in a shallow metal container with perforations in the bottom and allowed to stand in water until the soil becomes saturated. Surplus water is drained off and the amount of water retained is determined by drying and weighing. It is apparent that this value is not a measure of the water held under field conditions. The reason for this is that soil is usually pulverized before testing. Also the drainage of a sample of soil in the laboratory is certainly different from that of the sample in its natural environment. However, it affords a means of obtaining quantitative comparative values for groups of soils and has been found to be generally satisfactory for experimental work.

Wilting Coefficient. This quantity is of considerable importance from the standpoint of the growing plant. It is expressed as the amount of water present (per cent of dry weight) when a test plant becomes permanently wilted. It is said to be permanently wilted if it will not recover its turgor when placed in a moist chamber overnight. It has been found that the wilting coefficient is a characteristic of the soil and not of the plant which is being employed in the test. The type of plant is therefore not important. The plant which is quite commonly employed for this test is the sunflower (*Helianthus annuus*). Seedlings are transplanted to a tin can with a top like that on a baking powder can. Soil is placed in the

can and the seedling is inserted through a hole in the lid. The pot is kept watered and the plant is grown until it is about six inches high. Watering is then stopped and the plant is observed until the lower leaves show definite signs of wilting. At this stage the plant is transferred to a moist chamber (100% relative humidity). If the plant fails to recover during



Physiologists may determine wilting coefficient of soil with the aid of sunflower seedlings. Left: normal sunflower plant. Right: sunflower plant at the point of permanent wilting. (Courtesy, Bureau of Plant Industry, U.S. Dept. of Agriculture.)

the night this is considered the permanent wilting point. The per cent of moisture present at this time is reported as the wilting percentage or the wilting coefficient.

Moisture Equivalent. Briggs and McLane introduced this term to indicate the per cent of water remaining in a soil after having been subjected to a centrifugal force 1,000 times that of gravity. Briggs and Shantz

developed a formula for converting the moisture equivalent into the wilting coefficient. The formula is as follows:

$$\text{Wilting coefficient} = \frac{\text{moisture equivalent}}{1.84 \pm 0.013}$$

This method is not quite as cumbersome as the one employing a test plant and lends itself readily to routine analyses.

Significance of the Water Content of the Soil

So far as the well-being of the growing plant is concerned, it is not the total amount of moisture that is important but rather the amount of available moisture which is present. Gravitational water is readily available to the roots but this type of water does not normally remain long in the root zone. It is therefore the capillary moisture which is of most importance, and the condition of the plant is not seriously affected until capillary water is exhausted. The concern of the farmer or the gardener during a drought, therefore, is with the degree to which the soil moisture is approaching the wilting coefficient. For instance, at field capacity a clay soil will contain more available moisture than a sandy soil. The wilting percentage in a clay soil, however, will also be higher than that in a sandy soil, so that during severe droughts the plants on heavier soils may start wilting before those growing on lighter soils. These statements are illustrated in Table 12.

Attempts have been made to apply the principle of relative conductivity of wet and dry soils in determining the moisture content. Field and soil conditions vary so greatly, however, that no one kind of instrument is suitable for all types of soils.

chapter 9

Plant Pigments

Nature has endowed members of the plant kingdom with a variety of colors. Green, yellow, red, blue, and purple pigments may be found in flowers, fruits, vegetables, and in different organs of growing plants. As the result of natural coloration certain plants appear attractive to man and lower animals. The questions arise as to whether pigments perform any particular function in the living plant, or whether they are of any economic importance to man. It is hoped that questions of the above type will be answered in this chapter after we have given consideration to the characteristics of the common pigments. We shall restrict the discussion to a consideration of pigmentation in green plants, because a study of the color of plants like bacteria and fungi more properly belongs in a more advanced treatise.

For convenience plant pigments can be classified according to their solubilities, and according to the manner in which they occur in the plant. The pigments will therefore be discussed under the following classifications:

I. Ether-soluble or plastid pigments
   A. Chlorophylls (green)
   B. Carotenoids (orange)
      1. Carotenes
      2. Xanthophylls
II. Water-soluble or vacuolar pigments
   A. Anthocyanins (red, blue, purple)
   B. Anthoxanthins (yellow)

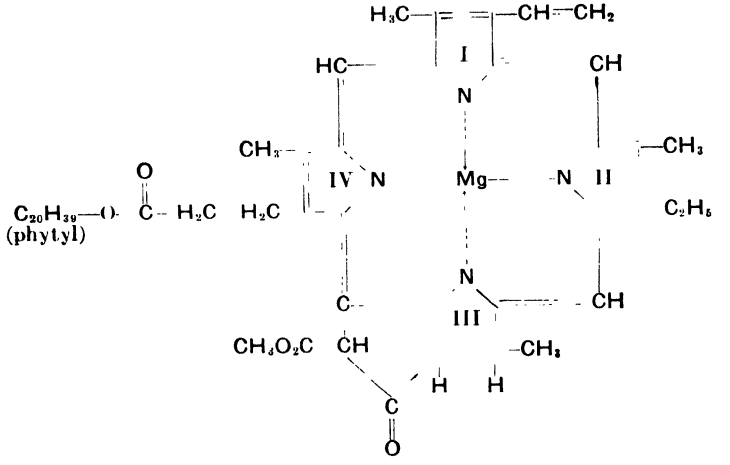
Chlorophylls

The bright green color in leaves, shoots, and succulent stems is produced by one or more of the *chlorophylls*. These green pigments occur in the chloroplasts, which are saucer-shaped inclusions in the cytoplasm. A microscopic examination shows that chlorophyll particles (*grana*) are imbedded in an amorphous matrix. The matrix consists of proteins and lipids.

As indicated in the outline, chlorophylls are soluble in ether. Because of the water present in succulent green leaves, the plastid pigments must

first be extracted with acetone. The pigments may then be transferred to ethyl ether or petroleum ether.

The green pigments in higher plants consist of chlorophylls *a* and *b*. The quantity of chlorophyll *a* is three times that of chlorophyll *b*. Willstätter analyzed over 200 species of plants and found the same ratio of *b* to *a* in all of them. The empirical formula for chlorophyll *a* is $C_{55}H_{72}O_5N_4Mg$; for chlorophyll *b* it is $C_{55}H_{70}O_6N_4Mg$. Structurally both molecules consist of four pyrrole rings with a molecule of magnesium in the center. Chlorophyll *b* differs from *a* structurally in having a *CHO* group in place of a CH_3 radical. Both are methyl-phytyl esters of a complex acid called chlorophyllin. Since chlorophylls are esters they may be saponified by



Chlorophyll A

Fischer's structural formula for chlorophyll A.

treatment with an alkali, like potassium hydroxide. The resulting product, potassium chlorophyllide, is soluble in water. Saponification is employed in order to separate the chlorophylls from the other ether-soluble pigments.

One of the most satisfactory methods for separating chlorophyll *a* from chlorophyll *b* is chromatographic analysis. This method was described in the discussion of adsorption. A concentrated, water-free, petroleum-ether solution of the two chlorophylls is filtered through a chromatographic column of confectioner's sugar under suction. If the tube has been properly packed, chlorophyll *a* will separate out on the column as a blue-green ring, and the chlorophyll *b* will appear as a pure green ring.

Treatment of chlorophyll with an acid results in displacement of the magnesium in the molecule by the hydrogen of the acid. The end product is a dull or muddy green color. When leafy vegetables lose their bright

green color during cooking, it is generally because natural plant acids have broken down the chlorophyll. Cooking vegetables with a pinch of soda prevents this discoloration, but it also accelerates destruction of vitamin C.

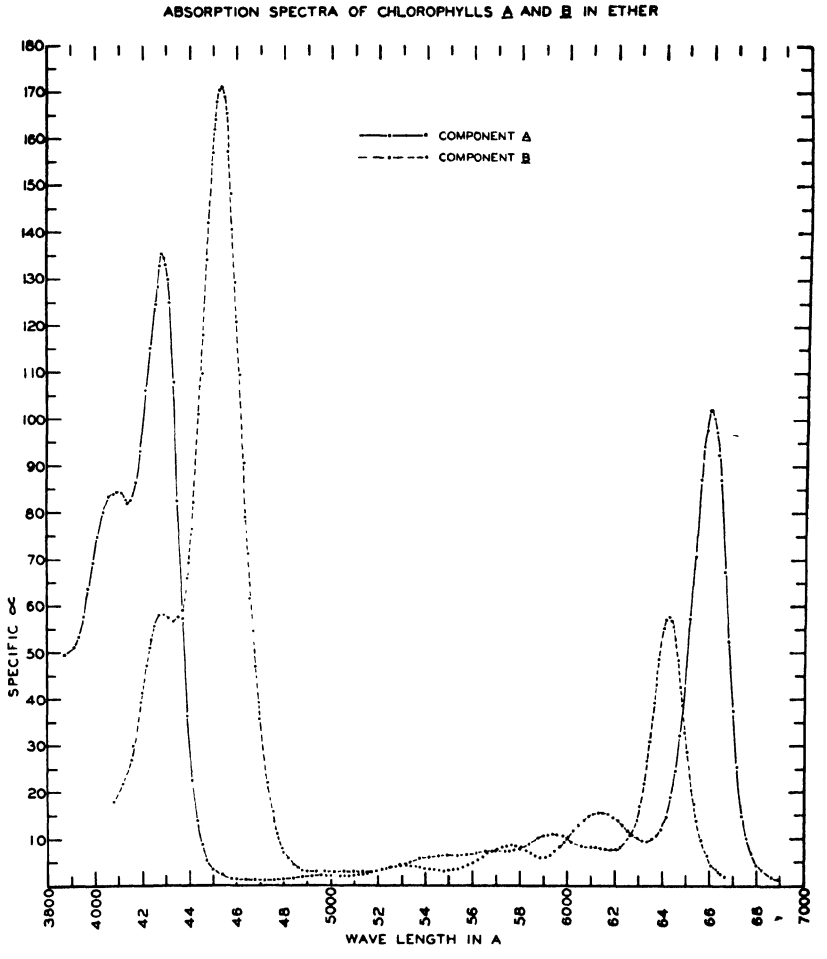
The magnesium in the chlorophyll molecule may also be replaced by copper, iron, or zinc. When copper is substituted for magnesium in the molecule, the color becomes a brighter green and is more stable. This principle is utilized in the common practice of preserving green leaves for subsequent use in the laboratory or classroom. The process consists of boiling the leaves in a mixture of acetic acid and copper acetate. The preserved leaves retain their color because copper has replaced magnesium in the chlorophyll.

Some years ago government officials seized a large shipment of canned peas which were being imported into the United States because the color of the product seemed too green to be natural. Chemical analyses showed that the peas had previously been treated with copper to enhance the green color. Introducing copper in place of magnesium in the chlorophyll molecule may have enhanced the appearance of the peas, but it also rendered them unfit for food.

Solutions of chlorophylls possess very definite characteristics in regard to specific wavelengths of light. Chlorophyll is green by transmitted light, that is, when a flask of dissolved chlorophyll is held between the observer and a source of white light, the color of the solution appears dark green. If the observer were to turn his back to the light and observe the solution by reflected light, the chlorophyll then would appear red in color. The property of re-radiating light of longer wavelength than that of light falling on a substance is termed *fluorescence*. Detailed studies have been made of the per cent of light of various wavelengths which is absorbed by solutions of chlorophyll. It will be recalled that the various colors of the rainbow, that is, the individual components of white light, are designated by their wavelengths. Thus the waves of blue light range between 430 and 470 millimicrons (a millimicron is one-millionth of a millimeter). Green light is represented by 500 to 560, and red light by 650 to 760 millimicrons. By the use of a spectrophotometer, light in narrow bands of wavelengths is directed upon colored solutions and the per cent of light absorbed at each wavelength is measured. Studies of this type have shown that both chlorophylls *a* and *b* exhibit a maximum absorption of light in the blue-violet and a secondary maximum in the short red regions. The significance of the absorption spectra of chlorophyll will be discussed under the topic of photosynthesis in chapter 10.

For the most part light is essential for the formation of chlorophyll in plants. When seeds are germinated in the dark, the color of the seedling becomes yellow rather than the characteristic green of normal seedlings. When boards or other articles have covered a green lawn for a week or

ten days, the grass under the opaque covering will have lost its chlorophyll. The speed with which chlorophyll appears in dark-grown seedlings after they have been removed to light is quite remarkable. In experiments in which germinating seeds are kept in the dark and then removed



Absorption spectra of chlorophylls A and B in ether. Wavelengths are expressed in angstroms (one-tenth of a millimicron). (Courtesy, Zscheile and Comar: "Influence of Preparation Procedure on the Purity of Chlorophyll Components as Shown by Absorption Spectra," *Botan. Gaz.* **102**:463–481, 1941.)

to the light for measurement, the seedlings will be observed to become green during the short time required to measure roots and shoots of 25 seedlings.

The statement that light is essential for formation of chlorophyll must be qualified because there are a few plants in which this is not true. For

example, chlorophyll will form in the dark in algae, ferns, mosses, and conifer seedlings. The same thing has been observed in the seedlings of at least two types of higher plants—water lotus and citrus seedlings. Cotyledons of citrus seedlings which have been grown in the dark contain chlorophyll, and it is a common experience to find germinating seeds with green cotyledons within the fruits of the grapefruit rather late in the season.

Physiological Significance of Chlorophylls

It is hardly an exaggeration to say that chlorophyll is the most important organic compound on this earth. As will be discussed in the next chapter on photosynthesis, it is this pigment which enables the green plant to utilize the energy of the sun in manufacturing carbohydrates from water and CO_2. All living organisms, with the exception of a few forms of bacteria, are directly or indirectly dependent upon chlorophyll for their existence.

A certain similarity exists between the chlorophyll of plants and the hemoglobin of the blood of animals. Hemoglobin consists of a chromophore, *hematin,* and a protein, *globin.* Hematin has a ring structure very much like chlorophyll, except that the metallic element is iron instead of magnesium. Both chlorophyll and hemoglobin may be broken down chemically to an identical compound, which is called *porphyrin.*

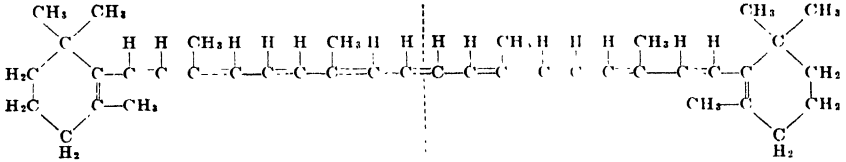
The blood of cephalopods, like squids and octopuses, and of crustaceans, like clams and oysters, does not contain hemoglobin. The corresponding pigment in the blood of these marine animals consists of a chromoprotein which is known as *hemacyanin.* This too has a chemical structure similar to that of chlorophyll. However, the metallic molecule in the center of the four pyrrole rings is copper, as distinct from magnesium in chlorophyll, and iron in the blood of mammals. Hemacyanin is not red, but blue. These two groups of animals can, therefore, be said to be the true "blue bloods."

Other Chlorophylls

Several other chlorophylls have been isolated from plants. These are chlorophylls *c,* and *d,* and *bacteriochlorophyll.* Sorby first observed chlorophyll *c* in 1873 and for a time several investigators considered this pigment to be an artifact. In 1942 Strain et al. confirmed the existence of chlorophyll *c* in nature. It has been isolated from diatoms and algae. Chlorophyll *c* is also called *chlorofucine.* The fourth chlorophyll, chlorophyll *d,* was reported by Manning and Strain in 1943. It was isolated from red algae. Since chlorophylls *b* and *c* are not present in the red algae, chlorophyll *d* may be prepared in pure form from these plants by means of chromotographic analysis. Chlorophyll *d* appears above chlorophyll *a* on the chromatogram. Bacteriochlorophyll, as its name suggests, is present in certain types of bacteria.

Carotenoid Pigments

As indicated in the outline, yellow, ether-soluble pigments are called *carotenoids*. They are present in all chloroplasts but are rendered invisible by the presence of chlorophylls. As chlorophylls in a green plant are destroyed, the plant assumes a yellow or orange color. A plastid which contains only carotenoid pigments is called a chromoplast. Carotenoids in leaves of deciduous trees become prominent in the fall when chlorophylls are being destroyed more rapidly than they are being manufactured. The color of many fruits undergoes a change from green to yellow during ripening. The processes are similar to those associated with yellowing of green leaves. Yellow color of seedlings which have been grown in the absence of light is an indication that carotenoids, but not chlorophylls, have developed in the plants.



β-Carotene (Symmetrical β,β Carotene)

Structural formula for beta-carotene, one of the many ether-soluble, yellow pigments.

Most yellow fruits and vegetables, and many yellow flowers derive their characteristic color from the presence of carotenoid pigments. Pumpkins, squash, sweet potatoes, and yellow varieties of Indian corn afford additional examples of foods which are relatively rich in carotenoid pigments.

Chemically the carotenoid pigments which occur in plants may be divided into two groups—*carotenes* and *xanthophylls*. Carotenes are long-chain hydrocarbon compounds whereas xanthophylls are alcohols. A few of the carotenoid pigments consist of acids and esters, but we shall confine this discussion to carotenes and xanthophylls because they are by far the most abundant in plants. The empirical formula for carotenes is $C_{40}H_{56}$ and for xanthophylls it is $C_{40}H_{56}O_2$.

The chemical formula for the xanthophylls suggests that they might be oxidation products of the carotenes. Chemists have not succeeded in converting carotene to xanthophyll in the laboratory but this does not preclude the possibility that this reaction may occur in the plant cell. Since the quantity of xanthophylls in the leaf is usually twice that of carotenes, it has been assumed that carotenes are converted to xanthophylls as fast as they are formed.

Four isomeric carotenes are found in plants: *alpha*, *beta*, and *gamma carotenes*, and *lycopene*. The carotene content of green leaves consists of

90% beta carotene and 10% alpha carotene. Gamma carotene is not so widely distributed. It is found in carrot roots and in marsh dodder (*Cuscuta salina*). Lycopene is the principal pigment in ripe tomatoes. It is also found in rose hips and is responsible for the color of the flesh of the watermelon and pink grapefruits.

The three carotenes are converted into vitamin A in the animal body. For this reason these particular pigments are called vitamin A precursors. Beta carotene is converted into two molecules of vitamin A, whereas alpha and gamma carotenes each yield but one molecule of the vitamin. Lycopene possesses no vitamin A potency. Only the precursors of this vitamin occur in plants.

It was once believed that the xanthophyll content of a leaf consisted of a single compound. It is now known that xanthophylls represent a group of isomeric compounds and the term is always used collectively. It was reported by Strain, for example, that twelve different xanthophylls occur in green leaves. The principal one of these is *lutein*. Another, *zeaxanthin*, is present in leaves to some extent and is rather abundant in maize, or Indian corn. *Violaxanthin* is the carotenoid pigment which produces the yellow color in pansies. *Cryptoxanthin* occurs in maize and in the peel of the orange. The last-mentioned pigment is the only xanthophyll thus far discovered which possesses intrinsic vitamin A potency.

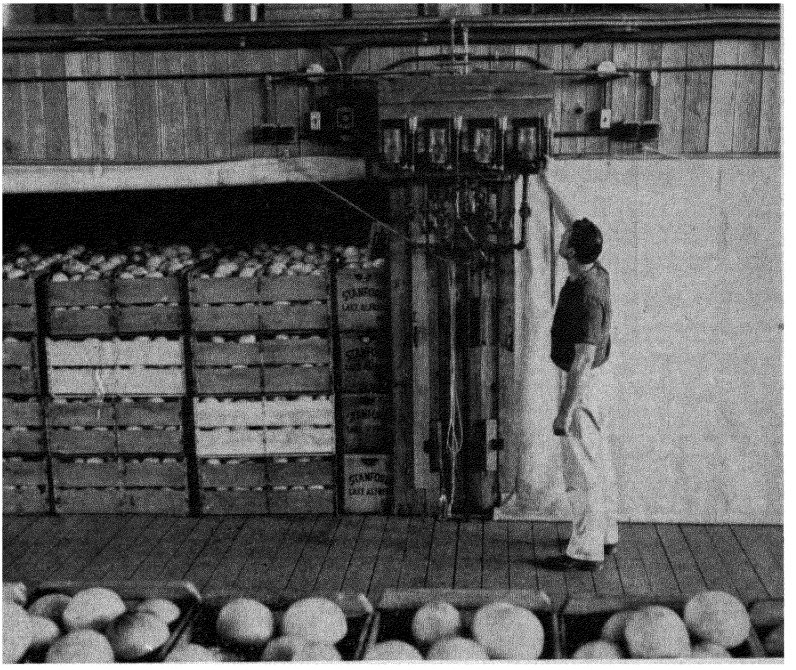
Plastid Pigments in General

The green and yellow pigments have figured in the economy of man in more ways than might at first seem apparent. Sometimes it becomes desirable to remove chlorophyll from a plant and at other times it is desirable to have the plant retain its chlorophyll content. Oranges which mature in the early fall before the arrival of cool weather may remain green after the flesh has become legally mature. Citrus growers have found that they can induce the fruits to lose their green color by treating them with ethylene gas. It does not ripen the fruits, it merely colors them. Ethylene may also be applied to mature green tomatoes and in this instance it both colors and ripens the fruits. The exact manner in which this gas acts on the physiology of these and other fruits is not known. Limes, as distinct from oranges, grapefruit, and lemons, are usually marketed in the green stage and the merchant dislikes to have these fruits lose their green color. Leafy vegetables, which have retained their green color after harvest, are both more attractive and more nutritious than those which have lost much of their original color.

Problems for the physiologist arise also in the field of the carotenoid pigments. We stated that oranges produced in the warmer areas of the citrus belts tend to retain their green color. Once the green pigments have been broken down, either naturally or artificially, the oranges may still not be as attractive as the merchant might wish them to be. Their color

is usually yellow rather than orange. At the present time the lighter-colored fruits are treated with a dye in order to impart a more attractive color to the rind. No method has been developed for inducing the oranges themselves to produce more orange pigments. Mandarin oranges (Temple, tangerine, King) are characterized by a deep orange color which is not affected by environment to the same extent as is true of the above-mentioned pale-yellow round oranges.

Mammals have a tendency to accumulate the carotenes, whereas birds retain the xanthophylls from their ingested food. This means that fats of



Treating early grapefruit with ethylene gas (C_2H_4) to hasten destruction of chlorophyll in the rinds of the fruits.

mammals, if yellow in color, can be assumed to contain vitamin A. Butter fat is a good example of this point. Furthermore, more carotene is absorbed by mammals if the pigment is ingested with fats. Thus the practice of cooking green leafy vegetables, which are rich in carotene, with bacon or the custom of adding butter to spinach may serve not only to make these vegetables more palatable but also to insure a greater retention of carotene, the vitamin A precursor.

That birds tend to accumulate xanthophylls rather than carotenes has been demonstrated in experiments with canary birds. When the yellow pigment in their diet is restricted to carotenes, the birds lose their yellow

color and appear more like English sparrows. Adding xanthophylls to the diet of these decolorized canaries soon restores the yellow color to their feathers.

Physiological Role of Carotenoids in Plants

Very little is known regarding the role of carotenoid pigments in plants. The fact that chloroplasts contain both green and yellow pigments suggests that the carotenoids play an important part in photosynthesis, but this has never been proved. Beta carotene absorbs light only in the blue-violet portion of the spectrum and it is possible that carotenoids serve to supplement the action of chlorophylls in the photosynthetic process. At least one photochemical reaction is known to be sensitized by carotenoids. This is the system in green plants which inactivates indoleacetic acid, a growth-promoting substance. When extracts of green plants are placed in the sunlight, the indoleacetic acid is destroyed, and the presence of carotenoid pigments tends to accelerate the reaction.

Claims have been made that carotenoids play a part in the reproduction of plants. These claims are based upon experiments in which it was found that the flowering of certain plants was accompanied by increase in the content of carotenoid pigments. But this too has not been verified by all workers, and the function of carotenoids in plants still remains a mystery for the most part.

Water-soluble Pigments

The water-soluble pigments are present in the vacuolar sap of plants. *Anthocyanins* account for the red, blue, and purple colors in flowers, fruits, and vegetables. *Anthoxanthins* produce only pale yellow colors in fruits and flowers, and are therefore not as conspicuous as the anthocyanins. Often the anthoxanthins occur in colorless form, as in white flowers. These colorless anthoxanthins may be converted to the yellow form by treatment with alkalies. This phenomenon may be observed by exposing the *albedo* (white inner peel) tissue of citrus fruits to the fumes of ammonia. The white tissue immediately turns to a deep yellow.

Anthocyanins

One of the interesting characteristics of anthocyanins is that they react as indicators in the presence of acids and alkalies. If no interfering substances are present, the anthocyanin will appear bluish red in acid, violet in neutral solution, and blue in alkaline solution. The red stain of a fresh strawberry may be turned blue by treating the spot with ammonia. Children of Yorkshire, England, it is said, used to change the color of bluebells to red by placing them in anthills where formic acid acted on the pigment. When only one anthocyanin is present in a flower, the shade

of color serves as an index of the relative acidity of the sap. This is demonstrated in the red rose (acid), the bordeaux-red cornflower (neutral), and the blue cornflower (alkaline). Sometimes a red flower turns green when treated with alkali. This transformation usually indicates that anthoxanthins are present in large quantities, and, as these turn yellow in alkali and the anthocyanins turn blue, the resulting mixture is green. The color of flowers may be the result of mixtures of several anthocyanins, of anthocyanins and tannins, or of carotenoids and anthocyanins.

Anthoxanthins

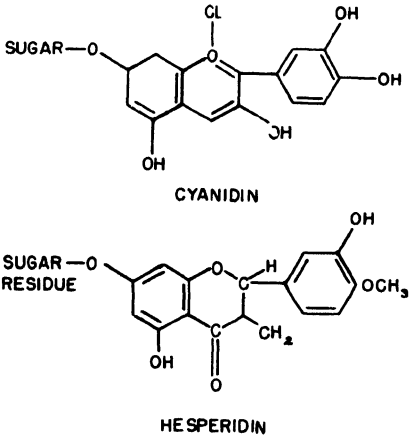
The water-soluble yellow pigments are often overlooked because they may be concealed by the anthocyanins or carotenoids, or by both, and because the color of anthoxanthins as a rule is not as intense as that of the other pigments. Nevertheless these substances are more widely distributed in different plants or in different parts of plants than are the anthocyanins, and it has been said that nature provides more variety among the anthoxanthins than among the red, blue, and purple pigments. Anthoxanthins have a limited though definite utility as dyestuffs, and some of them constitute the oldest known dyes. *Quercitrin*, a typical anthoxanthin, is extracted from the inner bark of the yellow-barked or black oak (*Quercus velutina*). This dye was introduced in 1775. Another anthoxanthin, *luteolin*, is found in a herbaceous plant, the European mignonette or weld (*Reseda luteola*), and is said to have been used for its yellow dye by some of the central European nations during the time of Julius Caesar. These substances possess a capacity for formation of lakes (pigmented precipitates) with certain metallic oxides, forming bright-colored, fast dyes. Other rich sources of these water-soluble pigments are tea leaves, the horse chestnut, the bark of apple trees, the wood of sumac (*Rhus sp.*), and the wood of osage orange (*Maclura pomifera*).

Chemistry of the Water-soluble Pigments

Anthocyanins and anthoxanthins are similar in chemical structure. Both are glycosides. This term is applied to compounds containing one or more sugar molecules and a nonsugar (aglycone) molecule. The nonsugar portion accounts for the color of the pigment. When glycosides are boiled with hydrochloric acid the sugar molecules are split off and the color of the solution is usually intensified.

The nonsugar or aglycone portion of these pigments consists of one pyrone and two benzene rings. Thus they are constructed from flavones, flavonols, or flavonones. Once they were called *flavone pigments* for this reason. The chemistry of the anthoxanthins was understood before much was known of the structure of the anthocyanins. Anthoxanthins have been converted to anthocyanins in the laboratory, and it is believed that this transformation occurs in nature. Thus far no experiments have been

reported which demonstrate the ability of the plant to bring about this conversion of anthoxanthins to anthocyanins, or vice versa. One observation suggests that this may occur in nature with the proper stimulus. In yellow-colored apples, like Grimes Golden or Yellow Newtown, excessive applications of copper sprays produce a type of red-spotting on the yellow peel. Treatment of these spots with alkali changes them to green, obviously indicating a mixture of blue and yellow pigments, which



Structural formulas of cyanidin, a water-soluble pigment and hesperidin, a glycoside found in oranges. Note close relationship.

in turn suggests that the spray injury caused a change from anthoxanthins to anthocyanins.

Significance of Water-soluble Pigments to Plants and to Man

Physiologists have pondered for a long time on the possible physiological significance of water-soluble pigments to plants. It has been suggested that the bright colors of flowers serve to attract insects, thus insuring pollination. This does not explain the need for coloring in fruits, however, unless it makes them attractive to animals and thus indirectly assists in the dissemination of seeds. Attempted explanations, however, suggest teleogical reasoning, that is, we tend to attach a motive to plants.

One observation might be cited here and offered for what it is worth. A dash of red color in the crown of a pineapple plant is evidence that it is shifting from the vegetative to reproductive type of growth, and that soon a flower will be produced. This phenomenon occurs prior to both natural and artificially induced flowering. The pineapple plant represents one of the few types of plants which can be caused to bloom by chemical treatment. (See chapter 19 on Growth-Modifying Substances.) A week or ten days after the pineapple plants have been treated to induce blooming, the grower usually looks for the red color at the base of the leaves making up the crown in order to determine the extent to which his treatment has been effective. With this type of plant at least, the formation of antho-

cyanin pigments precedes the development of flowers. It has been recently reported that development of anthocyanin pigments also followed the pollination of an orchid. In this connection it might be mentioned that in the late winter in temperate zones, the twigs of certain woody species of plants take on a yellow and some a red color as if the water-soluble pigments were being concentrated prior to the opening of the buds on these trees and shrubs.

It is much simpler to find examples of the usefulness of anthocyanin pigments to man than it was to determine their usefulness to the plant. The attractive colors of flowers certainly are of economic value to the florist. In like manner the sales value of fruits is enhanced by attractive color. The merchant knows that in red varieties of apples, the fruits with three-fourths red and one-fourth green color will outsell those in which there is more green than red. The horticulturist knows that poor coloration in varieties of apples which are normally red indicates that the fruits have not received an adequate amount of sunlight while growing. John M. Arthur found that development of red color in McIntosh and other varieties of red apples was accelerated by ultraviolet light. The effective region of the spectrum lay between 312 and 290 millimicrons. These wavelengths are filtered out by ordinary window glass. Ultraviolet light of shorter wavelength than 290 millimicrons killed the apple tissue and prevented formation of color.

Zilva reported that the flesh beneath the red side of an apple contained more vitamin C than that beneath the green or yellow peel. This does not necessarily indicate that anthocyanin pigments are always associated with vitamin C, but rather that both anthocyanins and vitamin C in the apple are produced abundantly in sunlight. Similar results in regard to vitamin C have been reported for citrus fruits.

Many years ago commercial canners experienced some difficulty in producing a satisfactory product with respect to fruits like blackberries and raspberries. It seems that in time the cans would start leaking and it was assumed that natural acids in the fruits were etching and dissolving the metal. Careful study indicated that anthocyanin pigments, and not acids, were causing the trouble. It was found that anthocyanin pigments were forming insoluble compounds with the metal of the cans and gradually eating away the walls. This was corrected by coating the inner walls of the cans with lacquer. Treating of cans in this manner not only solved the problem of the canners but it also reduced the chances of contamination of foods with metals from the walls of the cans.

During World War II another difficulty was experienced which concerned anthocyanin pigments. The problem arose as the result of substituting glucose for sucrose in *quick-freezing* fresh strawberries. It is necessary to add large quantities of sugar to acid fruits when preserving them by freezing. Glucose was much more plentiful than sucrose under war-

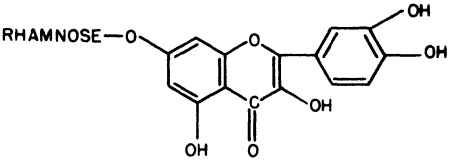
time economy. It was found, however, that when strawberries were frozen with glucose instead of sucrose, the frozen product assumed a purple color and not a typical characteristic red. This purple color was caused by a combination between the anthocyanin pigments and glucose. No other sugar which was tested produced these results. Normal color returned upon thawing the product, but since this type of product is sold in the frozen state, it was realized that glucose could not be substituted for sucrose in the process.

Variation in the color of hydrangea (*Hydrangea macrophylla*) was not entirely explained for a long time. It was common knowledge that the flowers were blue when the plant was grown in acid soil, and that they were pink when the soil was nearly neutral or alkaline. This could not be explained on the basis of the effect of acids and alkalies on anthocyanin pigments, because the color changes are just the reverse of what might be expected. Allen in 1943 found an explanation by showing that the color of the flower was dependent upon its aluminum content and that the effect of soil acidity was indirect. In an acid soil aluminum goes into solution and becomes available to the plants. Allen reported that blue flowers in sand cultures contained more than 250 p.p.m. of aluminum, mauve flowers (midway between pink and blue) contained 150 to 250 p.p.m., and pink flowers contained less than 150 p.p.m. of aluminum. Bright blue flowers from the field contained 800 to 900 p.p.m. of aluminum. It was discovered that color of the flowers could also be controlled by spraying with a 0.5% solution of aluminum ammonium citrate. When grown in nutrient solutions, pink flowers were produced on a solution containing 1.34 p.p.m. or less of aluminum, mauve with 13.4 p.p.m., and blue with a nutrient solution containing 134 p.p.m. of aluminum.

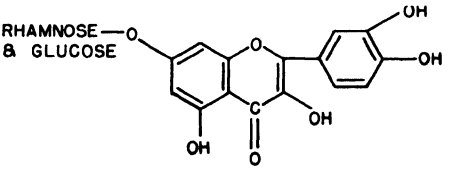
The importance of anthoxanthin pigments to man and to plants themselves is not readily apparent and not well understood. We have already recounted the part played by these yellow, water-soluble pigments in the dye industry of former years (before the era of synthetic dyestuffs). Let us now consider the possible physiological significance of these pigments either to plants or to man. The fact that anthoxanthin pigments occur in practically every living plant cell suggests that they may play an important role in the functions of the cell, although this role is unknown at present. Many of them are combined with molecules of sugar to form glycosides. These compounds generally have a bitter taste, and the bitterness becomes more pronounced when the sugar molecule is split off by hydrolysis. An example of this type of compound is *naringin*, the substance which accounts for bitterness in immature grapefruit.

One outstanding example of a water-soluble yellow pigment which is of physiological significance to man may be cited by relating the story of *rutin*, sometimes called *vitamin P*. In 1936 a Hungarian biochemist, Szent-Györgi, accidentally discovered in citrus fruits and red peppers a sub-

stance which possessed peculiar physiological properties. This strange substance served to restore weakened capillary blood vessels in animals. It was useful in reducing susceptibility to hemorrhage. Szent-Györgi announced that this new compound was *hesperidin*, the glycoside which produces the bitter flavor in oranges and which is similar to naringin in grapefruits. Further research, however, indicated that vitamin P activity of the extract was due to an impurity which was associated with hesperidin. Szent-Györgi and his associates next prepared a concentrate containing *eriodictin* (eriodictyol glycoside) which they believed to be the



QUERCETRIN



RUTIN

Structural formulas of quercetrin, a water-soluble pigment, and rutin (Vitamin P). Note similarity in structure.

active principle. By this time research workers in various parts of the world became interested in the problem of identifying vitamin P. It was now claimed that *quercetrin* was always present in the extracts which possessed greatest activity. It will be recalled that quercetrin is an anthoxanthin pigment which occurs in oak trees and other plants.

The final chapter of this search can be written as follows. As the result of the work of Scottish, English, and American investigators, it was learned that this new vitamin was related to but not identical with the pigment quercetrin. This glycosidal pigment contains a hexose sugar called *rhamnose*. It was found that vitamin P was similar to quercetrin in every respect except that it contains both rhamnose and glucose in the molecule. Now that the identity of the vitamin was known, it could be given a name rather than a symbol. It is now called *rutin*. This vitamin has found widespread usage in medicine. For instance, one of the best remedies commonly used for treating high blood pressure has the distinct disadvantage of tending to weaken the capillaries in many patients. If rutin is administered too, the tendency to weaken the capillaries is counteracted.

A large number of plant species are known to contain rutin and many more will doubtless be discovered. A very good source of this vitamin is buckwheat. Discovery of this fact has increased the value of this plant

so that whereas it was once used mainly as a source of food for domestic fowls, it can now be classed as more or less of a drug plant.

Riboflavin is another water-soluble pigment which possesses interesting physiological properties. The chemical structure of this pigment is not exactly like that of anthoxanthins which have been mentioned previously, yet it certainly fits in with our general classification of water-soluble, yellow plant pigments. Like rutin, riboflavin is also a vitamin. Almost all fruits and vegetables contain riboflavin, and the richest sources are the green leafy vegetables like turnip greens, spinach, carrot tops, and kale.

Autumnal Coloring

No discussion of plant pigments would be complete without mention of autumnal coloring. Deciduous trees and shrubs in temperate zones often terminate the growing season with an extravagant display of color which is familiar to every one. As the fall days become cooler, manufacture of chlorophyll is retarded until a day arrives when the rate of destruction exceeds the rate of production of this pigment. Yellow pigments (both carotenoid and anthoxanthins) begin to appear, having been previously concealed by the chlorophylls. In many species anthocyanin pigments develop with the onset of cool weather, producing the red and purple colors which are so pronounced in autumnal coloring. Examples of this type of plant are the red maple, sweet gum, sumac, Virginia creeper, dogwood, and the oaks. Leaves of the tulip tree or yellow poplar do not produce anthocyanins, and they therefore turn only yellow in the fall. Tannins in the yellow leaves produce a golden-brown effect, and in the red leaves the result is a purplish color. It should be borne in mind that cool weather and not frost is conducive to a development of autumnal color. Finally, as the pigments are all decomposed, only tannins remain in the leaves and the countryside attains a distinctly brown aspect. Frost hastens the arrival of the last stage.

chapter 10

Photosynthesis

Almost every schoolboy is aware that green plants "purify" the atmosphere in sunlight by absorbing carbon dioxide and releasing oxygen. The process whereby green plants utilize the energy of sunlight to convert water and carbon dioxide to energy-rich organic compounds, with the release of oxygen, is called *photosynthesis*. The exact manner in which they accomplish this has interested physiologists for nearly 200 years and is not yet entirely understood today. Pioneer work in photosynthesis was being done by European scientists about the time that the American colonists were engrossed in their struggles for freedom from Great Britain. In 1772 Joseph Priestley, an English divine of London, reported that plants were capable of purifying air which had been "injured" by the respiration of animals or by the burning of candles. Priestley set up his experiment by placing a lighted candle under a bell jar. Next he showed that after the candle had burned a while, the air under the bell jar would not support a living mouse. However, after a sprig of mint had been kept growing under the bell jar for several months, the air within permitted a candle to burn or a mouse to live. At that time no one knew much about oxygen or carbon dioxide. The terms employed in discussions of the day consisted of *purified air* and *injured air*.

In subsequent experiments Priestley obtained erratic results, probably because he was not aware that light is essential in the process of purification of air by green plants. The Swedish chemist, K. W. Scheele, also was unable to repeat Priestley's experiments, and he denied that plants had the power of assimilating carbon dioxide. Then began a long controversy on the subject between Priestley and Jan Ingenhousz. Unfortunately the scientific investigations of Priestley came to a sudden end when his religious views brought him into conflict with the citizens of Birmingham, England. The Priestley house was burnt down and the family fled to America where they settled in Northumberland, Pennsylvania. The problem of the purification of the air by plants was considerably clarified by the Dutch physician, Jan Ingenhousz in 1779. He demonstrated that light was necessary for the purification of the air by plants. He demonstrated, furthermore, that only the green parts of plants produced this beneficial effect on the air, whereas those tissues which lacked

green pigments made the air impure, just as was done by animals. In 1781 the French chemist Antoine Lavoisier demonstrated that "fixed air" is carbon dioxide. This contribution paved the way for a better understanding of the exact gaseous exchanges which occur during photosynthesis. F. Senebier during the period from 1782 to 1788 showed that carbon dioxide is absorbed during photosynthesis and that this gas is essential to the process. The Swiss botanist and physicist, Nicolas T. de Saussure, in 1804 found that the volume of oxygen evolved is equal to the volume of carbon dioxide absorbed. He also found that water is utilized in the process, and that the plant shows an increase in weight as the result of photosynthesis.

Despite the valuable contributions which have just been listed, it was not recognized by these investigators that photosynthesis is actually a food-manufacturing process. In fact about 60 years elapsed before this fact was known. A detailed study of photosynthesis was undertaken by Jean B. J. D. Boussingault during the years 1860 to 1891. Plants were placed in a jar in a mixture of carbon dioxide, hydrogen, and nitrogen, and it was shown definitely that carbon dioxide is decomposed and oxygen is released as soon as the plant is illuminated, and that this interchange of gases ceases as soon as the light is withdrawn. A piece of phosphorus was exposed within the jar for the purpose of indicating the presence of oxygen. As soon as the apparatus was exposed to light, dense white fumes (phosphorus pentoxide) appeared. This constitutes a simple yet delicate test for oxygen. When the apparatus was darkened the fumes disappeared because phosphorus pentoxide dissolves in water which is in the soil or which has collected on the inside walls of the jar.

The question was raised regarding the ability of plants to utilize the slight amount of carbon dioxide which is normally present in the atmosphere (0.03 to 0.04%). Under the conditions of Boussingault's experiments a larger concentration of carbon dioxide was present than would ordinarily occur in the atmosphere. To answer this criticism Boussingault repeated the experiment by placing a plant in a jar through which a stream of air was passed. Analyses of the air before entering and after leaving the jar again showed that carbon dioxide was assimilated only while the plant was illuminated.

The following interesting anecdote is told about these experiments. The investigation was conducted jointly by Boussingault and his colleague, Dumas. Records were made independently by each worker in order to obtain more reliable results. For a while all went well. The plants in the jar absorbed carbon dioxide in the sunlight and evolved this gas in the dark. Then something happened. On one bright, sunny day the plants appeared to produce carbon dioxide instead of absorb it. The two investigators stared at each other in amazement. They recalled the misfortune that had attended Priestley's experiment when he had attempted to re-

peat it. Finally Regnault, the famous physicist, who had been watching the experiment with considerable interest, began to laugh. He admitted that he had been to blame for this apparent failure of the experiment. For several days, while Boussingault and Dumas were at lunch, Regnault had *breathed* into the apparatus in order to convince himself that these physiologists could determine such small amounts of carbon dioxide.

Organs of the Leaf Involved in the Photosynthetic Process

Carbon dioxide participitating in photosynthesis enters the plant through the stomates. The released oxygen is lost through the same apertures. Carbon dioxide of respiration, when not immediately used in photosynthesis, also passes out through the stomates. For a number of years differences of opinion existed regarding the means by which the gaseous exchange occurs in plants. One school claimed that the gases moved through the cuticle of the leaf, whereas another school claimed that the stomates facilitated the passage of the gases into and out of the leaf. It is now known that the stomates constitute the organs of both entry and exit of these gases. This can be demonstrated by making a test with a leaf in which the stomates are located on only one surface. Photosynthesis may be stopped by coating the stomates with grease. It must be understood that we are discussing terrestrial plants. In submerged aquatics the gaseous exchange takes place through the epidermis because stomates are not present in this type of plant.

The water required for the photosynthetic process in land plants enters the roots and moves up through the tracheae, as was explained during the discussion of transpiration. At the terminus of the vessels in the spongy tissues of the leaf water diffuses across the adjoining cells, moistening the surfaces of cells which border the air spaces in this tissue. The carbon dioxide which has entered through the stomates is dissolved and then diffuses into the chloroplasts in the chlorenchyma (chlorophyll-bearing cells). *Less than one per cent of the water taken in by the plant is utilized in photosynthesis.*

First Product of Photosynthesis

It will be recalled that de Saussure's main contribution to the subject of photosynthesis was the discovery that plants increase in dry weight when assimilating carbon dioxide. This was in 1804. The significance of this discovery was not realized by plant physiologists for nearly 40 years. In fact the belief was held that carbon in the dry matter of the plant came from the humus in the soil. But largely as the result of the writings of Liebig in 1804, the so-called *humus theory* was dropped and leading physiologists accepted de Saussure's claim that the plant's carbon came from the carbon dioxide of the atmosphere.

It was generally considered that carbohydrates constitute the first

products of photosynthesis but no one had proved this prior to 1840. In this year Julius Sachs reported that starch was the "first visible product" of photosynthesis. The method employed to prove this statement was the well-known iodine test for starch. The characteristic blue color of the starch-iodine complex appeared only when the test was made on leaves which had been exposed to the light. Negative tests for starch were shown for leaves which had been held continuously in the dark.

With our present knowledge of the chemistry of carbohydrates it is quite obvious that starch is not the first product of photosynthesis. Starch is a polysaccharide of rather large molecular weight, and is composed of a number of glucose molecules. It is logical to conclude that a simpler compound would be the forerunner of starch. Also starch is not found in the leaves of all plants. Many species of plants in the lily, amaryllis, gentian, composite, and umbellifer families do not synthesize starch in their leaves.

A. F. W. Schimper, a student of Sachs, advanced the hypothesis that the first sugar formed during photosynthesis is glucose. This theory became widely accepted and was given impetus when Willstatter reported that the photosynthetic ratio (O_2/CO_2) is equal to unity under all circumstances. That is, the quantity of oxygen released is always equal to the quantity of carbon dioxide absorbed. This was convincing proof to many physiologists that a simple sugar is formed according to the following equation:

$$nCO_2 + nH_2O \rightarrow nO_2 + (CH_2O)_n$$

However, Schimper's theory that glucose is the first product was challenged as early as 1893. It was claimed by the challengers that sucrose, and not glucose, is the first product of photosynthesis. The controversy continued for more than 50 years. During the half-century just mentioned perhaps more physiologists held to the glucose rather than to the sucrose theory. E. C. Barton-Wright and M. C. Pratt studied the accumulation of sugars in the leaves of the daffodil (*Narcissus pseudo-narcissus*). This plant was employed because, except for the guard cells, the leaves contain no starch. These studies showed that during a period of illumination the hexose content of the leaves underwent a steady decline in quantity. Sucrose showed a steady increase, reaching a peak at four o'clock in the afternoon. Results of this type convinced many physiologists that a hexose is first formed during photosynthesis, and that it is converted to sucrose as fast as it is formed. However, opponents of the hexose theory interpreted results of this type as proving that sucrose and not a hexose is the first sugar of photosynthesis. According to these physiologists the steady increase of sucrose during the daylight hours indicated that this disaccharide is formed first and that the hexoses found in plants result from hydrolysis of sucrose.

A significant contribution to the subject of the first sugar of photosynthesis was made by Calvin and Benson in 1949. These investigators studied photosynthesis in the single-celled alga, *Chlorella*. The carbon dioxide which was introduced into the experimental plants contained radioactive carbon. The atomic weight of the carbon was 14 instead of 12 and the chemical formula is written as $C^{14}O_2$. The carbon in this instance is called a *radioactive isotope* and its course through the plant can be traced because of its radioactivity. Calvin and Benson determined the different sugars appearing in their plants following photosynthesis and looked for the one with radioactivity. In these tests the first sugar to possess radioactivity was sucrose and not glucose or fructose. As a result of this work and that of other investigators the pendulum has now swung back to the sucrose theory. The widespread distribution of sucrose in plants and its rather consistent fluctuation between day and night add considerable weight to the theory.

Hexose Phosphates

Significant advances were made in physiology following the discovery that inorganic phosphorus plays an important role in the enzymatic degradation of starch and that hexose phosphates appear as active intermediate products. These phosphoric acid esters of simple sugars (glucose-phosphate, fructose-phosphate, and others) will be discussed more completely in chapter 17 on Respiration. It seems logical to assume, however, that if phosphate esters play so important a role in the breakdown of starch (and sucrose) they are probably just as essential in the synthesis of the higher carbohydrates. In fact synthesis of sucrose from hexose esters has been accomplished *in vitro*. Furthermore, Calvin and Benson identified glucose- and fructose-phosphates in Chlorella cells in their investigations *and these esters were radioactive*. It is fairly evident that hexose esters, and not hexoses themselves, are first formed and are directly converted to a higher carbohydrate. In all probability sucrose is formed directly from hexose phosphates.

Formaldehyde Theory

Johann F. W. Baeyer, a German chemist, in 1870 formulated the hypothesis that during photosynthesis carbon dioxide and water combine to form formaldehyde (CH_2O). This theory held the interest of physiologists for years but at the present time it has few followers. Perhaps the once wide acceptance of this hypothesis was a result of its convenience as much as anything else, because carbohydrates appear to be polymers of formaldehyde $(CH_2O)_n$.

A certain amount of evidence exists to suggest that still simpler substances are formed before the appearance of the hexose phosphates. There is strong reason to believe that the first product is phosphoglyceraldehyde or phosphoglyceric acid. A triose (three-carbon sugar) is in reality a

glyceraldehyde. The empirical formula is $C_3H_6O_3$ but it may also be written as $CH_2OH{\cdot}CHOH{\cdot}CHO$. So in all probability the first product of photosynthesis may be an aldehyde, though one not quite as simple in composition as formaldehyde, a probability which was originally proposed by Baeyer.

Light

The importance of light to the functioning of higher plants has been mentioned previously and we shall have occasion to refer to the subject again. Subsequent discussion of this topic will be facilitated if the reader understands something about the nature of light.

If a beam of sunlight be directed through a glass prism, the light will be dispersed or separated into its components: that is, into red, orange, yellow, green, blue, and violet light. These are frequently referred to as the colors of the visible spectrum. Colors can also be observed when white light is reflected from thin, transparent films, like soap bubbles, the effect in this instance being produced by interference. Isaac Newton (1667), who was the first to demonstrate that light could be dispersed by a prism, also conducted studies with reflection by films. As he sat at his window for hours, blowing bubbles and observing the colors in them, townspeople shook their heads and said that the great Newton had now become childish!

It has been found that the particular color of different portions of the visible spectrum is determined by the wavelength of the light at that position. These wavelengths have been measured by physicists and are customarily expressed in millimicrons as follows: red, 650 to 760; orange, 600 to 650; yellow, 560 to 600; green, 500 to 560; blue, 430 to 500; violet, 390 to 430. Shorter waves beyond the violet are called ultraviolet. Beyond the ultraviolet are *x-rays*, *gamma rays*, and *cosmic rays*. At the other end of the spectrum, the waves longer than the visible red are called *infrared* or *heat rays*. Radiations longer than the infrared fall into the category of *radio waves*.

Up to the present time in this discussion we have been assuming that light is entirely undulatory. Ample evidence exists to suggest that light is particulate, that is, made up of particles. When light falls on a photographic plate certain chemical changes are induced which indicate that the plate was struck by particles. This *particulate theory* of light is not new. Pythagoras, the Greek philosopher, proposed it during the 6th century B.C. Isaac Newton was also a proponent of the particulate theory of light although he admitted the possibility of wave motion. However, by the beginning of the 20th century the *wave-theory of light* had superseded the particulate theory. But in 1905 Albert Einstein suggested that light is propagated through space as corpuscular radiation, each corpuscle or *photon* having the energy $h\nu$. The h in the formula is Planck's constant

(6.55×10^{-27} erg seconds) and the ν stands for frequency of the emitted light. The term *quantum* is used to designate the energy of a photon.

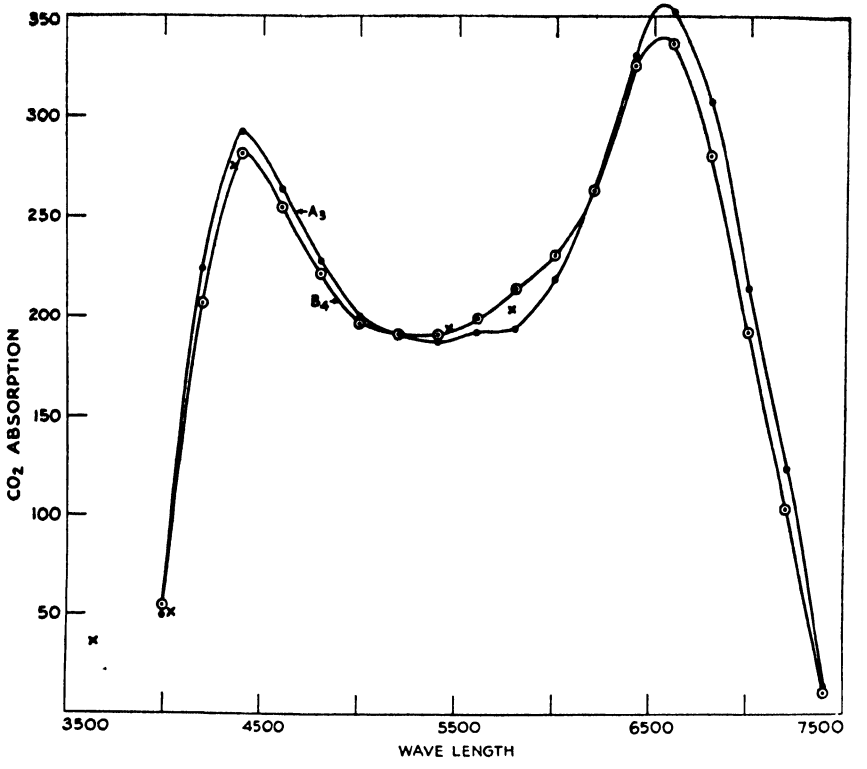
The belief now exists that radiant energy is both undulatory and particulate. That it may possess these dual characteristics is conceivable and tenable in view of recent discoveries in the science of physics. *Electrons*, which are minute particles, are capable of describing wave motions, and it is not unlikely that photons of light behave in the same manner.

The energy value of a quantum of light varies with its frequency. Rays in the red portion of the spectrum contain less energy than those at the violet end.

Using Einstein's formula, red light, with a wavelength of 600 millimicrons has an energy value of 3.3×10^{-12} (0.0000000000033) ergs per quantum; violet light, with a wavelength of 400 millimicrons is equal to 4.9×10^{-12} (0.0000000000049) ergs per quantum. We shall have occasion to refer to these energy values of light when we discuss the quantum efficiency of photosynthesis. First let us consider which particular wavelength is most effective in photosynthesis.

Effective Wavelength of Light

About the close of the 18th century and the beginning of the 19th, interest was manifested in the portion of the spectrum which is most efficient in photosynthesis. Light filters were constructed with the aid of double-walled bell jars which contained colored solutions between the walls. Orange-red light was produced by means of a solution of potassium dichromate. An ammoniacal solution of copper oxide transmitted light in the blue end of the spectrum. Even with these rather crude methods the investigators of that period observed that a maximum rate of photosynthesis occurs in the red end of the spectrum. In subsequent investigations white light was separated into its components by means of prisms or with specially designed glass filters. What is often overlooked in studies of this kind is that not all wavelengths are transmitted by the filter in use with the same intensity. Many of the published results do not mean a great deal because of the existence of two variables, i.e., both the quality and quantity of light. In 1937 W. H. Hoover conducted studies with wheat plants in which all wavelengths studied were transmitted with equal intensity. These results indicated one maximum rate in the red (655 millimicrons) and another in the blue (440 millimicrons). All wavelengths of visible light were effective to a certain extent. It is interesting to note that the maxima for photosynthesis practically coincide with the maxima for absorption of light by chlorophyll. However, in the curve of absorption the maximum in the blue-violet end is slightly greater than that in the red end. The reverse is true in regard to photosynthesis. In addition to this, the curves for absorption and for photosynthesis do not coincide in the portions of the spectrum between the red and the blue-



Wavelength and photosynthesis (expressed as CO_2 assimilation). Wavelengths are expressed in angstroms (one-tenth of a millimicron). (Courtesy, Hoover: "The Dependence of Carbon Dioxide Assimilation in a Higher Plant on Wave Length of Radiation," *Smithsonian Miscellaneous Collections* **95**:1–13, 1937.)

violet. A very small portion of green light is absorbed by chlorophyll. The rate of photosynthesis in this region, though lower than that in the red and blue, is only slightly lower. Perhaps the energy is absorbed by some other pigment.

Factors Affecting the Rate of Photosynthesis

It is obvious that under natural conditions the rate of photosynthesis in plants may show considerable variation. The process may be limited by one or more factors and these factors may be either internal or external.

Carbon Dioxide. We have stated that the raw products of photosynthesis are carbon dioxide and water. Under natural conditions carbon dioxide may frequently be the limiting factor in photosynthetic activity. The atmosphere normally contains 0.03 to 0.04 per cent by volume of this gas. This percentage is maintained rather constantly. It has been

shown experimentally that an increase in CO_2 content of the atmosphere up to about one per cent will produce a corresponding increase in photosynthesis. Experiments have been reported in Germany in which carbon dioxide was introduced into greenhouses to enrich the atmosphere. According to these reports the yield of crops under glass was increased 100% to 150%. Similar results were reported by M. B. Cummings and C. H. Jones in the United States. However, "fertilizing" with carbon dioxide in open fields would not be practicable.

Carbon dioxide which is removed from the atmosphere by plants is replaced from several sources. Respiration of plants and animals, the combustion of fuels, and gaseous discharges from volcanoes, all produce carbon dioxide. In certain habitats a relatively large portion of this gas is supplied by plants, particularly those of the lower orders. Bacteria and fungi in the soil, as well as other microorganisms, produce large quantities of carbon dioxide as the result of respiration. This is what is meant by the term *soil respiration*. It is possible that cultural practices which encourage the growth and reproduction of microorganisms in the soil serve to exert an indirect benefit upon crop plants; that is, the atmosphere above ground is enriched with CO_2 as the result of increased soil respiration.

One wonders why the carbon dioxide of the atmosphere as a whole remains so nearly constant. It has been suggested that the balance is maintained by a constant exchange of carbon dioxide between the atmosphere and the waters of the oceans. Water in the ocean contains 30 to 40 times as much carbon dioxide as the atmosphere. When the carbonates and bicarbonates are included in the calculations, sea water contains five per cent carbon dioxide, or 125 times as much as that in the atmosphere.

Light. To say that light is a factor affecting photosynthesis is a gross understatement. It is the sole source of energy for the process. With few exceptions, all life on this planet owes its existence to the fact that green plants are able to store up the energy of the sun.

The light of the sun at noon on a clear day is equal in intensity to 8000–10,000 foot-candles. (One foot-candle has been defined as the intensity of light at a distance of one foot from a standard sperm candle.) The intensity of the sunlight which reaches the earth is reduced by particles in the air, such as those in clouds, fogs, and dust. Light intensity is reduced also by the presence of water vapor in the atmosphere. Furthermore, not all leaves on a plant receive the same amount of light because of shading produced by the leaves themselves. Laboratory experiments have shown that, other conditions being favorable, the rate of photosynthesis increases with increase in light intensity. It should be noted, however, that in most of these experiments the maximum light intensity employed was 1000 foot-candles or only one-tenth that of maximum normal sunlight. In all probability the maximum intensity of sunlight

under natural conditions is necessary for normal growth in most plants because a certain amount of compensation must be made for those parts which do not receive full sunlight.

Not all green plants require full sunlight for survival. Wood sage, violets, ferns, and azaleas are examples of the so-called shade-loving plants. On the floor of the forest and jungle will be found many species of plants growing in reduced sunlight. When one is attempting to grow both shade trees and flowers in a limited area, he will do well to select shade-loving plants for the flower beds. Likewise lawns may be maintained in shaded areas by planting grasses like the fescues whose normal habitat is in shaded spots.

Experiments have been conducted to determine the minimum amount of light required by certain plants to maintain themselves. In order to survive, they must manufacture enough food (photosynthate) to compensate for that consumed in respiration. The tests in question showed that some of the conifers (yellow pine, spruce, Douglas fir) required from 1 to 5 per cent of full sunlight to maintain themselves. Deep-water algae, mosses, and algae which inhabit caves can grow in very weak light, often under intensities no greater than that of moonlight. Myers has reported that the alga *Chlorella pyrenoidosa* can grow at a light intensity of less than 10 foot-candles, and attains a maximum growth-rate at less than 100 foot-candles.

Temperature. In plants of the temperate zones photosynthesis is carried on in a temperature range of between 10°C. and 35°C. It is apparent, however, that plants which remain green during winters in the temperate regions must be able to carry on photosynthesis at relatively lower temperatures. Photosynthesis has been demonstrated in the leaves of the cherry laurel (*Prunus laurocerasus*) at −6°C. and in certain conifers at −35°C. In tropical species of plants the lower limit of photosynthesis is around 5°C. Some species of algae have been found to be able to grow at relatively low temperatures, whereas others survive at unusually high ones. Certain marine algae are known to complete their life cycle in water which is below 0°C. Contrariwise, algae are found growing in hot springs where the temperature of the water is 93°C. Since these species of algae complete their life cycles in these extremes of temperatures, it follows that they are capable of carrying on photosynthesis under such conditions.

It is not possible to establish an optimum temperature for photosynthesis without adding certain qualifications. Are we interested in the initial rate or a continuing rate? Experiments conducted with plants at 25, 30, 35, and 40°C. have indicated that the initial rate of photosynthesis increases with each increase in temperature. But at the end of 30 minutes in these experiments, only the plants at 25°C. were maintaining the original rate of photosynthesis. All of the others had fallen off at the

end of this period, and the rate of the lots at 40°C. was now lower than the initial rate at 25°C.

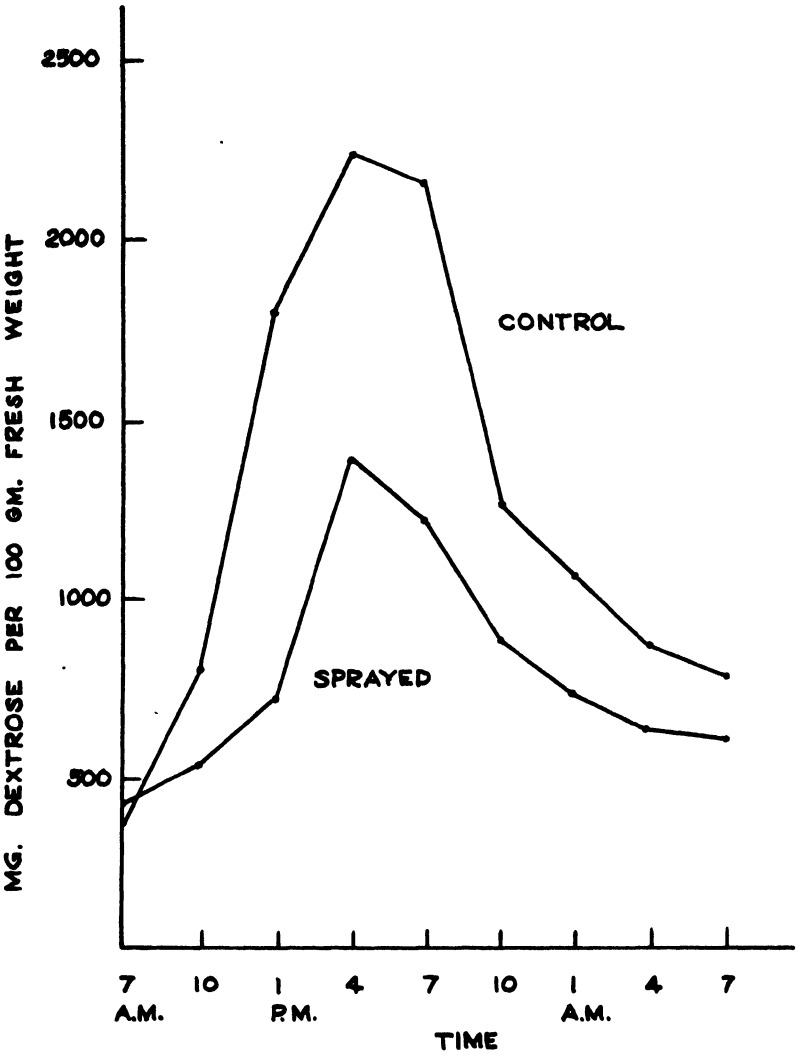
Water. The amount of water required for photosynthesis is not large when compared to the quantity which normally passes through the plant in the transpiration stream. Actually it is less than one per cent of the total water absorbed. Nevertheless, as shown in the equation for photosynthesis, water is essential for the process. When a plant is deprived of water, the amount of photosynthesis is reduced. Some of the effects of drought may be secondary since stomates tend to close when the plant is deprived of water. A more specific effect of drought on photosynthesis results from dehydration of protoplasm. This was demonstrated by H. Walter who worked with the water plant, *Anacharis canadensis*. The plant was grown in varying concentrations of sucrose. The rate of photosynthesis was retarded when the concentration of sucrose was high enough to cause *plasmolysis*. This concentration was between 0.3 and 0.4 weight molar. Photosynthesis was almost stopped when the plants were grown in 0.7 molar sucrose solutions. These results rather clearly indicate that lack of water per se may reduce the rate of photosynthesis.

Oxygen. Laboratory experiments have indicated that oxygen is necessary for photosynthesis although the quantity required is not large. Willstätter and Stoll reduced the oxygen pressure to one-hundredth of an atmosphere without affecting the rate of photosynthesis of a number of species of plants. Physiologists suggested that respiration constitutes an essential part of the over-all photosynthetic process, but they failed to explain why so little is required. Investigations of Burk, Warburg, and others have clarified the matter by showing that at a certain stage in photosynthesis the plant consumes (in respiration) some of the oxygen which it has previously released. Thus slightly more oxygen is required than was first realized. This is discussed later in this chapter.

Other Factors. Photosynthetic activity may be affected by other factors. A retarding effect is caused by deficiency of certain essential elements such as magnesium, potassium, iron, and phosphorus. Since many mineral deficiencies interfere with the production of chlorophyll, the effect on photosynthesis may be indirect. On the other hand, some of the essential minerals are required in enzymatic processes, and they doubtless influence the rate of photosynthesis irrespective of their effect on chlorophyll production.

Various internal factors may affect photosynthetic activity. A few of these are the chlorophyll in the leaf, degree of hydration of the protoplasm, and the structure of the leaf itself.

Cultural Practices. Instances have been recorded in which certain agricultural practices have reduced photosynthetic activity in plants. Mention has been made of the cracking of cherries during dry weather.

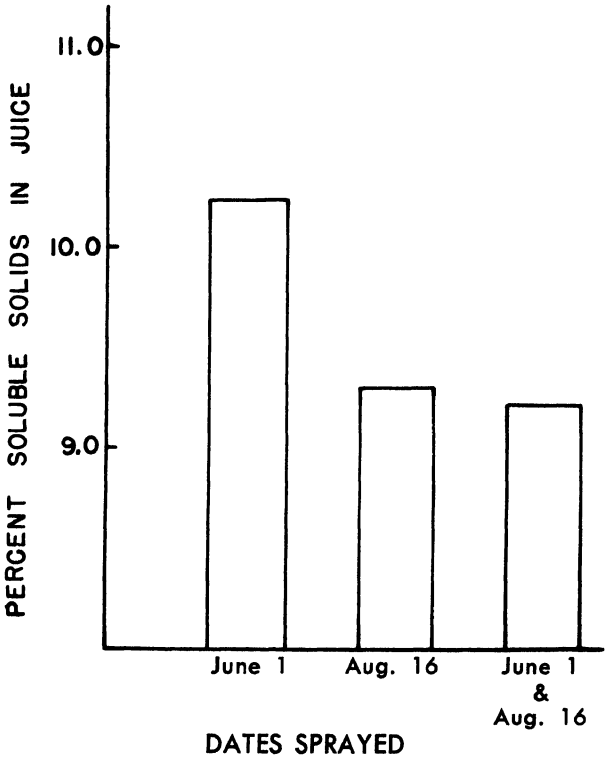


Effect of transpiration-reducing spray on dextrose content of maize leaves. (Plotted from data of Barr: "Photosynthesis in Maize as Influenced by a Transpiration-reducing spray," *Plant Physiol.* **20**:86–97, 1945.)

In Michigan a wax-oil emulsion was developed for the purpose of reducing loss of water from cherry trees during droughts, the ultimate objective being to prevent excessive cracking of the fruits. The question was raised regarding the effects of such a spray on photosynthetic activity. Tests were then made on corn plants, and the effect of the spray on photosynthesis was evaluated by determining the amount of sugar which

accumulated in the leaves during the day. Results showed a much lower quantity of total sugar in sprayed leaves than in samples from untreated plants, indicating a reduction in the rate of photosynthesis.

Somewhat similar results have been reported in areas of citrus production where it is customary to employ oil sprays for controlling infections



Comparison of soluble solids in relation to timing of two oil sprays on pineapple oranges. Photosynthetic activity of leaves is reduced by late oil spray. Oil applied in June was washed off by summer rains. (Redrawn after Thompson and Sites: "Relationship of Solids and Ratio to the Timing of Oil Sprays on Citrus," *Proc. Fla. State Hort. Soc.*, 1945.)

of rust mites. Experiments with orange trees in Florida showed that ripe oranges in November and December had lower quantities of total soluble solids (mainly sugars) when oil sprays were applied in late summer (July and August) than when trees were sprayed in June. Results indicated that a thin film of oil on the leaves reduced the rate of photosynthesis during a critical period in the ripening of oranges. Spray residues left on the leaves following application of oil in early summer were evidently washed off by summer rain.

The Photosynthetic Reaction

The importance of the photosynthetic reaction can hardly be overemphasized. Since the latter part of the 18th century, plant physiologists have realized that during photosynthesis organic matter is produced from inorganic chemical compounds and that animals are dependent upon this process for their food supply.

More recently interest has centered on photosynthesis for another reason. The reaction represents a mechanism whereby light energy is converted into chemical energy with a degree of efficiency which is unequaled in any other known photochemical reaction.

According to Whittingham (1952) photosynthesis consists of the following processes:

"(a) Absorption of light in a photochemical primary process.

(b) Formation of a hydrogen donor with hydrogen ultimately derived from water.

(c) Reduction of carbon dioxide by the hydrogen donor, directly or indirectly."

In short, it is an oxidation-reduction reaction. Removal of hydrogen from water is oxidation, and the combining of hydrogen with carbon dioxide is reduction.

We now know that oxygen produced during photosynthesis comes from water and not from carbon dioxide. This was demonstrated by growing the alga Chlorella in water which contained radioactive oxygen. It was found that the oxygen which was released during photosynthesis contained the same proportion of radioactive isotopes as were present in the original water. This reaction is presented in the following equation. Radioactive elements are indicated by asterisks.

$$CO_2 + 2H_2O^* \rightarrow (CH_2O)_n + H_2O + O_2^*$$

It is apparent therefore, that one of the steps in photosynthesis consists of the breaking up of the water molecule. This part of the process is termed the *Hill reaction*. It is the only part of the process which has thus far been demonstrated with isolated chloroplasts. R. Hill and others have shown that preparations of chloroplasts isolated from leaves will evolve oxygen in the presence of light, providing a hydrogen acceptor is present. Ferric oxalate or quinone have been employed as hydrogen acceptors in these experiments.

Little is known regarding the mechanics of carbon dioxide fixation in photosynthesis. Reduction of CO_2 has not as yet been demonstrated with isolated chloroplasts, as was true in the Hill reaction. The work of A. W. Frenkel (1941) suggests that carbon dioxide uptake occurs in the cytoplasm, and that carbon dioxide reduction is associated with the chloroplasts.

The exact role of chlorophyll in reduction of carbon dioxide is not well understood. That absorption of light is one important function of chlorophyll is apparent from the fact that neither water nor carbon dioxide absorb the visible rays of the spectrum. It has been suggested that, in addition to this, chlorophyll may form a temporary combination with CO_2 in a reversible reaction. It seems fairly certain that the first reaction of CO_2 in photosynthesis involves the combination of an organic molecule, R—H, to a C=O bond in CO_2. Identity of this organic molecule is still to be determined. Studies with radioactive carbon (C^{14}) have shown that the C^{14} first appears in the carboxyl groups of certain organic acids, and then in a number of other apparently intermediate products. In all probability the formation of carbohydrates in photosynthesis proceeds through a number of steps, like those which have been found to occur in the oxidation of sugar during respiration, except, of course, in the reverse order.

Two distinct types of reactions have been demonstrated in the photosynthetic process. One of these is a *photoreaction*. Light is necessary for this step. The other is termed the *Blackman reaction* and is not dependent upon light. (The latter is also referred to as the *dark reaction*.) The photoreaction is also dependent upon carbon dioxide and chlorophyll. The Blackman reaction is concerned with water but does not require chlorophyll or carbon dioxide.

The two types of reactions were demonstrated by Emerson and Arnold, who exposed cultures of Chlorella to intermittent light at the rate of fifty flashes per second. Duration of the period of illumination was 0.0034 seconds, and that of the dark period was 0.0166 seconds. With intermittent illumination the photosynthetic yield per unit of light was increased approximately 400%. These results were interpreted to mean that under continuous lighting the products of photosynthesis are formed faster than they can be utilized. More will be said about the importance of the dark period in our discussion of efficiency of the process which follows.

Efficiency of Photosynthesis

A controversary once existed regarding the efficiency of the photosynthetic process. A vast difference of opinion prevailed with respect to ratio of the intake of energy to the output. On one side of the question was Otto Warburg in Germany and on the other was a group of American investigators. In discussing this topic in detail we must again refer to the physics of light. As was mentioned previously, the individual particles of light are termed photons, and the energy of each quantum of light varies with its wavelength. In 1921 Warburg claimed that between three and four quanta of light were required for the release of one molecule of oxygen. The American group (J. Franck, R. Emerson, and H. Gaffron)

reported that ten quanta of light were required for the production of one molecule of oxygen. In 1948 Emerson invited Warburg to come to America to join forces with the American team of workers. The invitation was accepted and the German-American team of investigators began studying photosynthesis in earnest. S. B. Hendricks and Dean Burk joined the group of workers and in 1949 it was reported the amount of light required per molecule of oxygen was three to four and not ten as the American group claimed.

All of the experiments just cited were conducted with red light. If one molecule of oxygen is produced by the absorption of four quanta of red light then the photosynthetic process represents an efficiency of 65%. Compared to machines this is rather high. The gas turbine, which is one of the most efficient of modern machines, has an efficiency of only 30%. In 1911 Einstein, stimulated by the earlier experimental work of Emil Warburg, proposed what was subsequently to become the law of chemical equivalence. According to this law, chemical substances affected by light must react through the precise equality of one molecule altered per one quantum of minimum energy content absorbed. In 1911 Einstein stated that the law of photochemical equivalence should apply to the photosynthetic reaction. In other words one quantum of red light and not three or four should be required to produce one molecule of oxygen. Let us consider the energy values involved in such a reaction. In the oxidation of hexose the products are CO_2 and H_2O, and 110,000 calories of heat per mole have been dissipated. It is assumed then that when the reaction is reversed, as in photosynthesis, 110,000 calories are required for the synthesis of a mole of sugar. The energy which is supplied by four quanta of red light may be computed as follows:

$$\text{One quantum of red light (600 millimicrons)} = 3.3 \times 10^{-12} \text{ ergs}$$

$$\text{Number of molecules per mole} = 6.06 \times 10^{23}$$

$$\text{One calorie} = 4.186 \times 10^{7} \text{ ergs}$$

$$\frac{(3.3 \times 10^{-12})(6.06 \times 10^{23})}{4.186 \times 10^{7}} = 47{,}700 \text{ calories per mole}$$

From this calculation it is evident that one quantum of red light supplies only 47,700 calories per mole, or a little over one-third of the 110,000 calories which are necessary for the production of one mole of hexose and the release of one mole of oxygen. The solution to the problem was offered by Burk, Cornfield, and Schwartz in 1951. Warburg's technique included, among other things, the inclusion of intermittent lighting in his experiments so that the experimental plants were exposed first to light and then to darkness. It then was revealed that during the dark period two-thirds of each oxygen mole formed was oxidized back to CO_2 with release of energy. In other words the approximately 70,000 additional calories were produced for the process during the dark reaction. The actual quantity

of oxygen which was measured represented only one-third of what was produced in the light. This meant that the three quanta of red light were instrumental in producing not one molecule, but three molecules of oxygen. The law of photochemical equivalence therefore holds for photosynthesis, and the production of one molecule of oxygen requires one quantum of light.

It is difficult to appreciate the extent to which green plants are producing photosynthate from day to day throughout the year. The world production of steel is something like 100,000,000 tons a year. World production of material by green plants is more than 2,000 times as great, or 200,000,000,000 tons per year. But this amount is still not enough. We continue to draw on photosynthate from the prehistoric past for much of our energy. In the United States the average daily per-capita consumption of energy is 150,000 kilogram calories. This includes energy required for heating and lighting of buildings, transportation, and the operation of machines, as well as the energy required for keeping our own bodies running. For the latter we consume about 3,000 kilogram calories per day. This is the only part which is drawn entirely from current supplies of photosynthate. The remaining 147,000 kilogram calories are derived from fossil fuels such as coal, oil, and gas. (We are neglecting slight quantities of wood which are used for fuel.) It is apparent that we are rapidly exhausting our surplus of stored energy. In addition to this, not every individual on this earth is able to obtain food enough to supply him with 3,000 kilogram calories per day. In other words, some day we may be short of fuel, but our shortage of food actually exists today.

All of this adds up to the fact that a better understanding of the process of photosynthesis is essential to the survival of the human race. It would be a boon to humanity to learn the secret of the green plant or the means by which the energy is stored in the chloroplasts. Dreamers predict that some day this process will be carried out in the laboratory independently of the green plant. The next step would be the pilot plant, and then mass production. Such a set up, however, may be far in the future. But what may *not* be very far off is the more efficient utilization of some of the lower plants. We have stated that plants like the algae may carry on photosynthesis in light of no greater intensity than that of moonlight. These plants may be cultivated without depending upon full sunlight and are suitable for growing indoors. Lack of sufficient light hampers the growing of higher plants indoors, therefore most crop plants must be produced under natural conditions only during the growing season. Even in glasshouses during the winter the light is not sufficient for normal growth. The problem of adding to our food supply is not solved by the use of algae, but they suggest one approach.

Still another possibility exists for supplementing our food supplies. At the present time man has not learned to utilize very much of the

photosynthate from the sea. Over the oceans lies a layer of plankton extending 300 feet below the surface. Microscopic organisms of this kind carry on photosynthesis like their relatives on the land. They supply the fish and other marine organisms with food. The fact that man might live on plankton has been demonstrated at least experimentally. Explorers have collected these organisms by means of "plankton nets" and have found them edible, if not palatable. The area of the oceans in which plankton exist has been estimated at 90 billion acres. If we assume that the rate of photosynthesis per acre in the ocean is twice that on land (which is not an unreasonable assumption) then the annual output of carbon for the 300 foot layer of phytoplankton would be 90 billion tons. This corresponds to 225 billion tons of glucose. Still higher forms of plant life exist in the sea. We have called them seaweeds but they are not necessarily "weeds." Some of them are used as foods and there must be others also that are edible.

Some day the biologist may discover a method for the economic utilization of lower forms of green plants on both land and sea. And the physiologist may unlock the secrets of the chloroplast in the living plant. In the meantime research workers from all countries are directing their efforts to these problems.

chapter 11

Carbohydrates

In the chapter on photosynthesis it was stated that green plants produce carbohydrates and other organic compounds from carbon dioxide and water. *Carbohydrates* have taken their name from the fact that many of them consist of carbon, hydrogen, and oxygen, with the last two elements combined in the same ratio in which they occur in a molecule of water. We know of course that carbohydrates are not actually "hydrated carbons" and that the type formula $(CH_2O)_n$ can be found in other organic compounds, but the term is still generally accepted for the group of substances which we are about to discuss.

Carbohydrates may be classified into three large groups: sugars, polysaccharides, and compound carbohydrates. In Table 13 will be found a list of carbohydrates commonly encountered in plants.

Sugars

Sugars represent the simplest form of carbohydrates. Included in this group are mono-, di-, tri-, and tetra-saccharides. Sugars are white crystalline compounds which are soluble in water and slightly soluble in alcohol. A common characteristic is sweetness to the taste. Sugars may be classified according to their optical activity and also as to whether they are reducing or nonreducing. Optically active compounds rotate the plane of polarized light to the right or left, and they are described as being *dextrorotatory* or *levorotatory*, depending upon the direction of rotation. Ordinary, or nonpolarized light, vibrates in all directions in a plane perpendicular to the direction in which light is traveling. By passing light through a filter of oriented molecules, light waves vibrate in only one direction and are said to be polarized. The following analogy may help one to understand the principle of polarized light. If a person is more than an arm's length behind the iron bars of a high fence he may wave his arms in all directions. If he steps forward and extends his arms between the bars he is then able to wave his arms only vertically. This is the way in which the waves of light are polarized by the proper filter. They vibrate only in one direction. When a beam of polarized light is directed through a solution of an optically active compound, the beam will be rotated either to the right or to the left. The amount of rotation is specific for

each substance and is proportional to both its concentration and to the length of the column through which the light is passing. This principle is utilized in an instrument which is called a *saccharimeter*. A solution containing a gram of sugar per milliliter of water is placed in a column 10

Table 13

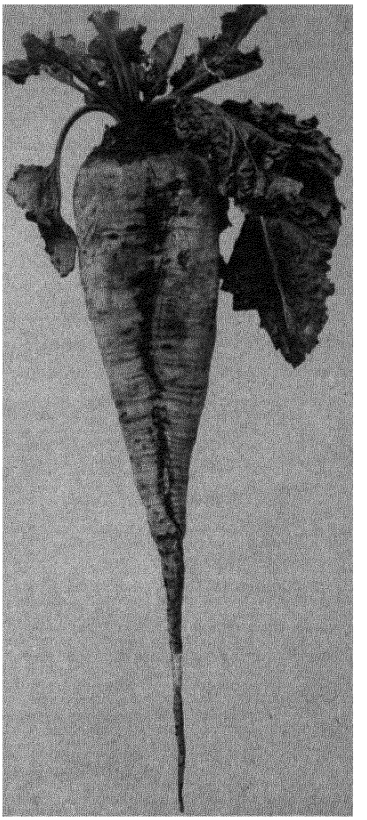
CARBOHYDRATES COMMONLY FOUND IN PLANTS

- Sugars
  - Monosaccharides
    - Pentoses ($C_5H_{10}O_5$)
      - Arabinose
      - Xylose
      - Ribose
    - Hexoses ($C_6H_{12}O_6$)
      - Glucose
      - Fructose
      - Galactose
      - Mannose
      - Sorbose
  - Disaccharides ($C_{12}H_{22}O_{11}$)
    - Sucrose
    - Maltose
  - Trisaccharides ($C_{18}H_{32}O_{16}$)
    - Raffinose
    - Gentianose
    - Melezitose
  - Tetrasaccharides ($C_{24}H_{42}O_{21}$)
    - Stachyose
- Polysaccharides
  - Pentosans
    - Araban
    - Xylan
  - Hexosans
    - Dextrin
    - Starch
    - Glycogen
    - Cellulose
    - Inulin
    - Lichenin
  - Hemicelluloses
- Compound Carbohydrates
  - Gums
  - Mucilages
  - Glycosides
  - Tannins
  - Pectic compounds

centimeters long and maintained at 20°C. Under these conditions, the angle of rotation for glucose is 52.7° to the right and that of fructose is 92.5° to the left. These values may also be expressed as plus 52.7° and minus 92.5°.

A *reducing sugar* is one which contains either an aldehyde (—CHO) or a ketone (C=O) radical in its molecule. *Nonreducing sugars* are compound sugars in which the aldehydic or ketonic groups have lost their usual reactivity as a result of the manner in which the component mono-

saccharides are linked. Since the aldehydic and ketonic groups are easily oxidized, they will reduce oxidizing agents, such as Fehling's solution. This reagent consists of a mixture of copper sulfate, sodium hydroxide, and sodium potassium tartrate. Alkaline cupric hydroxide, which results from the mixture, is acted on by reducing sugars to form cuprous oxide, a red precipitate. The quantity of reduced copper bears a definite relationship to the amount of reducing sugars present, and the reaction is the basis of most quantitative methods for sugar analysis.



The sugar beet of commerce represents a root in which large quantities of sucrose are stored by the plant. (Courtesy, Bureau of Plant Industry, U.S. Dept. of Agriculture.)

A simple qualitative test may be employed for indicating sugar in plant extracts. It is called the *Molisch test.* It serves to determine the completeness of an alcoholic extraction of plant tissue. Molisch reagent consists of a 15% solution of alphanapthol in alcohol. Five milliliters of concentrated H_2SO_4 are placed in a test tube. Then two drops of Molisch reagent are added to the extract which in turn is poured carefully into the test tube of acid. If a violet ring forms at the junction of the two liquids, it is an indication that sugar is present in the extract.

The simplest sugars are the monosaccharides. They are absorbed directly into the blood of animals without further digestion. Specific monosaccharides are named according to the number of carbon atoms in the molecule. Thus five-carbon sugars are termed *pentoses* ($C_5H_{10}O_5$) and six-carbon sugars are *hexoses* ($C_6H_{12}O_6$). Simple sugars containing more than six carbon atoms are known but they apparently do not occur in plants. It is highly probable that *trioses* (three-carbon sugars) occur either in slight quantities or are transitory products because triose phosphates have been reported as possible precursors of higher sugars in plants (see chapter 10).

Pentoses ($C_5H_{10}O_5$). The pentose sugars are widely distributed in plants although they do not occur in large amounts. They are found more commonly as their condensation products or *pentosans.* Pentoses rarely

represent more than one per cent of the dry weight of plants. The hydrolysis of such compound polysaccharides as gums, mucilages, and pectins yield pentoses. Five-carbon sugars may be prepared also from bran, straw, wood, and shells of apricot seeds. When pentose sugars are boiled with 10% hydrochloric acid or 10% sulfuric acid, they yield furfuraldehyde. They may thus be distinguished from hexoses which yield the above-mentioned aldehyde only by means of treatment with concentrated mineral acids.

Pentoses seem to originate in plants under conditions of abnormal metabolic conditions such as drought, exposure to low temperature, or wounding. At least one example has been recorded in which tendrils, when subjected to tension, produced woody elements containing pentoses.

The following are some of the pentose sugars which have been identified in plants. *Xylose* is obtained from bran, wood, straw. *Arabinose* is found in gum arabic, cherry gum, etc. *Rhamnose* is a constituent of the water-soluble pigment, *quercitrin*, which occurs in oaks. *Ribose* does not occur in very large quantities, but it apparently performs rather important functions in the physiology of plants. This pentose is a constituent of nucleic acid, and also of riboflavin, the latter being one of the so-called *B complex vitamins*. Both nucleic acid and riboflavin perform very important roles in essential functions like respiration and reproduction. *Desoxyribose* is another important pentose sugar, and its functions are somewhat similar to those of ribose.

Hexoses ($C_6H_{12}O_6$). The six-carbon sugars most commonly found in plants are *glucose* and *fructose*. Glucose is an aldehyde. It is also known as *dextrose*, *corn sugar*, *grape sugar*, or *blood sugar*. This sugar is dextrorotatory, that is, it rotates polarized light to the right. Fructose, which is also called *levulose* or *fruit sugar*, is chemically a ketone. It is levorotatory. The prefix *d* or *l* on organic compounds indicates that they are dextro or levorotatory, respectively. Sugars, however, constitute an exception to this rule. The *d* and *l* in this case merely indicates a structural relationship to *d* and *l* glyceric aldehyde.

Mannose and *galactose* are aldohexoses which occur in plants only in traces. They occur more abundantly in their condensation products, mannosans and galactans, from which they may be released by boiling with acids.

Galactose crystallizes on the surface of the berries of Virginia creeper (*Parthenocissus*) after the fruits have been frozen. This sugar also forms in the sugar beet under abnormal conditions.

Disaccharides. Disaccharides are formed by the union of two molecules of hexose sugars. Both hexoses may be identical or they may be different sugars. The commonest disaccharide in plants is *sucrose* ($C_{12}H_{22}O_{11}$). In fact the bulk of the sugars found in higher plants generally consists of glucose, fructose, and sucrose in varying proportions. Re-



Field of sugar cane nearing maturity on plantation of United States Sugar Corporation in the Florida Everglades. (Courtesy, United States Sugar Corporation, Clewiston, Fla.)

sults of analyses are customarily expressed as reducing (glucose plus fructose) and nonreducing sugars. (See Table 14.)

Table 14

SUGAR CONTENT OF SEVERAL TYPES OF PLANT TISSUE*

Product	*Reducing Sugar* %	*Nonreducing Sugar (Sucrose)* %	*Total Sugar* %
Field corn leaves at 3:00 p.m.	0.81	3.54	4.59
Pineapple orange in December	3.30	3.07	6.37
Jonathan apple	6.41	2.89	9.30
Peas	.00	3.00–6.00	3.00–6.00
Lima beans	.00	3.00–4.00	3.00–4.00

* Per cent fresh weight.

Certain plants, like sugar cane and sugar beets, have been bred for high sucrose-content. Plants of this type supply most of the sucrose in commerce today. Other sources of sucrose are the sugar maple tree and certain sorghums. Another natural source of sugar, honey, contains large

quantities of reducing sugars (see Table 15). *Maltose* is found rather

Table 15

SUGAR CONTENT OF CERTAIN COMMERCIAL SOURCES OF SUGAR

Sugar cane stalks		12–20% sucrose
Sugar beet roots		15–20% sucrose
Honey	Levulose	40.5%
	Dextrose	34.0%
	Sucrose	1.9%

widely distributed in plants. But it is not found in as large quantities as is sucrose. Maltose is an intermediate product in the digestion of starch.



The trunk of a sugar maple (*Acer saccharum*) tapped for sap. (Courtesy, Gibbs: *Botany*, New York, The Blakiston Co., Inc., 1950.)

Cellobiose is another disaccharide which occurs in small quantities. This disaccharide results from the enzymatic degradation of cellulose. (Table 16 shows the sugar content of a number of natural foods.)

Other Sugars. Trisaccharides ($C_{18}H_{32}O_{16}$) and tetrasaccharides ($C_{24}H_{42}O_{21}$) are not as widely distributed in plants as are the simpler sugars. The trisaccharide *raffinose* is found in cotton seeds, in sugar beets in appreciable quantities, and to a lesser extent in other plants. *Melezitose*, another trisaccharide, occurs in the European larch, Douglas fir, scrub pine, and other conifers. It is a very sweet sugar. It is a rather common occurrence to find aphids attacking trees which contain this trisaccharide.

So much of the sugar is ingested by sucking insects that it oozes from their bodies, covering the leaves of the tree and falling on objects below.

An example of a tetrasaccharide is *stachyose*, which is found in the hedge nettle (*Stachys tubifera*). Another example is *lupeose*, which is a constituent of the seeds of *Lupinus luteus* and other legumes.

Table 16

SUGAR CONTENT OF A NUMBER OF FRUITS AND VEGETABLES*

Product	*% Total Sugar*	*Product*	*% Total Sugar*
Greenbeans	0.4	Cranberries	4.2
Broccoli	1.9	Blackberries	6.1
Cabbage	3.5	Grapefruit	6.5
Peas	5.2	Orange	8.8
Sweet corn	4.3	Peaches	8.8
Sweet potatoes	5.4	Sweet cherries	11.6
Carrots	7.5	Pineapple	11.9
		Apples	11.1
		Bananas	19.0
		Dates	61.2

* Chatfield, Charlotte, and Georgian Adams: *Proximate Composition of American Food Materials.* (U.S. Department of Agriculture Circular #549.) Washington, D.C., 1940.

Polysaccharides

Sugars in excess of the plant's immediate requirement for respiration are converted into polysaccharides. These compounds are actually polymers of the simple sugars; that is, they consist of a large number of sugar molecules and are represented by such substances as starch, dextrins, cellulose. They are insoluble or slightly soluble in water and most of them form hydrophillic colloids. They are important to the plant because they serve as storage materials, and they also constitute the structural materials of the cell wall. When the fundamental unit of the polysaccharide is a pentose sugar it is called a *pentosan*. When the unit is a hexose it is called a *hexosan*.

Pentosans ($C_5H_8O_4$). The best known pentosans are *arabans* and *xylans*, which are formed from arabinose and xylose, respectively. They are spoken of as condensation products of these sugars. A pentosan consists of a long chain of dehydrated pentose molecules. Compounds of this type occur mainly in cell walls where they serve principally as reserve foods. Arabans are found in cherry, peach, and plum gums. Xylans occur in wood, straw, bran, and the shells of apricot seeds.

Pentosans possess the ability to imbibe large quantities of water and they surpass hexosans in this capacity. The water-holding power of cacti and other drought-enduring plants is traceable to the presence of pentosans which are constituents of mucilaginous materials. Pentosans also retard ice formation in tissues which have been exposed to low temperatures.

Pentosans may serve as reserve foods after the more readily utilizable materials (starch and dextrins) have been exhausted. Woody tissues of

some trees contain as much as 15% xylans. It has been found that these substances are frequently digested in the spring with the formation of gums. A number of wood-rotting fungi, like *Xylaria sp.*, regularly utilize pentosans as a source of energy.

Hexosans. Polysaccharides of this type are similar to pentosans in that they consist of long chains of simple sugars. They differ in that the sugar molecule in the hexosan is a hexose rather than a pentose.

Cellulose, a hexosan which occurs in the cell wall, is said to be the most abundant organic compound on the earth. When the primary wall of a plant cell is first laid down it consists largely of cellulose. As the cell matures, the cellulose layers may become impregnated with substances like lignin, cutin, or suberin.

Cellulose is the basis of all wood fibers. Cotton and linen are nearly pure cellulose. Concentrated sulfuric or hydrochloric acids convert cellulose to glucose. In dilute sulfuric acid, cellulose swells and becomes hydrocellulose. A 15% solution of sodium hydroxide produces similar results. Cellulose is insoluble in water but may be dissolved by Schweitzer's reagent (ammoniacal copper hydroxide) providing this reagent is made up with proper precautions.

It is apparent from what has been said that cellulose serves as structural material in the plant rather than as a reserve food. Its general insolubility and its relatively inert characteristics indicate this. Nor is it of any value as a food to man. We must depend upon lower organisms, such as bacteria and fungi, to digest the cellulose of plant residues so that they may be returned to the earth. Termites perform an important function in digesting cellulose, but actually the work is done by a protozoan in the intestinal tract, and not by the digestive juices of the termite. Some of the higher animals are capable of utilizing cellulose for food but they also depend upon microorganisms for actual digestion of the compound.

In contrast to cellulose, *starch* is an important food for both plants and animals. Starch may be observed in both leucoplasts and chloroplasts. Starch grains appear in the chloroplasts soon after photosynthesis takes place and they continue to accumulate during the daylight hours. At night starch is converted to sugar and translocated to other parts of the plant. It may thus be synthesized from glucose in the leucoplasts, the process being independent of light. As a food reserve, starch is stored in roots, tubers, seeds, and other storage organs, as well as in the stems of plants. Man has learned to utilize many of these storage organs for his own food such as white potatoes (tubers), sweet potatoes (roots), and cereal grains (seeds). The starch content of white potatoes ranges from 50% to 70% dry weight; in cereals it ranges from 15% to 30%. The shape of starch grains is constant within a given species so that the source of the grain may be determined by microscopic examination. Starch deposited on the grain in the daytime appears to be amorphous and to

contain a different amount of water from that deposited at night. This produces a difference in the refractive index and as a result the starch grains display concentric rings or bands. Starch grains are not soluble in cold water. If they are heated with water the grains swell and burst. An opalescent dispersion will then be formed which is called starch paste. When this colloidal solution is filtered it is known as soluble starch. A blue color appears when soluble starch combines with iodine.

Starch is hydrolyzed to glucose by heating it with dilute mineral acids. The enzyme amylase carries the reaction only to maltose. A common procedure for quantitative estimation of starch in plant tissues consists of treating the tissues with *diastase*, which is a commercial preparation consisting of amylase and maltase. With this treatment the end product is glucose.

The starch molecule consists of two components, amylose and amylopectin, both of which are condensation products of d-glucose. Amylose is made up of straight chains of glucose, whereas amylopectin is composed of branched chains. The two components differ in their physical properties.

Dextrins may be produced by heating starch to about 202°C. These compounds have a slightly sweetish taste, apparently the result of having fewer glucose units in the molecules than does starch. Dextrins are intermediate products in the digestion of starch. Progressive steps during the hydrolysis of starch are as follows:

$$\text{starch} \rightarrow \text{amylodextrin} \rightarrow \text{erythrodextrin} \rightarrow \text{achroodextrin} \rightarrow \text{maltose} \rightarrow \text{glucose}$$

Glycogen is a hexosan found in the liver and muscles of animals. It is found in fungi but has not been reported in green plants.

All of the hexosans thus far considered are glucosans. Only one levulosan, *inulin*, occurs in plants. Tubers of the Jerusalem artichoke contain this compound. Other sources of inulin are chickory, dahlia, dandelion, salsify, and goldenrod.

Hemicelluloses. Until rather recently the word *hemicellulose* could not be adequately defined. The term was once employed to indicate insoluble polysaccharides and related compounds which are hydrolyzed by boiling with dilute mineral acids. This would include pentosans, pectins, galactans, and mannosans.

Although the structure of hemicelluloses has not been completely worked out, we do know that polyuronide hemicelluloses, which are cell wall components, are composed of glycosidic chains containing molecules of both uronic acid and pentose (Bonner, 1950). One type of polyuronide hemicelluloses contains glucuronic acid and xylose. Another type contains galacturonic acid and arabinose. The former appears to be more common.

Hemicelluloses are readily hydrolyzed by dilute mineral acids and they may be isolated from plant tissues in colloidal form by means of weak

caustic solutions. Hemicelluloses differ from pectins in being soluble in alkali and insoluble in water. This also distinguishes them from gums and mucilages, which are soluble or dispersable in water. Hemicelluloses differ from cellulose in being hydrolyzable by dilute acids. Hydrolysis of cellulose requires concentrated acids.

Hemicelluloses are found as constituents of cell walls. They occur in seed coats, nut shells, woody tissues, and are usually associated with lignin. Gerhardt reported that the hemicellulose fraction of one-year-old apple wood contained 15% lignin, 70% pentose sugars, 5% ash, and 5% moisture.

The question has often been raised as to whether hemicelluloses are structural or metabolic materials. It is possible that they are both. Hemicelluloses in the woody tissues of trees are digested in the spring, supplying energy for growth before the tree is manufacturing photosynthate. Endosperms of many seeds contain hemicelluloses which are utilized as food during germination. Vegetative organs of wheat contain very small amounts of starch. It has been assumed that hemicelluloses take the place of starch in these tissues. These compounds tend to accumulate in wheat plants during drought.

Compound Carbohydrates

The term *compound carbohydrates* has been applied to compounds composed of both carbohydrates and noncarbohydrate groups. Examples of compound carbohydrates are gums, mucilages, pectic substances, tannins, and glycocides.

Gums are more or less transparent, amorphous substances, some of which dissolve (or disperse) in water, whereas others merely imbibe water. They may be precipitated by alcohol or by basic lead acetate. Upon hydrolysis they yield hexoses, pentoses, and complex organic acids. Gums usually occur in nature as salts of potassium, calcium, or potassium combined with an organic acid radical in the molecule. Gum arabic and gum tragacanth are examples of gums which are soluble in water. The type which only swells in water is represented by the gums which exude from the bark of species of Prunus (cherry, plum, peach). Gum arabic is used as a stabilizing agent in emulsions and tragacanth is used to improve the consistency of ice cream.

A disease of fruit trees, called *gummosis*, is characterized by the exudation of gum at the surface of the bark. Although several species of fungi have been identified as causal agents, it is highly probable that gummosis is a symptom of several diseases. Gums are also exuded by trees when injured or when subjected to other abnormal conditions such as low temperatures. J. Grüss found that the inner part of wood vessels is digested in the spring with the formation of gum. In the early spring, while snow is still on the ground, relatively large masses of gum sometimes

exude from the bark of wild cherry trees and may be observed on the ground under the tree. Not a great deal is known regarding the physiological importance of these complex carbohydrates to plants.

Mucilages are probably similar to gums in their constitution although information on this subject too is fragmentary. They form slimy, colloidal dispersions in water and this is the reason for their name. Mucilages are obtained from seed coats of flax, mustard, Plantago and from the bark of slippery elm (*Ulmus fulva*). Mucilage from seeds of *Plantago psyllium* is used medicinally as a bulk-forming material. Agar is a mucilage-like material which is prepared from marine algae. Similar material is prepared from Irish moss (Gigartina sp.) and is useful in increasing smoothness of ice creams.

Pectic substances include *pectic acid*, *pectin*, and *protopectin*. The average person is probably more familiar with pectin than with the other members of this group. This compound forms hydrophillic colloids with water and under proper conditions may be caused to gel. The gelling property of pectin has been utilized for many years in the making of jelly although the process has never been thoroughly understood. Protopectin is the parent material of pectic compounds and occurs in the cell wall. Hydrolysis of protopectin with mineral acids yields pectin. The process is similar to the conversion of starch to sugar. Enzymic degradation of pectin yields pectic acid. We now know that pectic acid consists of long chains of galactose molecules with several molecules of arabinose and galacturonic acid. These three compounds are obtained upon hydrolysis of pectic acid. Pectin differs from pectic acid in that it has several of the acid or carboxyl groups combined with the methyl (CH_3) radical. In other words, pectin is a methyl ester of pectic acid. Jellies are made with pectin and not with pectic acid or protopectin, and the gelling ability of pectin depends upon the degree of "methylation."

Cell walls of fruits like apples, peaches, pears, and others contain large quantities of protopectin. During ripening, protopectin is converted to pectin which appears in the fruit juice or vacuolar sap. Ripening is therefore accompanied by a certain amount of softening. Periodic analyses of maturing fruits of the above type usually show a decrease in protopectin and an increase in soluble pectic substances.

Pectic acid, in the form of its calcium salt, occurs in the middle lamella of plant cells and serves as cementing material. The soft and mealy condition of overripe fruits is caused by disintegration of pectic substances in the middle lamella. Pectic acid in the juice is also broken down into its components in overripe fruits.

Jelly-making requires pectin, sugar, and acid. It is apparent then that overripe fruits cannot be utilized for this purpose. In fact "mature green" fruits are better suited for jelly-making because they supply both pectin and the necessary amount of acid. It is always necessary to add sugar in

the process. Not all fruits contain sufficient pectin for making jelly. Peaches, strawberries, blackberries, and raspberries belong to this group. Apples, pears, and quinces make excellent jelly. The albedo (inner white peel) of citrus fruits is rich in pectin and serves as a source for commercial supplies of pectin. Apple peel from canneries is also a common source of commercial pectin. Commercial supplies of pectin are employed for making jellies from pectin-poor fruits, for thickening desserts, and in medicine.

Tannins represent a group of complex compounds which are rather widely distributed in plants. Chemically they are glycosides yet they differ so markedly from other glycosides that they are usually considered separately. Upon hydrolysis these compounds produce a sugar, complex acids, and often other substances. Tannins account for the astringency in immature fruits like the pear, banana, persimmon, and olive. During ripening of the fruits astringency is lost, not as a result of the destruction of tannins, but because these substances become insoluble. The details of this process are not completely understood. Tannins also occur in tea leaves, in nutshells, and in the bark of oaks, chestnut, hemlock, sumac, and others. In the curing or tanning of hides, the process depends upon the capacity of tannins to form insoluble compounds with the proteins in the animal skin.

The role of tannins in the physiology of plants has never been completely understood. We know that they play a part in oxidation processes and are apparently important in the function of respiration. The conversion of green tea to black is largely the result of the oxidation of tannins. In the respiratory process, tannins are apparently the respiration pigments first described by Palladin.

Glycosides. A *glycoside* is a compound formed by the reaction between a sugar and one or more nonsugar compounds. The components are usually linked by an oxygen atom so that, structurally speaking, a glycoside is an etherlike compound. This type of linking characterizes other substances such as anthocyanin and anthoxanthin pigments, tannins, and even sucrose; this indicates that glycoside is therefore a general term. The other groups of substances, however, have already been discussed under topics to which they more appropriately belong. Under the heading of plant glycosides we shall discuss substances whose physical characteristics at least are quite similar. They are colorless, crystalline compounds which are soluble in water, alcohol, and a few other organic solvents. A common characteristic is bitterness and it is this property which impresses the average person in his contact with such substances. Glycosides may be hydrolyzed either by the enzyme *emulsin* or by dilute mineral acids into sugar and the nonsugar or *aglycone* component.

The physiological role of glycosides in plants is not thoroughly understood. They have been considered to be reserve foods because they often accumulate in certain woody species of plants during the winter and dis-

appear in the spring. One school of thought assumes that plants remove injurious materials by combining them with sugars to form the glycoside molecule. This theory is based largely upon the fact that when animals are given camphor it is combined with sugar, eventually oxidized, and subsequently excreted by the kidneys.

A number of the more common glycosides are listed in Table 17 along with their source and also their hydrolytic products. Included in the list

Table 17

SOME OF THE COMMON GLYCOSIDES AND THEIR HYDROLYTIC PRODUCTS*

Glycoside	*Plant in Which Commonly Found*	*Products of Hydrolysis*
		Alcohols
Coniferin	(*Coniferae, Beta, Asparagus, Scorzonera*)	Glucose + coniferyl alcohol
Salicin	(*Salix*, Populus)	Glucose + saligenol
		Aldehydes
Amygdalin	(*Prunus, Pyrus*)	Glucose + benzaldehyde + hydrocyanic acid
Prunasin	(*Cerasus, Prunus*)	Glucose + benzaldehyde + hydrocyanic acid
		Phenols
Arbutin	(*Ericaceæ*)	Glucose + quinol
Hesperidin	(*Citrus*)	Glucose + rhamnose + hesperetin
Naringin	(*Citrus*)	Glucose + rhamnose + narigenin
Phloridzin	(*Rosaceæ*)	Glucose + phloretin
		Coumarin derivatives
Esculin	(*Æsculus*)	Glucose + esculetin
		Mustard Oils
Sinigrin	(*Brassica nigra*)	Glucose + allyl isothiocyanate + potassium hydrogen sulphate
		Flavene and Flavonal Pigments
Quercitrin	(*Quercus, Fraxinus, Thea*)	Rhamnose + quercetin
		Anthocyan Pigments
Cyanin	(*Centaurea, Rosa*)	Glucose + cyanidin
Delphinin	(*Delphinium*)	Glucose + oxybenzoic acid + delphinidin
Pelargonin	(*Pelargonium, Centaurea*)	Glucose + pelargonidin
		Various Constituents
Digitalin	(*Digitalis*)	Glucose + digitalose + digitaligenin
Indican	(*Indigofera*)	Glucose + indoxyl

* Onslow, Muriel Wheldale: *Practical Plant Biochemistry*, 3rd ed., New York, Cambridge University Press, 1929.

are some of the water-soluble pigments, even though these were discussed in a previous chapter. It must be remembered that this list is not all-inclusive, because over 100 glycosides have been identified. It will be observed that many different chemical groups are represented in the agly-

cone part of the molecule, such as alcohols, aldehydes, phenols, as well as more complex substances.

Salicin (which occurs in willow trees) and *coniferin* (which is found in conifers) both belong to the group which contain alcohols. *Amygdalin* and *prunasin* are similar, not only because they belong to the aldehyde group, but also because they contain hydrocyanic acid. They are also called "cyanophoric" glycosides. Amygdalin is present in the seeds of the bitter almond and in the seeds of the cherry. Cyanophoric glycosides sometimes occur in grasses, especially if the nitrate content is high or if the plants have been subjected to extensive drought. Occasionally livestock are poisoned by grazing on plants which contain large quantities of this type of glycoside.

The bitterness of immature oranges is caused by *hesperidin*. In immature grapefruit the bitter principle is *naringin*.

Mustard oil glycosides are present in plants of the *Umbelliferae*, a common example being *sinigrin* of black mustard (*Brassica nigra*). Hydrolytic products, in addition to glucose, are potassium hydrogen sulfate and allyl isothiocyanate (C_3H_5CNS), i.e., mustard oil. Isothiocyanates give pungency to the members of the umbellifer group, such as mustard, radish, etc.

Interesting Facts Regarding Sucrose, Glucose, Fructose

As mentioned previously, sucrose, glucose, and fructose make up the bulk of the sugars in plants. There may be varying amounts of these three sugars present in different organs of the plant. Sometimes the quantity of reducing sugars (mainly glucose and fructose) is equal to that of sucrose, and sometimes no reducing sugars are present at all. Sucrose is probably present to some extent, however, in every living cell.

When sucrose is hydrolyzed, either by acids or by enzymes, the resulting mixture of glucose and fructose is called *invert sugar*. This term springs from the fact that the direction of rotation of polarized light is changed or inverted as the result of hydrolysis. The degree of rotation for sucrose is +66.5. The rotary power of glucose is +52.7 and that of fructose is −92.5. In a mixture of equal parts of glucose and fructose the resulting degree of rotation of polarized light is −39.8 (52.7–92.5).

Sucrose is the principal sugar in plant nectars. In the manufacture of honey, the bee inverts most of the sucrose so that the final product contains 40.5% fructose, 34.0% glucose, and only 1.9% sucrose. This more or less refined "natural sweet" has been called a supersaturated solution of different substances in noncrystallizable sugar. Although sucrose had been identified for many years it was not until 1927 that its correct chemical structure was elucidated. Another important event occurred in 1944 when W. Z. Hassid, M. Doudoroff, and H. S. Barker accomplished the

synthesis of sucrose from glucose and fructose with the aid of an enzyme which was secreted by the bacterium *Pseudomonas saccharophila.*

Other interesting results have been obtained in experiments with sucrose. I. M. Rabinowitch reported that this disaccharide, when taken by mouth, provides glucose to the blood more rapidly than does glucose itself when taken in the same manner. Both W. J. Robbins and F. W. Went found that excised tomato roots grew better in nutrient solutions containing sucrose than when the solutions contained equivalent amounts of dextrose, levulose, or of mixtures of the two.

The interrelationship between glucose and fructose in the plant is not well understood. Glucose has been considered to be the sugar which is most readily oxidized in the cell, but there is evidence that fructose is the more active physiologically. For example, in the synthesis of sucrose in the phosphorolytic reaction, glucose must first be converted to glucose-1-phosphate, whereas the other reactant, fructose, apparently enters into the reaction without previous transformation.

W. E. Martin has reported that glucose is present in largest amount in the skin region of the pear, whereas fructose is found in all parts of the fruit except the skin. Why this particular distribution should exist is not clear. Fruits are essentially storage organs and are not particularly active physiologically.

Sugar-Starch Transformation

Starch grains appear in leaves during photosynthesis as a result of the accumulation of sugars. During the night the starch is converted to sugar again and is translocated to other parts of the plant. When sugar reaches storage tissues, such as seeds, tubers, and roots, it is changed to starch. In deciduous trees carbohydrates are stored during the winter in the wood, pith, and medullary rays as starch, hemicelluloses, and pentosans. In the spring the insoluble carbohydrates are converted to sugars which in turn supply energy during early spring growth. In evergreen trees insoluble carbohydrates in the leaves are converted to sugar during the winter, thus enabling the cells to withstand relatively low temperatures.

Certain fruits and vegetables, which may be harvested in the "mature green" or "market-ripe" stage of maturity, contain large quantities of starch in their cells. Examples of this type are the apple, pear, banana, peach, and tomato. As they become "full ripe," or edible, the starch is converted to sugar. In tropical fruits, like bananas and tomatoes, this transformation of starch to sugar will not occur at temperatures below 10°C. (50°F.). With fruits from temperate zones the conversion will occur at low temperatures, though slowly.

Vegetables like peas, beans, and corn are often consumed in the "immature-seed" stage, at which time they contain a maximum amount

of sugar; but as these seeds mature, the sugar is converted to insoluble polysaccharides. As mature seeds, these vegetables are not quite as desirable for food, though they are entirely satisfactory for planting. Loss of sugar in peas, beans, and corn is very rapid at relatively high temperatures, i.e., 25°C. to 30°C. Refrigeration temperatures retard this conversion of sugar to insoluble polysaccharides.

The white potato represents a different category regarding transformations of carbohydrates. If this commodity is held at 0°C. to 5°C., starch is converted to sugar with the result that the tubers become too sweet for the average person. A high sugar content is undesirable in potatoes intended for preparing potato chips and french fries because the excess glucose causes a darkening of the final product. Holding the potatoes at 10°C. to 15.5°C. (50°F. to 60°F.) for several weeks prior to processing will eliminate this difficulty because experiments have shown that an enzyme inhibitor prevents the conversion of starch to sugar when potatoes are held at this temperature.

chapter 12

Lipids (Fats and Related Compounds)

The term *lipid* is applied to fats and related compounds, such as waxes, sterols, phosphatides, and others. The physical characteristics of lipids make them easily recognizable. They are insoluble in water but dissolve in fat solvents such as ether, petroleum ether, chloroform, carbon tetrachloride. Lipids are greasy to the touch and make grease spots on paper. These spots are dark by reflected light but translucent in transmitted light.

A number of systems of classification of lipids have been proposed, one of which is that suggested by Bloor (1943). The following simplification of Bloor's system includes only types commonly found in plants:

1. Simple lipids
   Fats (esters of fatty acids and glycerol)
   Waxes (esters of fatty acids and alcohols other than glycerol)
2. Compound lipids (esters of fatty acids containing additional groups)
   Phospholipids or phosphatides (substituted fats containing phosphoric acid and nitrogen)
3. Derived lipids
   Fatty acids
   Sterols (alcohols of large molecular weight)

It will be seen from this outline that a true fat consists of an ester of glycerol and fatty acids. Since glycerol is a trihydric alcohol, three acid molecules are combined to form a fat. It was once believed that all of the acids making up a fat were identical but it is now known that each may be different.

The structural formula for a simple fat, *palmitin*, is as follows:

$$\begin{matrix} C_{15}H_{31}COO \\ C_{15}H_{31}COO- \\ C_{15}H_{31}COO \end{matrix} \!\!\!\

more important saturated fatty acids are shown in the following list. This group is also called the *acetic acid series.*

formic	$HCOOH$
acetic	CH_3COOH
propionic	$CH_3(CH_2)COOH$
butyric	$CH_3(CH_2)_2COOH$
caproic	$CH_3(CH_2)_4COOH$
caprylic	$CH_3(CH_2)_6COOH$
capric	$CH_3(CH_2)_8COOH$
lauric	$CH_3(CH_2)_{10}COOH$
myristic	$CH_3(CH_2)_{12}COOH$
palmitic	$CH_3(CH_2)_{14}COOH$
stearic	$CH_3(CH_2)_{16}COOH$

The first four acids in this series are not ordinarily found in fats. Caproic, caprylic, and capric acids are constituents of palm and coconut oils. Butterfat consists of glycerides of oleic (unsaturated acid), palmitic, and stearic acids, but the flavor is due to a small amount of butyrin.

In unsaturated fatty acids all of the valence electrons are not "tied up" so that the compound is capable of combining with additional elements. It is convenient to represent the degree of unsaturation by additional "valence bonds." Thus some of these acids are shown with one or more double bonds. Oleic acid, a double-bond compound, is represented by this formula:

$$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$$

Linoleic acid ($C_{18}H_{32}O_2$), a constituent of cottonseed oil, contains two double bonds. Linolenic acid ($C_{18}H_{30}O_2$) is a highly unsaturated acid which is found in linseed oil. This acid contains three double bonds. We shall have more to say about unsaturated fats and fatty acids later.

Occurrence of Lipids

Lipids occur in the cytoplasm of the cell both as microscopic globules and as emulsions. In the vacuole these substances are found as emulsions. Fats are stained red by Sudan III and black by osmic acid although these reagents are not specific for fats. When fats occur as colloidal dispersions, it is probable that they are more readily available for metabolic processes both because of their instability and because of their increased surface area. The fact that they abound in meristematic protoplasm in this form tends to corroborate this statement.

In the storage organs of plants, fats occur usually in the form of globules; this indicates that their function in this state is to serve as reserve foods. If the oil globule is surrounded by a membrane it is called an *elaioplast.*

Storage organs of plants constitute common sources of fats for man. Seeds of the cotton, peanut, sunflower, castor bean, and soy bean are rich

in fats, as are also the nuts. The oil content of certain natural products is presented in Table 18. Seeds of cereals (wheat, corn, rice) are not as rich

Table 18

OIL CONTENT OF CERTAIN NATURAL PRODUCTS*

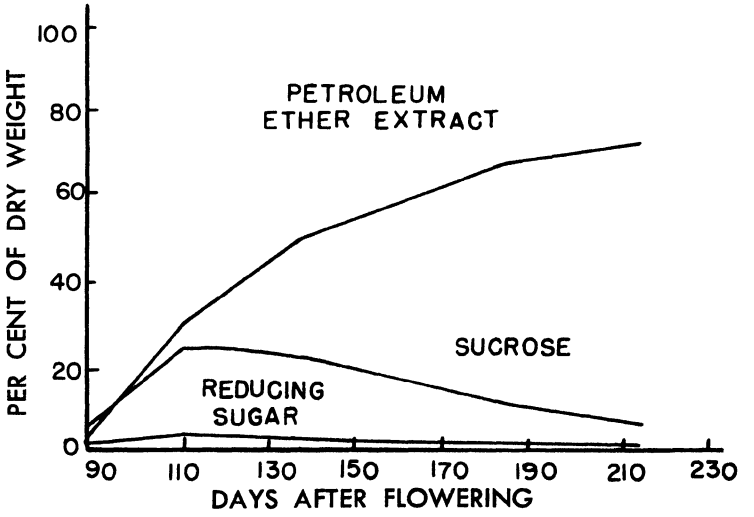
Product	*% Oil*
Brazil nut	70
Tung nut	60-70
Coconut	65
Castor bean	60
Olive pulp	50
Avocado pulp	50
Sunflower seed	45–50
Peanut	40–50
Cotton seed	15–20
Corn seed	5–10
Wheat seed	2–4

* Miller, Edwin C.: *Plant Physiology*, 2nd ed., New York, McGraw-Hill Book Co., 1938, and other sources.

in fat as are those mentioned above. The highest oil content is found in nuts, with Brazil and tung nuts containing as much as 70% of the dry weight. Fruits, as a rule, are not high in fat. Two exceptions are the olive and avocado, both of which contain on the average 50% oil in the pulp.

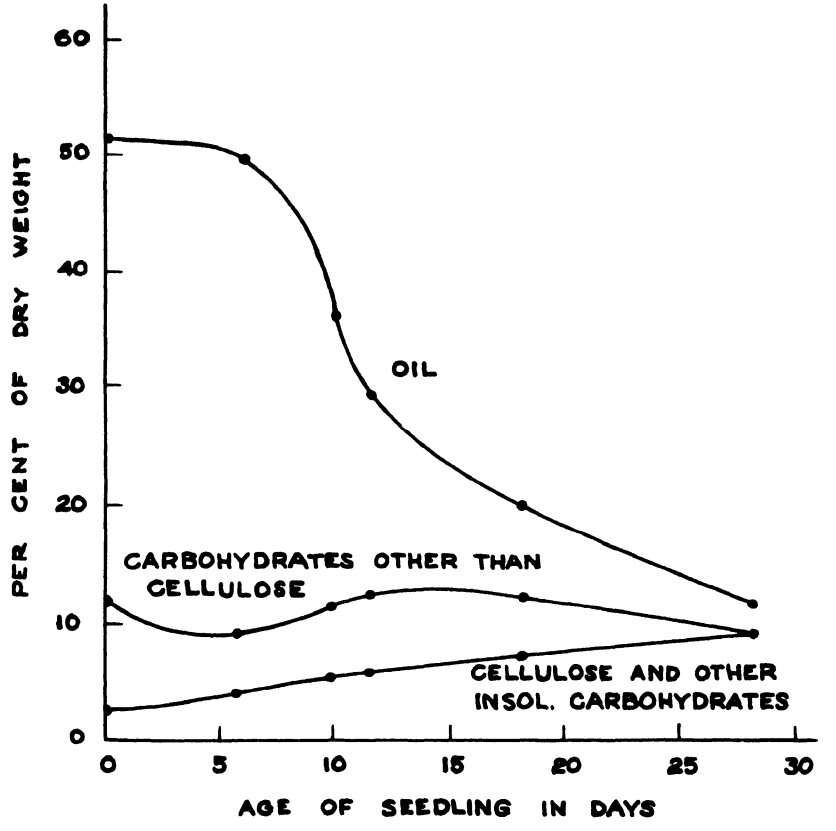
Synthesis of Fats in Plants

Investigators have known for a long time that as fats accumulate in plants, certain carbohydrates diminish in quantity. This has been ob-



Changes in reducing sugar, sucrose, and petroleum ether extract (oil) in the Macadamia embryo during maturation. (Plotted from data of Jones and Shaw: "The Process of Oil Formation and Accumulation in the Macadamia," *Plant Physiol.* **18**:1-7, 1943.)

served in seeds of the almond, Macadamia, pecan, and tung trees, and a number of other plants. In the kernel of the almond seed the fat has been found to increase from 2% to 46% between June 9 and October 4. At the beginning of the period, the glucose content was 6% and it had entirely disappeared when the seeds were mature. During the same period the



Changes in oil and carbohydrates in the peanut during germination. (Plotted from data of Harvey: *Plant Physiological Chemistry*, New York, The Century Co., 1930.)

sucrose content dropped from 6.7 to 2.5%. Studies of the changes occurring in the ripening of the Macadamia embryo have shown that reducing sugar constituted 1.47% of dry weight 90 days after flowering and dropped to 0.30% when mature (215 days after flowering). During this period sucrose changed from 6.07 to 5.50% and the oil content rose *from 3.46 to 73.44%*.

These results have given rise to the theory that carbohydrates are utilized in the synthesis of fats. It is believed that carbohydrates are con-

verted into both glycerol and fatty acids and the two are then esterfied to form fats.

The whole process has never been carried out *in vitro* although certain stages have been accomplished. Smedley succeeded in producing a limited amount of fatty acids from hexoses outside of the living cell by first breaking the hexoses down to three-carbon compounds. The conversion of fatty acids and glycerol to fats can be brought about in the laboratory with the aid of the enzyme which accomplishes the synthesis of fats in the living cell.

Fats and Oils

It will be observed that we have employed the terms fat and oil interchangeably. Chemically they are no different, provided of course that they are true fats or oils. If solid at ordinary temperatures they are considered *fats*, if liquid at these temperatures they are termed *oils*. The melting point of a fat is dependent upon the nature of the fatty acid present. Fatty acids of the saturated series containing 10 carbon atoms or less form glycerides which are liquid at 30°C. With the addition of each carbon atom to the molecule there occurs a rise of about 20°C. in the melting point. The state of the fat in the cell is important from a physiological standpoint because this determines its mobility in the protoplasm. On the whole, fats in plants are usually liquid at ordinary temperatures. This is in great contrast to the fats in animals. All plants are poikilothermal (cold-blooded) organisms so that the physical state of the fat or oil during periods of active metabolism is highly important. This brings us to the important topic of unsaturated fatty acids and fats.

Unsaturated Fats

Fats which contain acids of the unsaturated series are fluid at lower temperatures than those containing acids of the saturated series. A common example of this may be found in olive oil which contains nearly 80% of oleic acid. It will be recalled that oleic acid is unsatured. Cocoa-butter, which is solid at ordinary temperatures, consists of a relatively large proportion of palmitic and stearic acids, these being saturated acids.

The fats of most plants are liquid at the average temperatures of temperate zones (15 to 20°C.) yet there are many plants in climates where temperatures are as low as −30°C. Nevertheless, the fats in these particular plants remain liquid at such low temperatures. Conifers are typical of this group.

It has been claimed that plants growing in northern latitudes maintain their fats in the liquid state as the result of the fact that they contain mainly unsaturated fats and that the farther from the equator the plants grow the greater the degree of unsaturation of their fats. It appears now that this is not generally true. Data in support of the theory were pub-

lished a number of years ago by S. Ivanow who made a study of the fats of the same species of plants grown at different latitudes. Some of the results are given in Table 19. The degree of saturation of a fat is expressed as the iodine number because an unsaturated fat may be saturated with iodine. The chemist employs this method in order to estimate the degree of unsaturation. A large iodine number indicates a greater degree of unsaturation. In Ivanow's tests the iodine number of the fat in the sunflower (*Helianthus annuus*) was 117 at Aschabad, Siberia (37°–30′ N. Lat.), whereas at Moscow (55° 50′ N. Lat.) it was 135. At several stations between these two points the iodine number was represented by intermediate values.

Table 19

EFFECT OF LATITUDE ON IODINE NUMBER OF *Helianthus annuus* OIL*

Location	*Latitude*	*Corresponding Location in America*	*Iodine Number*
Moscow	55° 50′	Lower tip of Hudson Bay	135
Voronezh	51° 40′	Winnipeg, Canada	125–128
Kuban-Odessa	45°–46′	Bangor, Maine	120
Aschabad	37°–30′	Washington, D.C.	117

* Harvey, R. B.: *Plant Physiological Chemistry*, New York, The Century Co., 1930.

Animals in the arctics are characterized by having considerable quantities of unsaturated fats in their bodies. According to T. P. Hilditch, fats of marine animals contain a higher percentage of unsaturated acids than do fats of land animals.

Unsaturated oils from plants are of considerable economic importance. Quick-drying oils, like those of linseed, sunflower seeds, and tung nuts owe their quick-drying property to their state of unsaturation. In drying, they combine with oxygen of the air. The process may be accelerated by the addition of metals like zinc, copper, lead, barium, and calcium. When oils of this type are saturated with hydrogen instead of oxygen, they become solid. Commercial substitutes for butter and lard are made from vegetable oils in this manner.

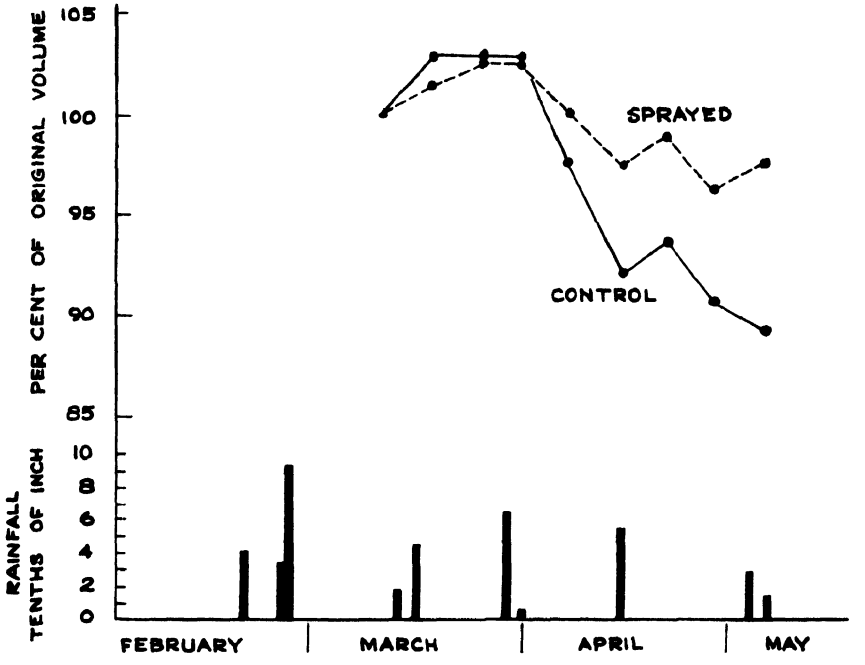
Oxidation and rancidification of fats is favored by lipases, which cause hydrolysis of the glycerides. In the presence of atmospheric oxygen and moisture, free fatty acids are oxidized. The greater the percentage of unsaturated fatty acids in the fats, the greater the tendency to become rancid.

Waxes, Cutin, Suberin

Waxes differ from fats in being esters of fatty acids and monohydroxylic alcohols of high molecular weight. Several examples of alcoholic constituents of waxes are as follows:

Carnaubyl alcohol ($C_{24}H_{50}O$) in wool fat
Neoceryl alcohol ($C_{25}H_{52}O$) in beeswax
N-octacosanol ($C_{28}H_{58}O$) in wax of apple cuticle

These waxes are not at all reactive. They are found mainly as constituents of the cuticle of leaves and fruits. Commercial waxes, like carnauba and candililla waxes, are obtained from palm leaves. Waxes are abundant in plants which are especially adapted to xerophytic conditions. If the waxy bloom is removed from apples, pears, oranges, and other fruits after harvest, they will lose water and become shriveled.



Effect of oil spray on water loss by oranges during a drought. Spray was applied April 1st at the time when the drought was becoming severe. Although both lots of oranges lost water and decreased in volume, the unsprayed (control) lot showed the greater loss. Normal rainfall at this time of year is between two and three inches per month.

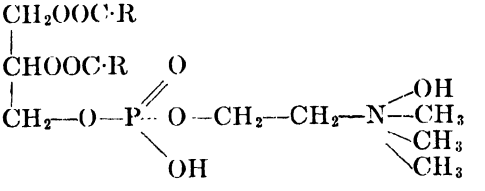
The cuticle of a leaf actually consists of two layers. A waxlike material is laid down on the external walls of the epidermis. This is called *cutin*. On top of the cutin is a waxy layer which has been deposited in scales. It is this layer which lends itself to polishing. The wax, but not the cutin, is soluble in ethyl ether. If the waxy layer is removed from fruits and vegetables during the processing, it is usually replaced by a commercial wax to prevent shriveling when these products are subsequently offered for sale.

Suberin is somewhat similar to cutin, but the main distinction is that glycerol is a constituent of suberin but not of cutin. Suberin is an important constituent of cork and is found in the bark and roots of trees, in seed

coats, and the epidermis of potato tubers. It is not quite as impervious to water as is cutin.

Compound Lipids

The only compound lipids occurring in plants are the *phospholipids*, sometimes called *phosphatides*. They are similar to true fats in that they are glycerides, but they differ in containing only two molecules of fatty acids. The third linkage is combined with phosphoric acid, one hydrogen of which is displaced by a nitrogenous base. The latter is usually choline, and less frequently it is amino-ethyl alcohol. *Lecithin* is a well-known phosphatide. Its structural formula is as follows, with the R representing the fatty acid radical:



The substituent in the phosphoric acid molecule is choline. It is interesting to note that choline is closely related to the poison, muscarine, which is found in the poisonous mushroom, *Amanita muscaria*.

Cephalin is a phospholipid which accompanies lecithin in plant tissues and is abundant in nervous tissues of animals. In cephalin the nitrogenous base is ethanolamine instead of choline as it is in lecithin.

It is quite evident that phospholipids are metabolic and not storage lipids. They are probably present in all living cells. One of the hydrolytic products of phospholipids is glycerophosphate, whether the cleavage be brought about by acids or enzymes. This fact alone suggests that compound lipids must play an important role in metabolism, because glycerophosphate (triosephosphate) is an intermediate product in photosynthesis, in fat synthesis, and no doubt in other processes. Lecithinases, the enzymes which hydrolyze lecithins, have been found in both plant and animal tissues. It has been reported that these enzymes are present in the venoms of snakes, and that it is the lysolecithin produced by the enzymes which destroys both red and white blood cells.

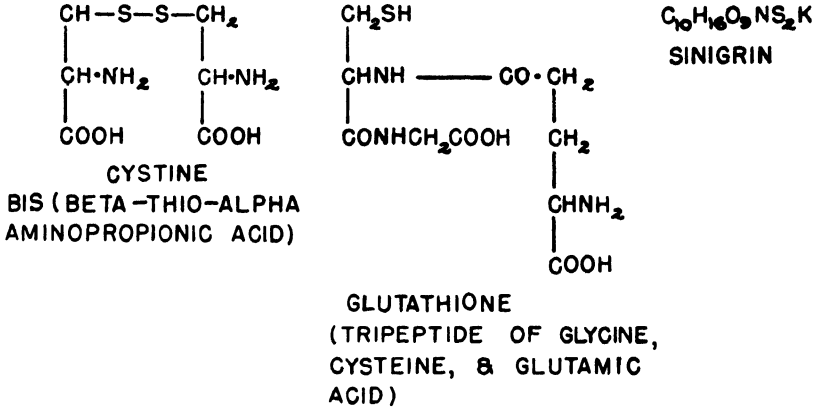
Derived Lipids

Since we have already discussed fatty acids we shall limit this discussion to the remaining group of derived lipids, i.e., *sterols*. These are monohydroxy alcohols of high molecular weight. The best known sterol is *cholesterol* ($C_{27}H_{45}OH$) which occurs in brain and nervous tissue of animals. It does not occur in higher plants. However, a number of plant sterols called *phytosterols* are known. *Ergosterol* ($C_{28}H_{43}OH$) was first discovered in ergot, a fungus disease of cereals and grasses. Ergosterol is

converted into the antirachitic vitamin (calciferol) by irradiation. A number of other sterols have been isolated from plants.

Essential Oils

To the average person the word *oil* may suggest any one of three types of substances: vegetable oils (liquid fats), petroleum oils (primarily



Examples of sulphur-containing compounds found in plants.

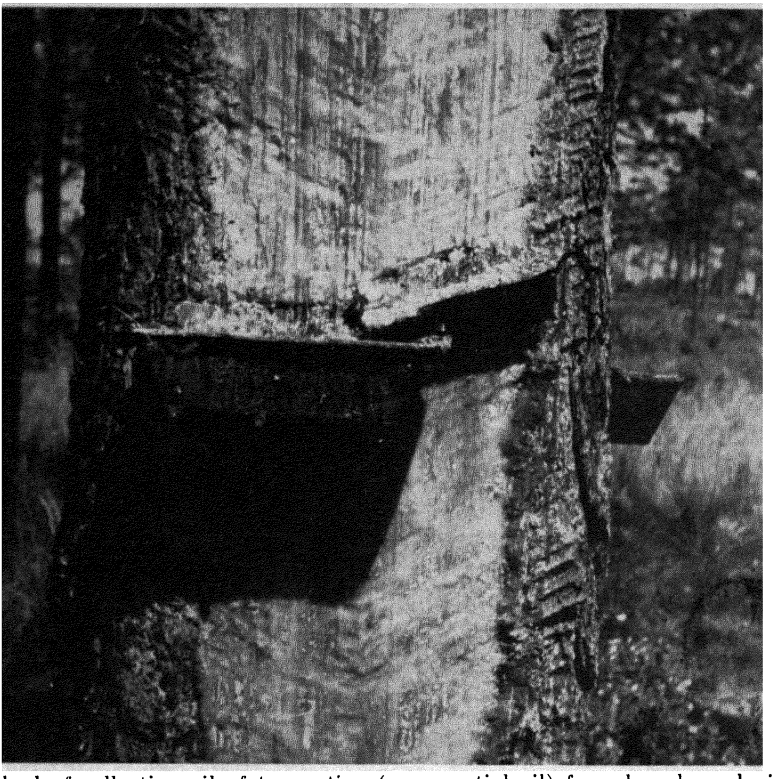
employed for fuels), and essential oils of plants. From a chemical standpoint, a true fat or oil is a glyceride of a fatty acid. Therefore, only the vegetable oils properly belong in this chapter. We shall include the essential oils, however, because their physical properties resemble those of liquid fats in many respects.

Essential oils consist of mixtures of many substances. In fact, it has been claimed that over 500 different chemical compounds have been identified in essential oils. Some of these compounds are terpenes, alcohols, aldehydes, ketones, organic acids, and esters. Many of the essential oils are pungent and aromatic, and they impart the distinctive odors and flavors to plants.

Terpenes ($C_{10}H_{16}$) constitute a large portion of the oils of turpentine, orange, lemon, pennyroyal, pine, eucalyptus, and others.

Another group of essential oils contain oxygen and some contain sulfur. Examples of oxygenated oils are camphor, thymol, menthol. The essential oils in onions, radish, garlic, mustard, and the like, owe their pungency to allyl isothiocyanate (C_3H_5CNS).

Man has found many uses for essential oils such as in making flavorings and drugs, but thus far their role in the plant is not understood. Some, like those found in flowers, may serve to attract pollinating insects. Some of the more pungent ones, like those in the rinds of tropical fruits, doubtless serve to repel predatory insects.



Method of collecting oil of turpentine (an essential oil) from long-leaved pine tree.

Ether Extract

Quite frequently the fat content of plant material is reported as *ether extract*, since the customary method of analysis consists of extracting dried plant tissue with ethyl ether. In view of the discussion in this chapter, it should now be apparent to the reader that an ether extract may contain substances other than true fats. Closely related substances, like fatty acids, sterols, waxes, and phospholipids will be extracted by the ether. But in addition to these lipoidal substances there may be essential oils, resins, and pigments. Furthermore Sando has pointed out that petroleum ether is a better extractant than ethyl ether because the former removes a lesser quantity of nonlipid material. By the use of refined analytical methods various factions of the ether extract may be separated and evaluated. When plant material has been extracted with ethyl ether, the results should be reported as *crude ether extract.*

Fats in General

The study of fats does not appear to have interested the plant physiologist to the same extent as it has the animal physiologist, judging from

the quantity of research in this field during the past 25 years. Although fundamental processes involving fats are similar in both plants and animals, there appear to be slight differences in regard to reactions of the organisms as a whole. Surplus foods in animals are usually stored in the form of fats, whereas in plants, stored carbohydrates tend to predominate over fats.

Fats are high-energy foods. The yield in kilogram calories per gram of fat is 9.3. The equivalent figure for protein is 5.7, and for carbohydrates it is 4.1. To the warm-blooded animal this is important, and is especially important to those living in arctic climates. But energy in the form of heat is of no use to the plant. It is wasted energy, like friction in a machine.

The question has been raised as to whether or not fungi secrete fat-splitting enzymes in any quantity. Harvey states that the cuticle of fruits and vegetables constitutes a complete protection against parasitic fungi and that these microorganisms gain access to the host only when the epidermis is broken.

The slight quantities of lipids that have been found in humus suggest that fats do not accumulate in the soil. It is apparent that lipoidal substances resulting from plant and animal residues are decomposed by soil organisms, but whether fungi or bacteria are the active agents is not known.

Lipids have been found to persist in certain types of soils in the form of insoluble soaps. At least one instance has been reported in which persistence of "dry spots" in a sandy soil was related to both the lipid content and certain cultural practices. When both magnesium and calcium had been added in the fertilizer, insoluble soaps were formed with the fatty acids in the soil. Areas containing these insoluble soaps remained dry even after prolonged periods of rainfall.

chapter 13

Nitrogen

The atmosphere which surrounds the earth and penetrates the pores of the soil contains 78% nitrogen. Since it is in the elemental form, atmospheric nitrogen is inert and of no immediate use to most plants and all animals. (Traces of ammonia and nitric oxides in the atmospheric envelope constitute an exception to this statement.) The nitrogen which is taken up by the roots of plants exists in the combined form such as nitrates, ammonium salts, or possibly in organic compounds. Rocks from which soils are derived contain no nitrogen. It is obvious therefore that there is no other source of nitrogen for plants save the atmospheric envelope which has just been mentioned. How then is the elemental nitrogen of the air converted into a form which is readily available to all plants?

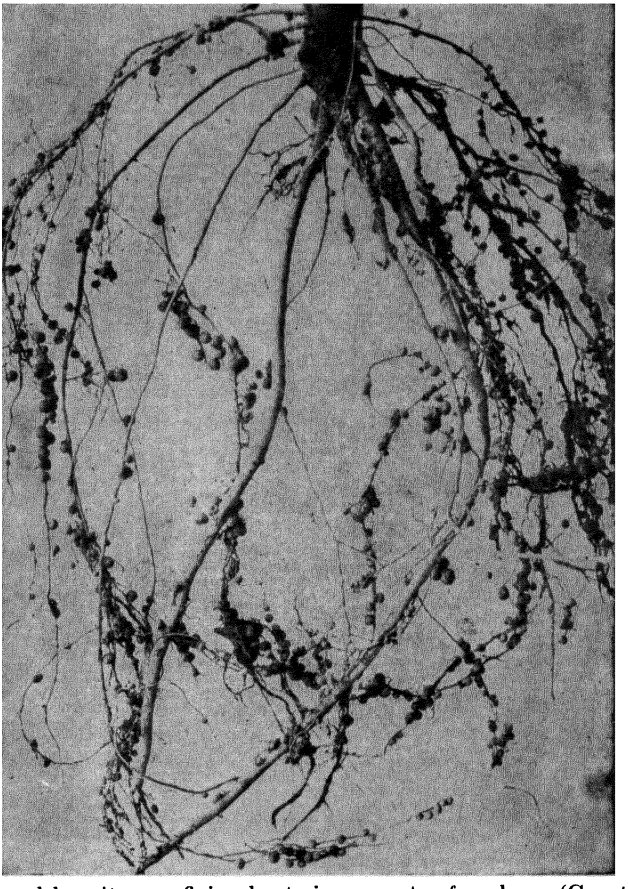
Fixation of Atmospheric Nitrogen

As early as 1882 it was observed that nitrogen accumulates in the soil when conditions are favorable for plant growth. The explanation of this phenomenon appeared not long afterward when it was discovered that certain microorganisms in the soil are able to utilize molecular nitrogen. S. Winogradski, a Russian scientist, isolated a bacterium which possessed these characteristics. It belonged to the genus *Clostridium*. Later another nitrogen-fixing bacterium was discovered. This one belonged to the *Azotobacter* group. These two groups of bacteria oxidize the organic matter which is present in the soil, utilizing part of the energy to "fix" atmospheric nitrogen. The exact mechanism by which this occurs is not known. Whatever the process is, these soil microorganisms are able to convert the atmospheric nitrogen into organic compounds which make up the protoplasmic structure of the cells. The compounds are probably amino acids, proteins, and other related substances. When the bacteria die and their cells disintegrate, organic nitrogen is returned to the soil.

For a number of years it was believed that nitrogen-fixation was a characteristic only of bacteria. Recently, however, it has been reported that the blue-green alga, *Nostoc*, possesses this characteristic too.

The two groups of nitrogen-fixing bacteria which have been described are both saprophytes, that is, they derive their source of energy from nonliving organisms. Another type of nitrogen-fixing bacteria holds

greater interest for the physiologist. This type is symbiotic. It enters into a mutual relationship with a living plant. Microorganisms of this type belong to the genus *Rhizobium*. Host plants to these organisms are the legumes, i.e., peas, beans, clover, alfalfa, etc. The bacteria enter the roots of the host through the root hairs. The presence of the symbiont in the



Nodules caused by nitrogen-fixing bacteria on roots of soybean (Courtesy, U.S. Dept. of Agriculture.)

plant is evidenced by the formation of nodules on the roots. Legumes are so nearly universally infected by these symbiotic organisms that some people are wont to include the presence of nodules on the roots in the definition of leguminous plants.

Within the nodule of the root symbiotic bacteria combine carbohydrates of the host with nitrogen of the air to form organic nitrogenous compounds. It is obvious from these facts that growing and plowing under leguminous cover crops serves to enrich the soil. Chinese farmers, before

the Christian era, were aware of this fact although they did not understand the reasons for it.

Ammonifying Bacteria

As both plant and animal matter decompose in the soil the nitrogen is first released in the form of organic compounds. Plants may absorb some of their nitrogen directly as organic compounds but most of the element is taken up in inorganic form. Here again we are dependent upon microorganisms for making the nitrogen more available to plants. The *ammonifying* bacteria act on the organic nitrogenous matter in the soil, converting it to ammonia. Certain filamentous fungi may act in the same capacity. The quantity of ammonia released by these organisms is dependent upon a number of conditions, such as acidity, aeration, moisture of the soil, and the nature of the organic matter present in the soil. For example, the ammonifying organisms appear to prefer carbohydrates to proteins for their source of energy so that the presence of large quantities of carbohydrates in the soil will tend to reduce the quantity of ammonia which is released. The gardener who plows under cellulosic material, like straw and leaves, without adding nitrogen, will not be enriching his soil as much as he believes. Legumes contribute much more to the soil because they add nitrogen as well as cellulosic material.

Nitrifying Bacteria

Analyses of soil rarely reveal significant quantities of nitrogen in the form of ammonia. The main reason for this is that nitrifying bacteria in the soil serve to convert ammonia to nitrites and finally to nitrates. The oxidation of ammonia to nitrites is brought about by species of *Nitrosomonas* and *Nitrosococcus*. These organisms do not carry the oxidation any further. Another genus, *Nitrobacter*, acts on nitrites, converting them to nitrates. Nitrifying bacteria obtain their energy from the oxidation of ammonia to nitrites or of nitrites to nitrates and thus differ from higher plants.

Not all of the inorganic nitrogen in the soil may have been produced by organisms. A certain amount of ammonia may be found in the air, originating in industrial fumes. Electrical discharges are also known to produce oxides of nitrogen. These compounds, though present only as traces, are washed down into the soil in rain and snow and may account for larger quantities of nitrogen than is generally realized. It has been reported at the Rothamsted Experiment Station in England that 4.4 pounds of nitrogen per acre per year were added to the soil in the rain water.

Absorption of Nitrogen Compounds by Plants

Under natural conditions nitrates constitute the major source of nitrogen for plants. In agricultural practice nitrogen fertilizers are supplied either as nitrates or ammonium salts. When grown in culture solu-

tions plants may absorb nitrogen as nitrates, nitrites, ammonium salts, amino acids, and other forms of organic nitrogen. It should be borne in mind, however, that nitrites in acid solutions are relatively toxic to plants.

In the plant cells nitrates are reduced, step by step, until ammonia is formed. Sophia H. Eckerson demonstrated the successive steps in nitrate reduction by the following experiment. Vigorously growing tomato plants were transferred from the soil to culture solutions which lacked nitrogen. The plants were maintained under these conditions until they gave negative tests for nitrates, nitrites, ammonia, and amino acids. Then calcium nitrate was added to the solution. With the aid of microchemical tests the following results were obtained:

In 24 hours nitrate ions were present in all parts of the plants.
In 36 hours nitrites were observed in stem tips and in certain other tissues. Traces of ammonia appeared.
In 48 hours nitrites decreased; ammonia increased and a small quantity of asparagine was detected.
In 3-5 days amino acids appeared and continued to increase.

Thus, nitrates are gradually reduced until the final product is ammonia. Ammonia combines with fatty acids to form amino acids.

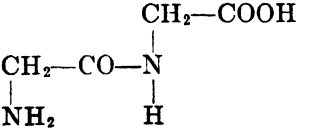
Amino Acids

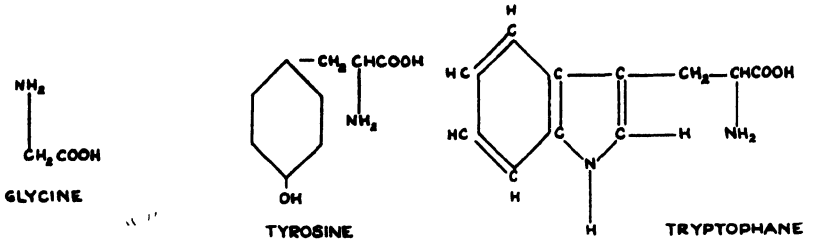
In Eckerson's experiments carbohydrates diminished while nitrates were being reduced. This is interpreted to indicate that fatty acids were being formed from the carbohydrates. It will be recalled that during the discussion of lipids it was stated that such a transformation is known to occur in plants. Combination of ammonia with a fatty acid yields an amino acid.

$$NH_3 + CH_3{\cdot}COOH \rightarrow CH_2{\cdot}NH_2{\cdot}COOH + H_2O$$

The amino acid depicted above is *glycine*, the simplest known representative of this group of acids. Structurally glycine is alpha-aminoacetic acid.

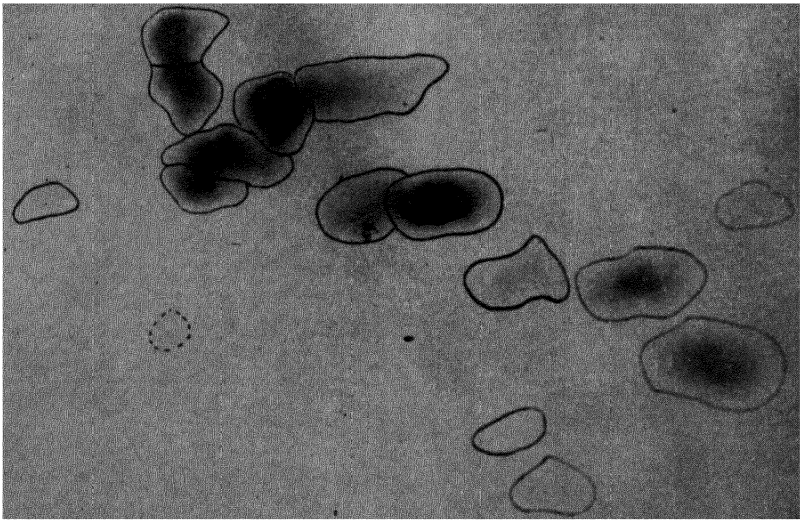
Twenty-two amino acids occur in nature as constituents of proteins and a number of others have been identified in hydrolysates. The amino acids *cystine* (also *cysteine*) and *methionine* contain sulfur. *Tyrosine* ($HO{\cdot}C_6H_4{\cdot}CH_2CH(NH_2){\cdot}COOH$) and *phenylalanine* ($C_6H_5{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH$) contain a benzene ring, and *tryptophane* contains an indole group. Amino acids constitute the building blocks of proteins and their cleavage products. The acid group of one unit is combined with the amino group of another, thus constituting what is known as the *peptide linkage.*





Structural formulas of several amino acids.

A combination of two amino acids in this manner is termed a dipeptide. When more than two acids are linked the compound becomes a polypeptide. A protein may contain from 300 to 3000 amino acids. The length of the chain and the specific acids in the molecule impart specific



Two-dimensional chromatogram of amino acids in the hydrolysate of the alcohol-insoluble fraction of the alfalfa leaf. Amino acids are identified by their relative positions. (Courtesy, Steward: "The Amino Acids of Alfalfa as Revealed by Paper Chromatography with Special Reference to Compounds Labelled with S^{35}," *Plant Physiol.* **26**:123–135, 1951.)

characteristics to each protein. Molecular weights of proteins may vary from about 10,000 to several hundred thousand or even several million.

A number of amino acids are now known to be necessary for normal growth and development of man and other animals. The essential amino acids therefore occupy as important a place in nutrition as vitamins. It is interesting, also, to realize that vertebrates depend upon the plant kingdom for the synthesis of amino acids.

Simple Proteins

Simple proteins are classified principally according to their solubilities, as follows:

Albumins: soluble in water and coagulated by heat.

Globulins: insoluble in water but soluble in dilute salt solutions.

Prolamines: insoluble in water, dilute salt solutions, dilute acids and alkalies, and absolute alcohol. They are soluble in 70% to 90% alcohol.

Glutelins: soluble in dilute acids and alkalies, insoluble in water or dilute neutral salt solutions.

Albuminoids, histones, and *protamines* are simple proteins which are not known to occur in plant tissues.

Conjugate Proteins

Conjugate proteins represent a type of compound containing a protein and a nonprotein group.

Nucleoproteins: one or more protein molecules combined with nucleic acid.

Phosphoprotein: a protein and a nonprotein molecule containing phosphoric acid.

Chromoprotein: a protein combined with a pigmented nonprotein group.

Lecithoprotein: a protein combined with a phospholipid.

Glycoproteins: combinations of proteins and carbohydrates.

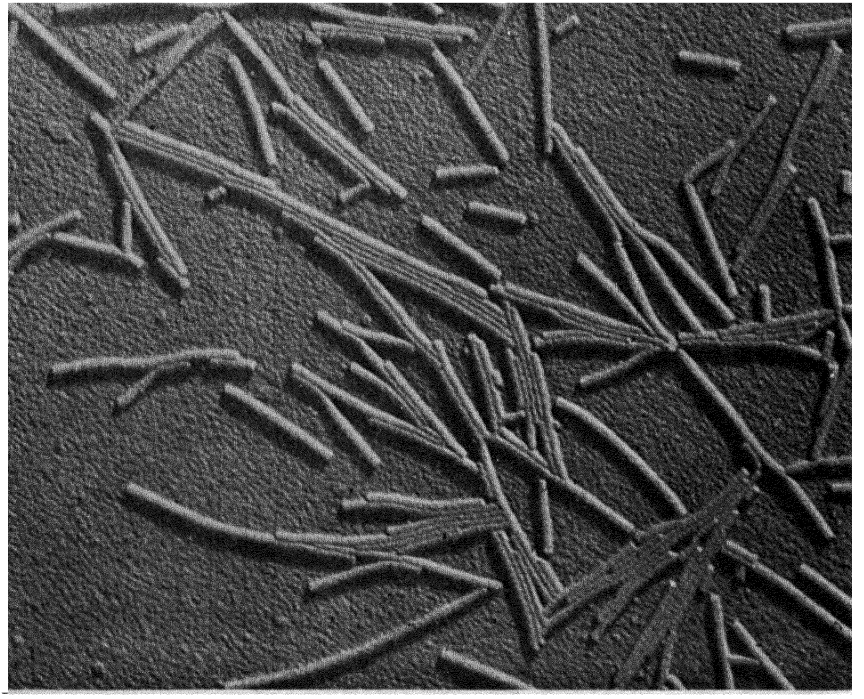
Protein Metabolism

The importance of proteins to the living plant is indicated by the fact that the organic matter in protoplasm is mainly protein or nitrogen-containing compounds. Proteins in plants may be grouped into two classes depending upon their roles in metabolism. Some of them serve as reserve foods and are found in great abundance in the cells of storage organs, such as seeds, roots, tubers. They are hydrolyzed to amino acids during periods of accelerated metabolic activity. The other type of proteins are sometimes called *functional proteins.* They constitute a part of the enzyme molecule, the matrix of the chloroplast, and are also found in the nucleus of the cell. In addition, all enzymes which have been identified have been shown to be protein in nature. One type of enzyme consists of only a protein molecule. In another type the protein molecule is attached to a nonprotein or prosthetic group. Functional proteins play active roles in the metabolism of the plant. When proteins are hydrolyzed, either by enzymes or acids, the degradation of the protein molecule proceeds through several intermediate steps with amino acids as the end product. Successive steps are as follows: Proteins → proteoses → peptones → polypeptides → dipeptides → amino acids.

It is in this manner that storage proteins are broken down as seeds begin to germinate. Amino acids are translocated to various parts of the plant, especially to the growing points. The specific roles of nitrogen in the plant will be discussed in the chapter on minerals.

Nucleoproteins

Nucleoproteins appear to occupy a foremost position in the activities of the living cell. The nonprotein portion of the molecule consists of



Characteristic particles of tobacco mosaic virus magnified 100,000 times. (Courtesy, Thomas R. Padden, reproduced by permission of Dept. of Biophysics, Univ. of Pittsburgh.)

nucleic acid, a compound of great molecular weight. Ribonucleic acid, for example, consists of a long chain of nucleotides, the latter being basic nitrogen compounds (like adenine) linked to a phosphate group by means of ribose. Nucleoproteins which occur in nuclei contain desoxyribose; those in the cytoplasm have ribose in the molecule.

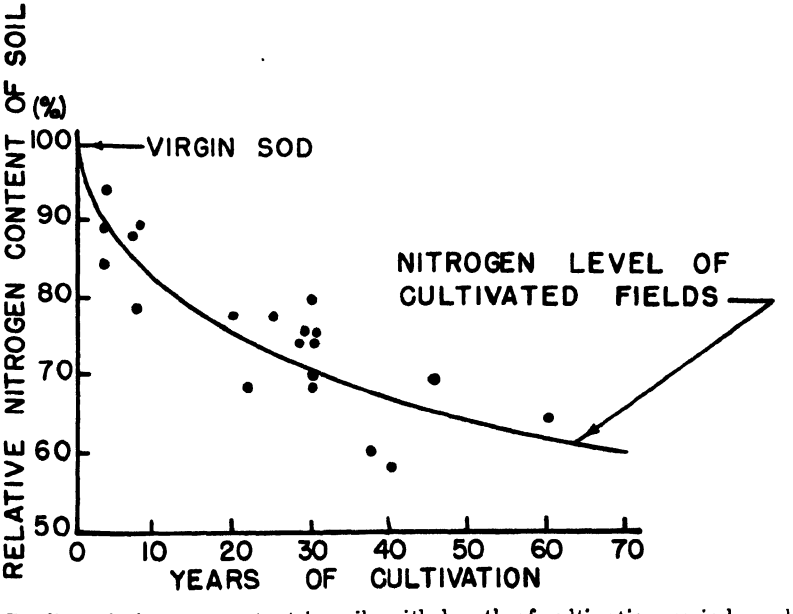
Nucleoproteins are so named because they were discovered in the nucleus of the cell. They are components of chromosomes. It is apparent, therefore, that these particular conjugated proteins are concerned directly with reproduction in the organism. This belief was given further support by the discovery that plant viruses are also nucleoproteins. Pathologists

were long puzzled by some of the characteristics of filterable substances (viruses) which cause diseases in higher plants. It was known that, once introduced into a plant, a virus is capable of increasing, much as a bacterium multiplies in its host. W. M. Stanley (1935) was the first to show that a virus is a protein, since he isolated and crystallized the virus which causes tobacco mosaic.

Virus proteins constitute a link between the animate and inanimate world. In the plant they are capable of growth and reproduction. In the test tube they act like pure chemical compounds. It has been suggested that virus proteins are nucleoproteins which have escaped from the nucleus, thereby causing abnormal conditions (disease) in the cytoplasm.

Nitrogen Balance in the Soil

Nitrogen content tends to remain constant in a soil in which the natural vegetative covering has not been disturbed. The accumulation of plant residues and the action of nitrogen-fixing bacteria serve to maintain this nitrogen balance. But when this orderly system of nature is changed as a result of agricultural practices, the nitrogen content of the soil is gradually reduced. Some is lost by erosion. More is lost by removal of crops from the land. In addition to all of this, farming may reduce soil nitrogen is still another way. Constant manipulation of the soil cover, as



Decline of nitrogen content in soils with length of cultivation periods under average farming practices in the Middle West. (Courtesy, U.S. Dept. of Agriculture Yearbook, 1938.)

in plowing, serves to destroy the biological balance, with the result that soil microorganisms no longer function as efficiently as under natural conditions. This is illustrated in a long-range experiment which was conducted at the Missouri Experiment Station. Records were kept of the nitrogen content of the soil in a plot during 70 years of cultivation. Nitrogen present in the virgin soil before cultivation began was expressed as 100. The results showed a steady decline in the nitrogen content during the period of cultivation until, at the end of 70 years, it had been reduced to nearly half of that present in the virgin soil.

It should be apparent from what has been said that good farming demands periodic growing and plowing under of leguminous cover crops and that application of mineral fertilizers should be supplemented with organic materials.

Organic Versus Mineral Fertilizers

It should not be inferred from preceding statements that organic fertilizers alone will necessarily supply all of the required elements to the average soil in a farm or garden. Good soil management requires the use of both organic and inorganic fertilizers. This is the view of most plant physiologists. One can find extreme enthusiasts, however, for either organic or inorganic fertilization alone. Perhaps the most avid group are the so-called "organic gardeners." This group adheres to the belief that only organic materials should be employed for restoring fertility to gardens. They claim that continued use of inorganic fertilizers poisons the soil and makes the plant more susceptible to fungous and bacterial diseases. This group of gardeners claim that certain organic diseases in man have been caused by his consuming foods which were produced on land supplied with mineral fertilizers.

An experiment which was conducted at the Rothamsted Experiment Station in England should allay the fears of the organic gardeners. J. B. Lawes and J. H. Gilbert, in 1843, began an experiment which was designed to determine the most suitable type of fertilizer for growing wheat. One plot received only organic fertilizers; another was given only commercial inorganic fertilizers; and the third received no fertilizer treatment at all. In 1951 the 108th crop of wheat was growing in these plots. At this time Sir William Ogg reported the results: one plot, which received no fertilizer at all, continued to produce 12 bushels of wheat per acre; the plot receiving 14 tons of farmyard manure each year produced three times as much wheat as the unfertilized plot; a third plot, which had received liberal supplies of complete fertilizers each year, yielded as much wheat as the organically fertilized plot. Crops produced on the fertilizer plots were in no way inferior to those produced on the plots which received farmyard manure. The fertilizer plots continued to maintain a normal population of earthworms and microorganisms, and the crops grown on

them were no more susceptible to disease than those receiving organic manures.

The following problem has been offered students on a number of occasions to determine if they are able to apply some of the principles expressed in the chapter.

Gardener's Dilemma

1. In preparation for a garden you clear a vacant lot by burning over the land. (Your reason for doing this is to remove the dense growth of weeds and briers and to leave a residue of ash or mineral matter for your crops.)
2. An organic-fertilizer enthusiast happens to see this and says: "Why didn't you plow under all the weeds and grass? All of your organic matter has gone off in smoke."
3. Next year, after having had a vegetable garden, you decide to turn under the organic matter. Corn stalks, tomato vines, bean plants, flowers, and weeds are all plowed under in the spring.
4. A plant pathologist appears. He says: "See what you have done! You have added infection to your soil. You have plowed under tomatoes with late blight fungus growing out of them; fruiting bodies of corn smut fungus are doubtless in the soil at the moment. These organisms may remain in the soil during the winter. How many Mexican bean beetle larvae did you plow under? You should have burned all of this plant residue and added clean organic matter."
5. The third year you burn all plant residue. You then plow under a bale of clean straw. Now a plant physiologist visits you. He says: "You are supplying carbohydrates to the ammonifying bacteria. It is true that these organisms normally consume proteins of the organic matter present, and that they release ammonia as a by-product. However, they prefer carbohydrates to proteins as a source of energy. Therefore the more carbohydrates (cellulose from straw, etc.), the less ammonia the organisms will add to your soil."
6. Finally a reader of *Plowman's Folly** arrives. He says: "Why plow at all? By doing so you break the continuity between topsoil and subsoil. The top layer will dry out faster. Under natural conditions there exists in the soil an equilibrium between the formation of organic matter (from vegetation) and its decomposition by microorganisms (bacteria, actinomyces, and filamentous fungi). Now you have destroyed this equilibrium and there will be less nitrogen."

Student of plant physiology, what would you do?

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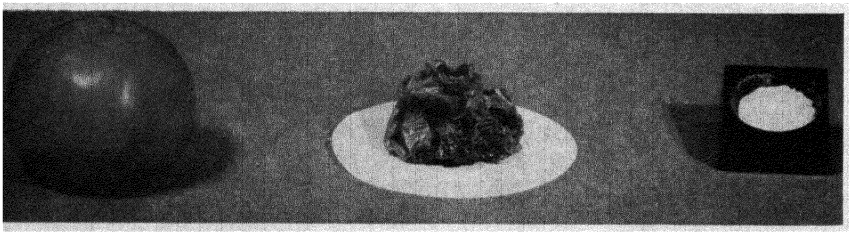
* Faulkner, Edward H.: *Plowman's Folly*, Norman, Okla., University of Oklahoma Press, 1944.

chapter 14

Mineral Nutrition

Ash in Plants

It is not difficult to demonstrate the presence of mineral matter in plants. If a dried plant is incinerated in a muffle furnace, the organic matter will be oxidized and driven off as water and carbon dioxide. The small amount of gray or white ash remaining after incineration is mineral matter. This residue is variously called *ash, mineral matter,* or *inorganic constituents.* All of the nitrogen and some of the sulfur and phosphorus will have been lost along with the organic matter so that it is not correct to say that all of the inorganic matter remains. Accurate analysis of plant



A 150 Gm. tomato was dried in the vacuum oven. Dry weight of this fruit (center) was 8.7 Gm. After ashing in the muffle furnace the remaining mineral matter (extreme right) weighed only one Gm.

tissue requires the determination of nitrogen, phosphorus, and sulfur before ashing. Nevertheless, it is often desirable to know the *per cent ash* of plants and plant materials, and analyses of foods usually include *total ash.* The quantity of ash which remains after incineration of a plant or plant product is surprisingly small. The amount of ash varies with the type of plant material under observation. Expressed in terms of fresh weight, the quantity of ash appears much smaller because living tissues contain so much water. The relative proportions of water, dry matter, and ash can be appreciated from the following results. A medium-sized tomato weighed 150 Gm. in the fresh state; when dried in a vacuum oven the weight was 8.7 Gm. Finally, upon incineration, the weight was 1.0 Gm.

The difference between leaves and fruit in regard to mineral matter is shown in Table 20. It will be seen that 100 Gm. of leaves of an orange

tree contained 1.71 Gm. of ash; the rind of the fruit contained 1.13 Gm.; and the flesh contained only 0.42 Gm. per 100 Gm.

Table 20

MINERAL CONTENT OF FRUITS AND LEAVES OF CITRUS

Tissue or Organ	*Gm. Ash per 100 Gm. Fresh Weight*
Leaves	1.71
Rind of fruit	1 13
Flesh of fruit	0 42

Culture Solution Methods

The mere presence of an element in the ash of a plant is not an indication of its essentiality. At least 40 elements have been found in plants, and it is probable that many more will be reported from time to time. But according to present knowledge a plant requires only 15 elements in order to complete its cycle of growth. Some idea of the mineral matter in a plant may be obtained from Table 21 which presents the results of an analysis of a whole corn plant.

Table 21

COMPOSITION OF WHOLE CORN PLANT*

Element	*% Dry Weight*
Oxygen	44.43
Carbon	43 57
Hydrogen	6 24
Nitrogen	1 46
Phosphorus	0 20
Potassium	0 92
Calcium	0.23
Magnesium	0.18
Sulfur	0 17
Iron	0 08
Manganese	0.03
Silicon	1.17
Aluminum	0.11
Chlorine	0.14
Undetermined elements	0.93

* Miller, Edwin C.: *Plant Physiology*, 2nd ed., New York, McGraw-Hill Book Co., 1938.

Our present knowledge regarding mineral requirements has been obtained largely by means of so-called *culture solutions*. In studies of this type, test plants are set in crocks containing inert materials, like quartz sand, and the crocks are watered periodically with aqueous solutions of mineral salts. Another method consists of placing nutrient solutions in crocks and supporting the plants so that their roots make contact with the solutions. The soil is too complex to serve as a medium for the roots in order to study the mineral requirements of plants. As we shall see a little later, absorption of salts from the soil by plants is not as simple as

was once thought, and even though adequate quantities of elements are supplied, there is no assurance that they will be readily available. The use of solution cultures not only supplies information on the mineral requirements of plants, but also enables one to determine some of the specific functions of individual elements in the metabolism of the plant. This method of growing plants has been popularized in recent years and has been given various names, such as *soilless growth*, *nutriculture*, and *hydroponics*. The method is not new, but has been employed by physiologists for hundreds of years. Perhaps the first true *culture-solution* experiment was performed in 1699. In that year J. Woodward of England, grew several species of plants in different kinds of water. He tried rain water, river water, spring water, conduit water, and distilled water. The results indicated that something besides water was required by plants. Woodward concluded that water is the carrier of terrestrial matter and that it is the earth and not the water which nourishes the plants.

In 1840 Justus von Liebig published a book entitled *Organic Chemistry in Its Application to Agriculture and Physiology*. This book appeared at the time when most physiologists believed that plants obtained carbon from humus in the soil. Liebig advanced the mineral theory of nutrition and was the first to recognize that the source of carbon in plants is the carbon dioxide of the air. Liebig had little patience with opponents of his theory and largely as a result of this attitude his views were not accepted at the time. Unfortunately, his attempt to put his mineral theory into practice was a complete failure. However, the old *humus theory* was dropped when other workers substantiated Liebig's theory. Boussingault (between 1837 and 1887) successfully grew plants in inert materials, such as quartz sand and charcoal, by watering them with nutrient solutions. Sachs, in 1860, grew a variety of land plants, such as Indian corn or maize in solutions containing dissolved mineral salts and no organic matter. W. Knop and F. Nobbe were also working on culture solutions during the same period (1860 to 1868) and we are indebted to the last three investigators for perfecting the synthetic nutrient solutions for plant growth. Over the period of years extending from 1882 to 1920 significant contributions were made to the subject of nutrient solutions by B. Tollens, A. F. W. Schimper, W. Pfeffer, G. Crone, W. E. Tottingham, J. W. Shive, and D. R. Hoagland.

Techniques

A knowledge of the technique of solution culture is essential to the study of plant physiology. The interest of laymen has also been aroused in recent years and many amateur gardeners have sought information on this method of cultivating plants. The practice of soilless culture was given additional impetus during World War II as a result of certain conditions which made it necessary to produce *hydroponic* vegetables in com-

mercial quantities. The following is a brief discussion of the methods for the preparation of solution cultures. Many precautions must be observed in order to insure satisfactory results. These are listed below, with elaborations when necessary.

1. The solution must contain an ample supply of all essential elements. A convenient method for supplying nutrients consists of preparing three types of solutions.*

SOLUTION A (MAJOR ELEMENTS)

	Ml per Liter of Water
Molar KH_2PO_4 (potassium acid phosphate)	1
Molar KNO_3 (potassium nitrate)	5
Molar $Ca(NO_3)_2$ (calcium nitrate)	5
Molar $MgSO_4$ (magnesium sulfate)	2

Molar solutions of the four compounds listed above are made up as follows:

KH_2PO_4	136.16 Gm. per liter of H_2O
KNO_3	101.11 " " " " "
$Ca(NO_4)_2$	164.09 " " " " "
($MgSO_4$	120.38 " " " " ")
or	
($MgSO_4 \cdot 7H_2O$	246.49 " " " " ")

SOLUTION B (MICRONUTRIENTS)

	Gm. per Liter of Water
H_3BO_3 (boric acid)	2.86
$MnCl_2 \cdot 4H_2O$ (manganese chloride)	1.81
$ZnSO_4 \cdot 7H_2O$ (zinc sulfate)	0.22
$CuSO_4 \cdot 5H_2O$ (copper sulfate)	0.08
$H_2MoO_4 \cdot H_2O$ (molybdic acid)	0.09

SOLUTION C (IRON)

0.5% iron tartrate

One ml of (B) is added to one liter of (A) when making up solutions. One ml of (C), the iron solution, may be added to each liter of nutrient solution just before using.

2. All solutions must have the same osmotic pressure.

3. A constant pH must be maintained (preferably pH 6).

4. Provision must be made for aerating the roots. If the plants are grown in media like sand or vermiculite, aeration of roots may not constitute a serious problem. If the roots are suspended in solution and the plant supported by wire mesh, then air must be bubbled through the nutrient solution.

5. Solutions must be changed periodically to assure that their concentration is not changing.

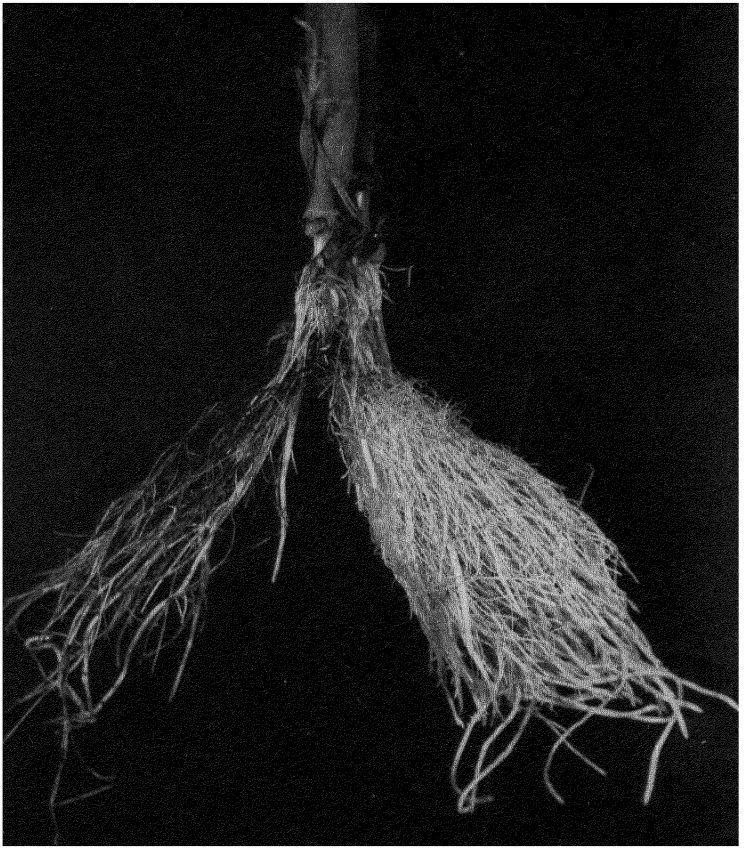
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* Hoagland, D. R., and D. I. Arnon: *The Water-Culture Method for Growing Plants without Soil.* (University of California Agricultural Experiment Station Circular #347.) 1938.

6. If salts have a tendency to crystallize and to "creep" up the stems of plants, they must be washed down with water occasionally.

7. If plants are growing in solutions rather than in solid media, they must be supported with stakes.

8. Other growing conditions such as light and temperature must be adequate.



Effect of minerals on root growth. Left half of corn roots was grown in distilled water; right half in nutrient solution. (Courtesy, Bureau of Plant Industry, U.S. Dept. of Agriculture.)

If all of these conditions are favorable, yields may be produced which are equal to, but not greater than, those grown out-of-doors under suitable conditions. Economic justification for commercial production of hydroponic crops depends upon several factors. These may be best appreciated by reference to the conditions under which the U.S. Army Quartermaster Corps approved the establishment of hydroponic gardens during World War II. These are as follows: (1) if no soil is available in

which vegetables can be grown safely in the normal agricultural manner; (2) if the installation is so located that no fresh vegetables can be supplied by shipping in an adequate quantity at reasonable cost; (3) if the post, camp, or station is permanent or is expected to be in existence for a considerable period; (4) if there is at least a four-month growing season each year; (5) if there is an ample supply of suitable water.

Under favorable conditions plants grown in solution cultures have been found to be similar to those produced in soil. Analyses have indicated no significant differences in the minerals absorbed or in the vitamin content of the plants produced.

Essential Elements

As a result of the work with mineral nutrient solutions previously described, we now know that 15 elements are essential to plants. Up to the beginning of the 20th century only ten were accepted as essential. These ten were carbon, hydrogen, oxygen, phosphorus, potassium, nitrogen, sulfur, calcium, iron, and magnesium.* They are often called the *major elements* because (with the exception of iron) they are required in larger quantities than are the five *minor elements* which have been added to the list rather recently. The minor elements are boron, copper, manganese, molybdenum, and zinc. They are also referred to as *trace* or *less common* elements, and as *micronutrients*. The reasons that trace elements were so long overlooked will be listed under the detailed discussion of these elements.

Test of Essentiality

To be considered essential an element must meet the following requirements:

1. Absence of the element must cause injury, abnormal development, including failure to complete its life cycle, or death of the plant.
2. No other element may be successfully substituted for the one being tested.
3. The test must be applied to a large number of species, in order to determine whether or not the element is universally required.

Arnon (1951) has made the suggestion that the essentiality of an element can be proved beyond doubt by demonstrating that it is a normal constituent of a plant or of a compound which participates in an essential function. Carbon, hydrogen, and oxygen have long been recognized as constituents of protoplasm and the cell wall. Nitrogen and sulfur are found in proteins, and phosphorus occurs in a number of important con-

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* To make the list easy for the student to remember Hopkins formulated a mnemonic device which reads: C HOPKNS Ca Fe Mg., i.e., C. Hopkin's Cafe, mighty good. The word *mnemonic* is derived from Mnemosyne, the Greek goddess of memory.

stituents such as phosphatides, the hexose phosphates, coenzymes, etc. Magnesium is essential for chlorophyll; calcium occurs in middle lamellae of cell walls. Iron, copper, manganese, and zinc are now known to form necessary parts of enzymes. Present knowledge is insufficient to warrant the assignment of a specific place for potassium, boron, and molybdenum in definite compounds although there seems to be no doubt of their essentiality. Perhaps as techniques become more refined we shall determine the exact function of all required elements.

Sources of the Elements

We have already noted that carbon, hydrogen, and oxygen are obtained from the carbon dioxide of the air, and from the water in the soil. The atmosphere is indirectly the source of the plant's nitrogen. The remaining elements are derived from the rocks which constitute the parent materials of the soil. This last group may be subdivided still further into metals (K, Ca, Mg, Fe, Mn, Cu, Mo, Zn) and nonmetals (P, S, B). Both metals and nonmetals are present in the soil in the combined state such as in chlorides, sulfates, nitrates, phosphates, bicarbonates, and borates.

A question may have arisen in the minds of the students regarding the means by which essential elements are retained in the soil. If the elements must be in soluble form before they can be absorbed by the roots of plants, why are not these same elements washed out of the soil with each driving rain? It is true that some compounds, like nitrates, chlorides, etc. are leached from the soil by rains, but fortunately many are retained, especially by those soils which contain colloids. A better understanding of this subject will be obtained from a discussion of cation exchange.

Cation Exchange and Exchange Capacity

It will be recalled that hydrophobic colloid particles carry electric charges, and that each charge attracts an oppositely charged ion in the dispersion medium. The micelle is stabilized by this double layer of electric charges. It has been found that clay micelles in the soil, being negatively charged, will attract and adsorb positively charged ions such as calcium, potassium, manganese, copper, zinc, hydrogen, and the radical NH_4. It is in this manner that certain elements are retained in the soil. These cations are not only held on the surface, but also within the crystal. (Clay consists primarily of silicates of aluminum and, although the particles in the soil are colloidal in size, they have a definite crystalline structure.) Although these cations are not easily leached out of the soil, they may be displaced by cations of another kind. For example, in a neutral soil there will be, among others, calcium ions adsorbed by the micelles of clay. If a sample of this soil is washed with potassium chloride, the calcium ions will be displaced by potassium. This substitution of one ion for another on the colloidal particle is termed *cation exchange* (base exchange).

It may occur as the result of the addition of minerals to the soil in fertilizing practices, or the ion may be introduced in another manner. The metals may be displaced by hydrogen ions in the following manner. Carbon dioxide, which is released by the roots during respiration, is dissolved in the soil water to form carbonic acid. When this acid ionizes, hydrogen ions are taken up by the clay particles and metallic ions are released.

The tenacity with which the cation is held depends upon a number of factors, one of which is the relative position of the element in the lyotropic series. Thus in the following group of elements, as we proceed from left to right, each element is held less tenaciously than the one preceding it:

$$Al \quad H \quad Ba \quad Sr \quad Ca \quad Mg \quad K \quad NH_4 \quad Na \quad Li$$

We have here a possible explanation for the increase in the acidity of a soil as the result of continual cropping. Hydrogen ions are displaced with more difficulty than any other cation except aluminum. Continued release of hydrogen ions from carbonic acid may soon saturate the soil with these ions unless corrective steps are taken.

The principle of cationic exchange has introduced a new concept in the study of plant-soil relationships. We now know that replaceable ions on colloidal soil particles are just as important as those which are in solution. And the plant physiologist, in estimating the fertility of a soil, makes use of methods which determine the quantity of replaceable cations. One of these methods consists of extracting a dry sample with a buffer solution like ammonium acetate. Cations on the micelles are displaced by ammonium ions, and the extract is then analyzed for essential elements. By analyzing the soil residue for ammonia, it is possible to determine the quantity of cations which has been displaced. The results are expressed as the *exchange capacity* of the particular soil.

We have mentioned only inorganic colloids in this discussion. Organic colloids (humus) are believed to possess even greater adsorbing capacity than the inorganic colloids, but not as much is known concerning the organic type.

This discussion of cation exchange is not meant to imply that true solutions do not exist in the soil. Nutrients are present in solution to some extent, and are adsorbed by the plant. Under the new concept the soil solutions are considered to be constantly in a state of change.

Ion Exchange

The phenomenon of ionic exchange, although known for over 100 years, has only very recently had widespread application in both research and industry. The practice of softening water consists of removing from solution undesirable ions like those of calcium or magnesium. Originally, silicates occurring in nature (zeolites or greensands) were employed as adsorbing agents. The exchange capacity of these natural substances was

improved first by chemical treatment and subsequently by development of synthetic zeolites. In 1935 new possibilities in the field were opened with the discovery by B. A. Adams and E. L. Holmes that certain synthetic resins also possess ion exchange capacities. Both anions and cations can now be removed from solutions with the aid of synthetic resins. In this case it is necessary to use the hydrogen form of exchanger so that when a compound like $CaSO_4$ is to be removed, calcium will replace hydrogen, which in turn will combine with the anion to form H_2SO_4 (in the solution). Treatment with another type of resin serves to bind the acid in an insoluble form with the result that the filtrate will be as free of ions as is distilled water.

Specific Roles of Mineral Elements in Plants

Let us now devote our attention to some of the specific roles played by essential elements. Fertilizers are sometimes referred to by combinations of numerals, such as 4-8-7, 5-10-5, etc., rather than by trade names. These numbers indicate the proportions of nitrogen, phosphorus, and potassium (in this order) present in 100 pounds of the fertilizer. (Actually phosphorus and potassium are expressed as oxides, P_2O_5 and K_2O.)

Commercial fertilizers contain other elements although the N-P-K content is usually stressed, because these three elements normally require replacement in the largest quantities in soils under cultivation.

Nitrogen

The physiological significance of nitrogen in plants has been discussed to some extent under the topic of nitrogen metabolism. From the standpoint of cellular physiology, nitrogen is of considerable importance because of its role in forming proteins. Young growing cells are full of protoplasm, and protoplasm is rich in proteins. From the standpoint of the plant as a whole, nitrogen stimulates vegetative growth. Plants which have been amply supplied with nitrogen are large and succulent, and the leaves are dark green. In contrast, plants growing on soils lacking nitrogen will be small and pale green or yellow-green. Crops respond to the application of nitrogen more quickly than they respond to potassium or phosphorus. Lack of nitrogen increases the production of fibers, whereas excess nitrogen interferes with the production of carbohydrates.

Phosphorus

A very large proportion of phosphorus in the mature plant is located in the seeds and fruit. This element is also abundant in young meristematic cells, however, and is a component of lecithin and nucleic acids. Large quantities of phosphorus are taken up by plants during maturation

of seeds. Gardeners generally try to meet the increased demand for this element at blooming time by applying phosphate fertilizer to the soil near the base of the plant.

Phosphorus is necessary for certain enzymatic processes such as the production of alcohol from sugars, and in the transformations of sugars to starch and starch to sugars.

Phosphorus-deficient plants are slow growing, and often dwarfed at maturity. Leaves are dark green in color, and the development of anthocyanin pigments may be enhanced. Plants of sweet corn on phosphorus-deficient soils have been observed to grow to the height of a foot or two and to produce small ears of corn without becoming any taller.

Potassium

Potassium differs from all of the other elements required in large quantities by plants in that it is not built into organic compounds of physiological significance. It occurs in plants almost entirely in the form of inorganic salts. It is present to a slight extent in the salts of organic acids.

Young and active regions of plants, like buds, young leaves, and root tips, are always rich in potassium. Actually potassium seems to act more as a catalyst than as anything else. Specifically, this element has been found to be necessary for the following processes:

1. Synthesis of simple sugars and starch
2. Translocation of carbohydrates
3. Reduction of nitrates
4. Synthesis of proteins
5. Normal cell division

Potassium-deficient plants are stunted at first and eventually dry up to a brownish color. Leaves are dull green, sometimes yellow, and the edges and tips are often scorched looking. Seeds may fail to mature. The fact that a small portion of potassium is naturally radioactive is of considerable interest, but it is not known whether or not this has any relation to the element's mode of action.

Magnesium

It will be recalled that the element magnesium is an essential constituent of the green pigment, chlorophyll. A deficiency of this element inhibits production of chlorophyll and therefore causes a yellowing of the leaves. It seems that magnesium performs other physiological functions in the plant. It occurs in seeds in relatively large quantities, and is more abundant in oily seeds than in those rich in starch. This fact and the results of experimental work suggest that magnesium is essential for the production of oil in plants.

Calcium

Although calcium is usually applied to soils for the primary purpose of reducing acidity, this element is necessary for normal metabolic processes in the plants and therefore plays an important role in plant nutrition. Calcium tends to accumulate in the leaves. It is found in the middle lamella of cell walls as calcium pectate.

Within the cell calcium frequently occurs as calcium oxalate. Because of this, it has been suggested that calcium functions in the plant by combining with organic acids which may be toxic to the plant in the uncombined state. This theory, originated by Schimper in 1888, subsequently fell into disrepute but has now been revived by F. W. Parker and E. Truog. It is pointed out that plants like legumes, which manufacture large quantities of proteins, release considerable amounts of organic acids in the process. It is supposed that under such circumstances calcium is used in the neutralization of these acids.

Sulfur

Sulfur is a constituent of the amino acid cystine, of glutathione, and of the mustard-oil glycosides. The last-mentioned compounds give the strong flavors to onions, radishes, cabbage, etc. Sulfur is essential for the formation of chlorophyll, for when plants are deprived of this element, they develop a pale-green color, much like that of nitrogen-deficient plants.

Iron*

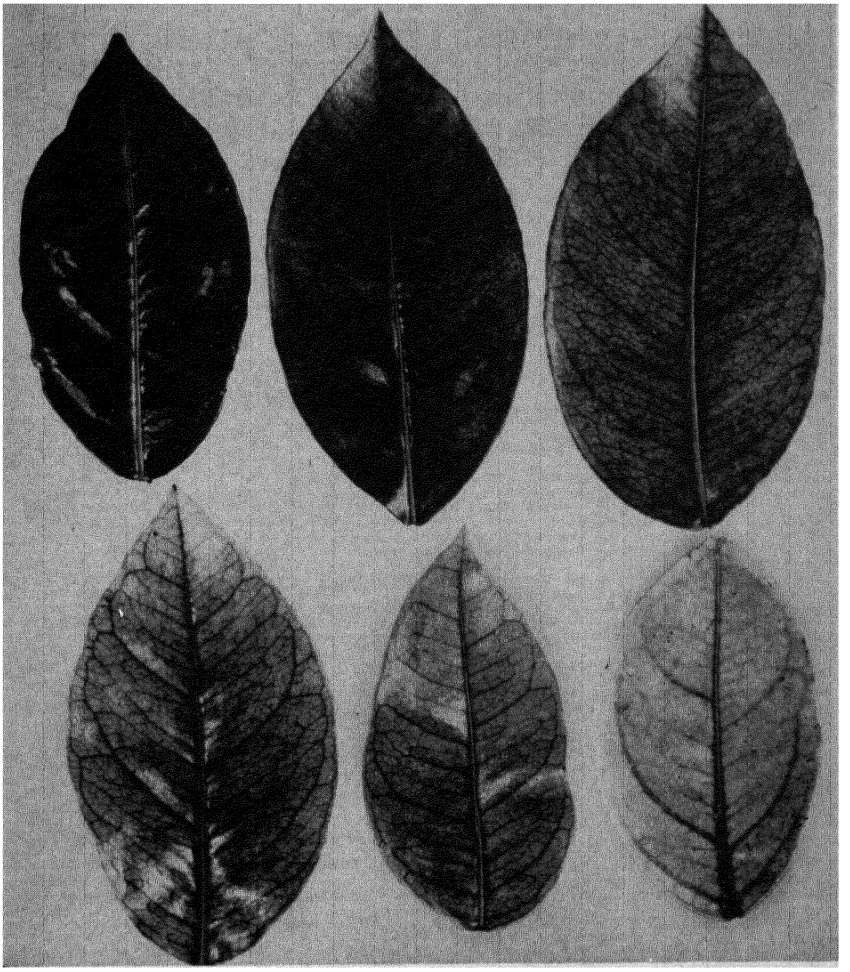
Iron is necessary for the formation of chlorophyll although it does not enter into the chlorophyll molecule. Iron apparently acts as a catalyst or oxygen carrier in oxidation-reduction processes in the cell, for it has been established that iron porphyrins play important roles in respiration.

Plants deficient in irons become chlorotic, i.e., they develop a pale-green, almost white color. Iron-deficient leaves in early stages of deficiency are mottled, whereas leaves lacking nitrogen are uniformly pale green.

One striking fact about iron in its relation to plant growth is its immobility. Iron-deficient plants are often found growing on soils containing ample amounts of iron yet the element is not absorbed because it has been rendered insoluble by certain compounds in the soil. Furthermore, iron may even be present in iron-deficient plants, having been precipitated and stored as insoluble compounds.

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* The element iron, because of the slight amount required by plants, rightfully belongs in the discussion of trace elements. It is considered here because it is usually listed among the ten major elements.



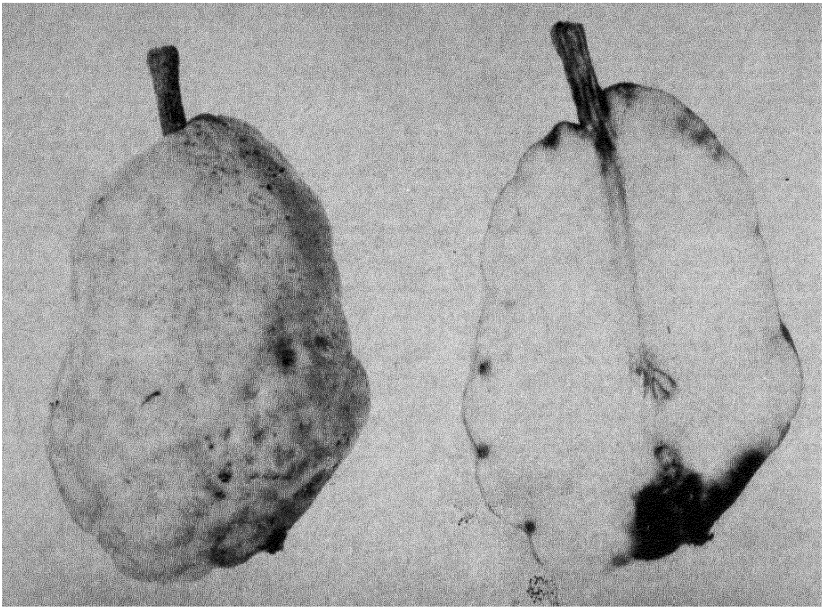
Orange leaves with varying degrees of iron chlorosis. The leaf in the upper left corner was taken from a tree treated with 20 Gm. of chelated iron. (Courtesy, Stewart and Leonard: "Iron Chlorosis—Its Possible Causes and Control," *Citrus Magazine*, June 1952.)

Micronutrients

Sometimes it is not easy to discover the originator of a new concept in science. Many of the early workers with nutrient solutions observed stimulating effects from adding elements not considered essential. As early as 1869 J. Raulin found zinc to be indispensable to the fungus *Aspergillus niger*, and was of the opinion that the element was required for higher plants also. M. Javillier (1912) was aware that zinc was a contaminant

of the purest chemicals obtainable, and that it might be introduced into solutions from the walls of glass containers. P. Mazé (1915) is usually given credit for establishing the fact that higher plants require small amounts of additional elements because of his work with corn. However, Mazé's work did not receive due recognition until several years later.

The essentiality of trace elements was long overlooked for the following reasons: (1) The so-called chemically pure salts, used in the earlier



Effect of boron deficiency on Bartlett pear. (Courtesy, Bureau of Plant Industry, U.S. Dept. of Agriculture.)

work, usually contained traces of the less common elements and it was not realized at the time that a trace was all that was required by the plant; (2) distilled water may contain small quantities of certain elements (zinc, copper), having been introduced as contaminants from containers and equipment which are used in distillations; (3) frequently the seed of a plant contains sufficient quantities of trace elements to last the plant through its complete life cycle.

Following the discovery of the essentiality of the trace elements there occurred a period of extensive investigation of the subject, but it is impossible to present here anything but a brief sketch of the results. Several points, however, seem to merit a certain amount of emphasis. First, many of these investigations resulted in the discovery that certain so-called physiological disorders of plants are actually symptoms of a lack of some

of the less common elements. Disorders of this kind subsequently became known as deficiency diseases. Second, whereas a lack of trace elements may cause a deficiency, a very slight excess may be toxic. The margin of safety is often very small. These two points will be elaborated in the individual discussions of the elements.

Boron

Although boron is widespread in the plant kingdom, its exact physiological role is unknown. Perhaps more has been written about its absence than its presence. A deficiency may cause abnormal growth or a blackening and death of internal tissues. There appears to be some relation between the functions of boron and calcium in the plant. Whenever the calcium-boron balance becomes upset, either by a shortage of calcium or by an excess of boron, the plant may show injury. When the imbalance is the result of lack of boron, a deficiency symptom appears.

Some of the physiological disorders now attributed to lack of boron are: internal cork of apples; brown heart of beets and other root crops; and browning of cauliflower.

Plants have different requirements and different tolerances toward boron. Haller and Batjer state that the optimum range of boron for apples is between 10 and 20 parts per million, and that with the Jonathan variety 30 to 40 parts per million may cause serious injury. Orange trees, on the other hand, if irrigated with water which is rich in boron, may contain as much as several hundred parts per million without showing injury.

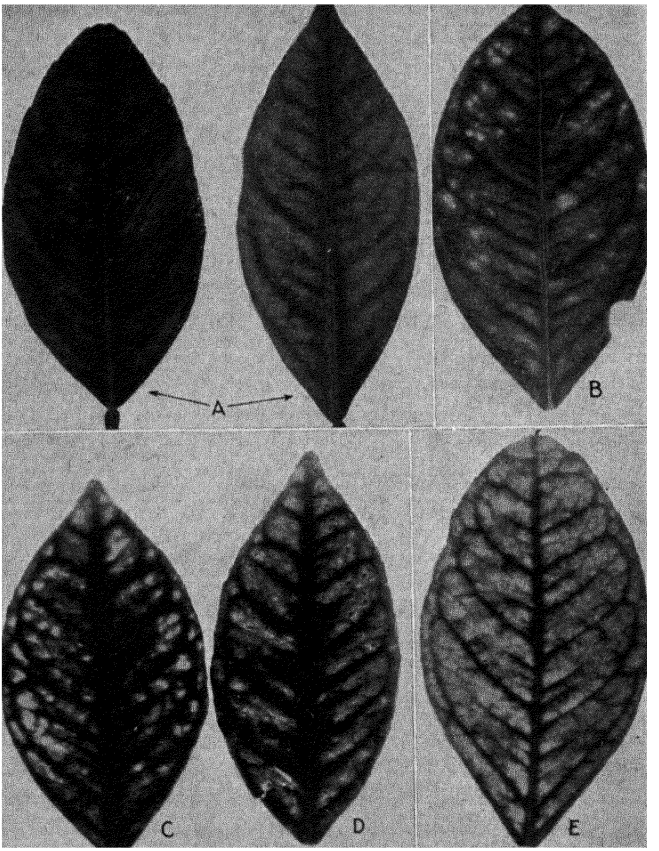
Copper

As early as 1894 it was reported that copper exerts a stimulating effect upon plants. Evidence for this was obtained from the results of the application of fungicidal sprays which contained copper (for instance, Bordeaux mixture). In 1927 the stimulating effect of copper was particularly noticeable in the muck lands of the Florida Everglades. It was reported that on the raw-peat soils no vegetables except potatoes could be grown. The potatoes were regularly sprayed with Bordeaux mixture as well as with arsenical compounds. Furthermore, certain agricultural crops grew fairly well when planted in soils in which potatoes had previously been grown. Subsequent detailed experiments showed that copper was lacking in the soils and was found to be the limiting factor.

It is now well established that copper constitutes the nonprotein portion of certain oxidizing enzymes, such as ascorbic acid oxidase and tyrosinase.

Copper, like boron, can be toxic in quantities which are slightly in excess of the plant's normal requirements. Lower plants like algae and fungi are much more sensitive to copper than are higher ones. This

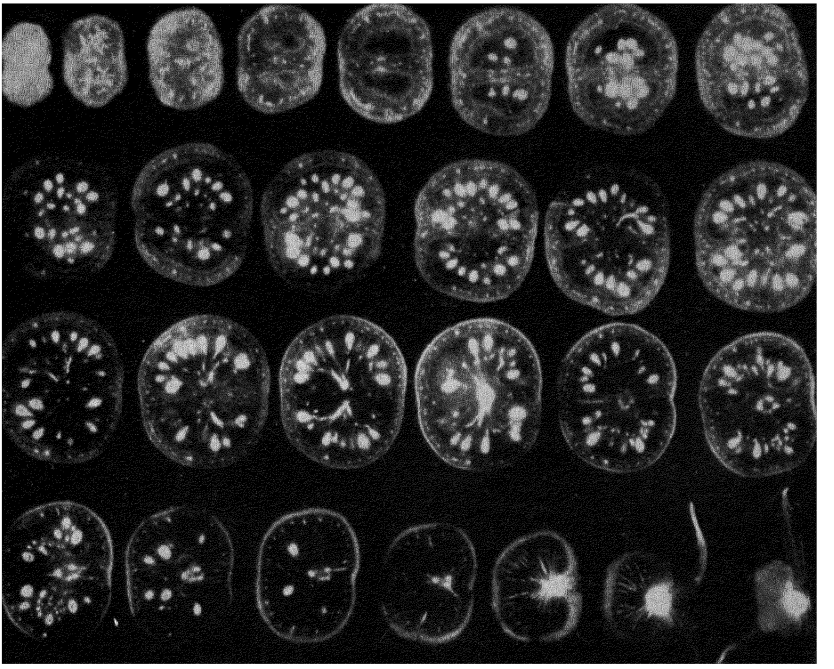
knowledge has been employed in the manufacture of fungicides and for removing algal growths from fish ponds and swimming pools. The United States Public Health Service has set a limit of 0.2 parts per million of copper (equivalent to approximately 0.8 p.p.m. of crystalline $CuSO_4$) for controlling algae in domestic water supplies.



Manganese-deficiency pattern in navel orange leaves from trees grown in cultures. (Courtesy of the California Agricultural Experiment Station, and The National Fertilizer Association, Washington, D.C., publishers of *Hunger Signs in Crops.*)

Manganese

Manganese, like iron, is essential for the production of chlorophyll, though it is not a constituent of this pigment. The element plays an important part in oxidation-reduction reactions because it acts as an activator in certain enzyme systems such as dehydrogenases and carboxylases.



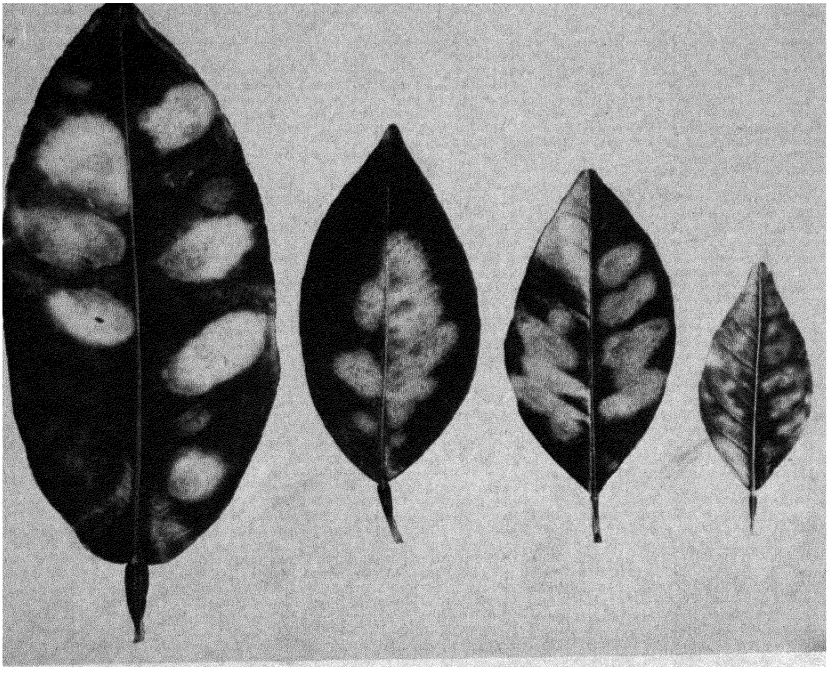
Radioautographs of zinc (Zn^{65}) in cross-sections of a green tomato fruit. Sections were made and exposed while maintained at 0°F. Light areas are caused by radioactive zinc. The total amount of zinc in one seed was found by analysis to be approximately 0.03 micrograms. (Courtesy, Dr. Perry R. Stout, Dept. of Plant Nutrition, Univ. of Calif.)

Zinc

Zinc is another element which has been proved to be essential to plants but whose exact role is unknown. Studies on tomatoes with radioactive isotopes have shown that this element tends to accumulate in the seeds. A deficiency of zinc, like boron, has been found to be the cause of several so-called physiological disorders. About 1930 J. R. Cole in Albany, Georgia, sprayed pecan trees with a zinc-lime solution instead of Bordeaux mixture because the latter often damaged the leaves. This treatment, though designed to control certain fungous diseases, produced distinctly beneficial effects on trees which showed symptoms of *pecan rosette*, a disease in which the leaves form in small bunches or rosettes on the twigs. Zinc sprays were applied to plants in other parts of the country with similar results. It was thus shown that a deficiency of zinc was responsible for diseases such as *bronzing* of tung trees, *mottle leaf* of citrus, and *apple rosette*.

Cobalt and Molybdenum

No evidence has yet been presented which suggests that cobalt is essential to higher plants. Lower plant forms (bacteria, fungi, algae) apparently require this element. It has been reported that *Lactobacillus lactis* and *Staphylococcus griseus* utilize cobalt to synthesize either a growth substance or cobalt-containing vitamin now known as B_{12}.



Citrus leaves showing molybdenum deficiency (yellow spot). Left to right: leaves from grapefruit, orange, tangerine, and temple orange. (Courtesy, Stewart and Leonard: "Molybdenum Deficiency in Citrus," *Proc. Fla. State Hort. Soc.*, 1951.)

Molybdenum has now been accepted as essential to higher plants. Tests have been made with many species of plants, and deficiency patterns are now recognized. The specific role of molybdenum is not known although it is apparent that it acts as more or less of a catalyst, especially in reduction of nitrates.

Mineral Deficiencies

Some mention was made of symptoms of mineral deficiency in the discussion of the individual elements. It must be borne in mind that specific deficiency symptoms vary with each species of plant, and if one wishes to be able to diagnose the deficiency, he should familiarize himself

with the individual symptoms. However, certain more or less general characteristics can be expected to appear in the absence of a specific element, and the student should familiarize himself with these general symptoms for at least the three elements (N, P, K) most frequently applied in fertilizer. These general characteristics are presented in Table 22.

Table 22

GENERAL SYMPTOMS OF CERTAIN MINERAL DEFICIENCIES

Deficient Element	*Whole Plant*	*Leaves*	*Fruits*
Nitrogen	Stunted growth	Small, thin, pale-green or yellowish color	Chlorotic, small when mature
Phosphorus	Slow-growing, dwarfed at maturity	Dark-green color, development of anthocyanins increased	Fruits slow in ripening, small when mature
Potassium	Stunted at first, later dried and brownish	Dull-green color with edges and tips often scorched looking	Fruit buds may fail to form; if they do, yields will be small

In many instances the leaves are the first to show signs of a deficiency. Here again the specific symptoms vary with the species of plant but in a general way chloroses may be divided into two groups: (1) chlorophyll content uniformly reduced over the whole leaf, producing a light-green, yellow, or white leaf; and (2) a mottled effect in which the veins tend to remain greener than the tissues between them. (See Table 23.)

Table 23

TYPES OF LEAF CHLOROSES

Deficiency Pattern	*Elements Lacking*
Leaves uniformly light green	Nitrogen Sulfur
Leaves mottled, with veins greener than tissues between them	Manganese Zinc Molybdenum Iron (in early stages) Magnesium (in early stages)

Nutritional Sprays

We have stated on several occasions that certain mineral deficiencies may be observed in plants even when adequate quantities of the deficient elements are present in the soil. The reason for this apparent paradox lies in the fact that the mineral is held in the soil in an insoluble or unavailable form. Such is often the case when plants are found to be deficient in the minor elements such as iron, manganese, copper, and zinc. It is now quite common practice in the United States to apply the above-mentioned elements to above-ground parts (aerial organs) of the plant by means of so-called nutritional sprays. In recent years it has been suggested that major elements might be applied in the same manner. Tests have shown, with

nitrogen at least, that such a practice may be practicable. Fruit trees which are deficient in this element have been reported to recover from the deficiency much more rapidly when sprayed than when fertilized through the roots. It has been claimed also that when nitrogen is applied as a nutritional spray (instead of by root fertilization) to apple trees in the spring, there is less chance of having excessive amounts of the element in the soil at the time of maturing fruits, when such a condition would be undesirable.

Chelating Agents

Still another method has been employed recently for overcoming deficiencies of certain elements in plants. This consists of combining the required element with a *chelating agent* and adding the combination to the soil as an ordinary fertilizer. The word *chelating* is derived from the Greek word *chēlē*, claw, and the agent serves its purpose by tenaciously holding onto an insoluble substance so that both go into solution. Stewart and others have reported success in treating iron-chlorosis of citrus trees by use of this method.

chapter 15

Enzymes

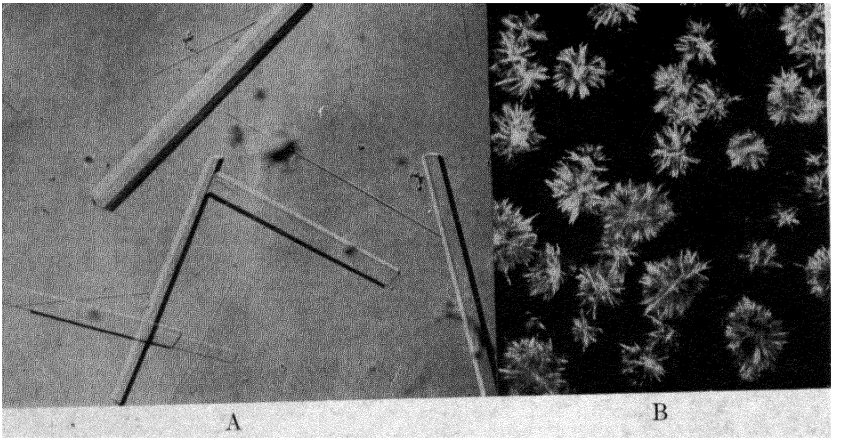
Within the microscopic green cell of the living plant there occur chemical reactions of an extraordinarily diverse nature. These reactions proceed smoothly and rapidly, at relatively low temperatures, and without the strong reagents employed by the chemist. Complex organic molecules are synthesized from simple substances, and these complex molecules are subsequently broken down again so that they may be transported in soluble form to other parts of the plant, or so that they may be metabolized in place. Many, though not all, of these reactions may also take place in the nongreen cell, as for example, when stored foods in the stems, seeds, and roots are being digested at the time of renewed growth.

The superiority of the living cell over the chemist in performing specific chemical reactions is traceable to the presence of organic catalysts called *enzymes*. Like any other type of catalyst, an enzyme changes the rate of a chemical reaction without being consumed in the process. Most of the discussion in this chapter is concerned with hydrolyzing enzymes because other types will be considered under the topic of respiration. Hydrolyzing enzymes participate in the digestion of complex food substances, cleavage of the large substrate molecule being caused by the addition of water. These efficient organic catalysts are not restricted to plant cells but are present in animal cells also. Consequently, many of the earlier contributions to our knowledge were supplied by animal physiologists. Let us therefore review briefly some of the early work in this field.

Pioneering work with enzymes began with a study of the process of digestion of foods in animals. Until early in the 18th century it was believed that the digestive system in animals was a kind of meat grinder and that complex foods were reduced to soluble forms by purely mechanical processes. Then a French scientist, René de Reaumur, made a falcon swallow a perforated metal tube containing pieces of meat. The report says that when the tube was pulled up a few hours later, the tube was intact but the meat was digested. Later an Italian physiologist, Lazarro Spallanzani, performed a similar experiment on hawks and subsequently on himself. In 1780, a year after Spallanzani died, his treatise was published. It suggested that food is digested by substances in the digestive juices. In 1835 Theodor Schwann, a German physiologist, discovered a

nonacid substance in the gastric juice which he called *pepsin*, although he was not aware of its chemical nature.

The next great discovery in this field was made by Louis Pasteur, who was commissioned by the French Government in 1860 to work on problems of the wine industry. Among other things Pasteur showed that alcoholic fermentation was merely "life without oxygen" or, more properly,



(A) Crystals of the enzyme hexokinase. (B) Crystals of the enzyme ribonuclease. (Courtesy, Dr. M. Kunitz.)

anaerobic oxidation of sugars by yeasts. For many years there was confusion among scientists mainly in regard to terminology. When an organism like yeast accomplished the breakdown of sugar it was called an *organized ferment*; a free substance like pepsin was considered an *unorganized ferment*. Later the term *enzyme* was given to the unorganized ferment, and the word *ferment* was restricted to living organisms like yeasts and bacteria.

In 1897 Eduard Buchner discovered that the enzyme could be extracted from yeast cells and that it could produce fermentation in the absence of the living cell. His discovery was purely accidental. He was interested in preserving an extract of yeast to be used subsequently on experimental animals. In one of his methods of preservation he added large quantities of sugar. This treatment failed to preserve the extract and the solution fermented, forming alcohol. It demonstrated for the first time, however, that alcoholic fermentation could occur in the absence of yeast cells and that the terms *enzyme* and *ferment* were one and the same. For this discovery Buchner was awarded the Nobel prize for chemistry in 1907.

Nature of Enzymes

Although enzymes generally carry on their activities within the cells in which they are produced, there are a number of instances in which this is not true. In the animal body hydrolytic enzymes are present in the digestive juices of the alimentary tract, having been secreted by cells which line the tract. In the plant world enzymes may be secreted by bacteria and fungi, and certain chemical processes are catalyzed outside the cell. This may occur in a few higher plants, such as the so-called carnivorous plants. But most enzymatic processes in the living plant take place within the confines of the cell. It has been estimated that a single plant cell may contain 100,000 enzyme molecules.

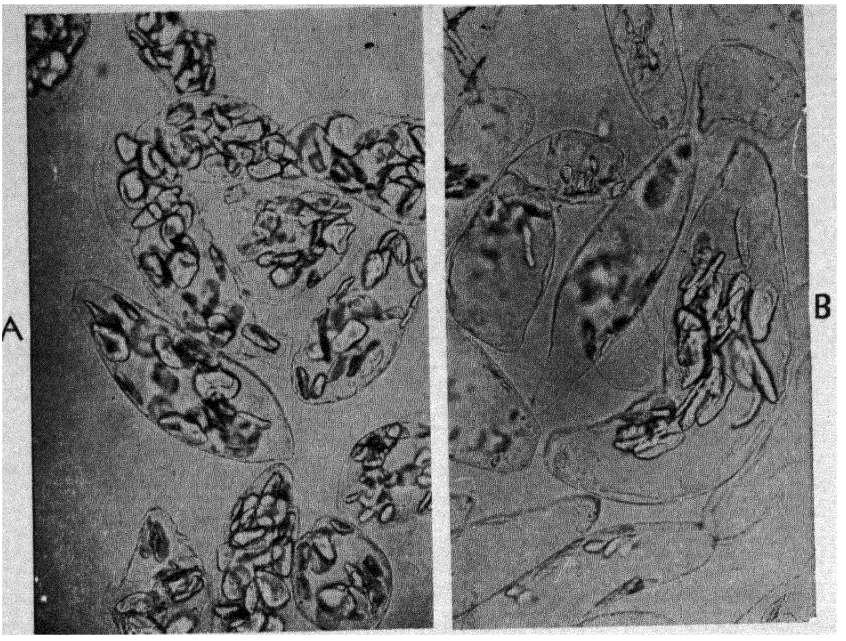
Enzymes are colloidal in nature. It was mentioned in our discussion of adsorption in a previous chapter that it is possible that these organic catalysts may function partly by concentrating the reacting substances on their surface. It has even been suggested that *surface energy* plays an important part in the reactions in which enzymes are involved. We shall soon see, however, that the chemical nature is important too.

The colloidal nature of enzymes is reflected in the method which is commonly employed in isolating them. Enzymes are first precipitated by alcohol or by a saturated salt solution. The precipitate is then purified by dialysis. This consists of enclosing the crude preparation in a cellophane bag and immersing it in running water for several hours. The precipitating agents diffuse out, whereas the large molecules of enzymes are retained within the bag.

Over 40 enzymes have been isolated in crystalline form and they appear to be either simple or conjugated proteins. The first enzyme to be crystallized was *urease*, which was prepared from the jack bean (*Canavalia ensiformis*) by J. B. Sumner. This enzyme is a globulin, having the molecular weight of 483,000.

Two types of conjugated protein enzymes have been isolated. In one type the prosthetic, or attached part, is a metal. Certain oxidizing enzymes (tyrosinase, ascorbic acid oxidase) have been shown to be copper proteins. Carbonic anhydrase is a zinc protein.

In the other type of conjugated protein the prosthetic portion is a complex molecule which may or may not contain a metal. The nonprotein portion of catalase consists of an iron porphyrin. It is structurally similar to the hemin of red corpuscles of blood. Some of the enzymes which participate in respiration and alcoholic fermentation contain a complex molecule which includes a vitamin. One of these (Warburg's yellow enzyme) is a riboflavin pyrophosphate. Nicotinamide is found in another one of these complex nonprotein molecules. The prosthetic groups in this type of enzyme have been termed *coenzymes*. They can be separated from



(A) Cells from pulp of unripe banana showing starch grains. (B) Cells from pulp of ripe banana, most of the starch having been hydrolyzed by enzymatic action (×125). (Courtesy, Gibbs: *Botany*, New York, The Blakiston Co., Inc., 1950.)

the protein portion by dialysis. The metalloproteins thus far have not been separated into their two components by this means.

Temperature and Enzymes

Up to a certain point the rate of enzymatic reactions increases with a rise in temperature. The optimum temperature usually lies between 40°C. and 50°C. This is assuming that other conditions are favorable for the reaction. The temperature coefficient (Q_{10}) for enzymes in the operative range is between 2 and 3. This means that for every ten-degree rise in temperature the reaction rate increases two or three times.

At temperatures above 50°C. most enzymes are inactivated, providing they occur in a liquid medium. Destruction of enzymes by heat is doubtless a result of coagulation of the protein portion. Preserving foods by boiling, pasteurizing, or autoclaving involves the principle of inactivation of enzymes, as well as that of destroying bacteria and fungi.

Temperatures below freezing serve to inactivate enzymes without completely destroying them. In the case of frozen foods enzymes may be free to begin acting following thawing, and it is for this reason that continual thawing and refreezing of these commodities is not recommended.

Frequently samples of plant tissue are preserved for subsequent analy-

ses by freezing them. Although, as has just been mentioned, enzymes are not destroyed by this process, it is possible that their activity may be significantly reduced by repeated thawing and freezing.

Classification of Hydrolytic Enzymes

Hydrolytic enzymes may be classified by reference to the structure of the substrate on which they act. Some of the more important examples of this type of enzyme along with their substrates are listed in Table 24. It

Table 24

CLASSIFICATION OF HYDROLYTIC ENZYMES

Name of Enzymes	*Substrate*
Carbohydrases	
1. Sucrase	Sucrose
2. Maltase	Maltose
3. Beta-glycosidases	All beta-glycosides
4. Amylase	Starch, dextrins
5. Cellulase	Cellulose
6. Inulase	Inulin
7. Protopectinase	Protopectin
8. Pectinase	Pectin, pectic acid
Esterases	
1. Lipases	Fats
2. Chlorophyllase	Chlorophyll a
3. Pectase	Pectin
4. Phosphatases	Esters of phosphoric acid
5. Tannase	Tannin
6. Phosphorylases	Glycogen, starch, sucrose
Enzymes which hydrolyze nitrogen compounds	
1. Proteinases	Proteins
2. Peptidases	Polypeptides
3. Desaminases	
a. Urease	Urea
b. Asparaginase	Asparagine
c. Arginase	Arginine

will be noted that there are three large groups: carbohydrases, esterases, and enzymes which hydrolyze compounds of nitrogen. We shall discuss a few examples of each of these large groups.

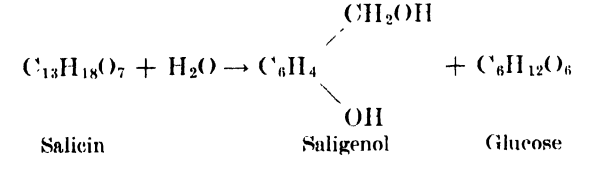
Carbohydrases

Enzymes in this group function by reducing complex carbohydrates to simpler forms. *Sucrase* splits sucrose into its component monosaccharides, glucose and fructose. This enzyme is sometimes called *invertase*, because the direction of optical rotation is inverted when sucrose is hydrolyzed. The reason for this inversion was given in the chapter on carbohydrates. The term *sucrase* is to be preferred to invertase since it is the custom in this country to name the enzyme after its particular substrate.

Amylases serve to break down starch into simpler forms. Two types have been isolated from plants: alpha-amylase and beta-amylase. Starch-

splitting enzymes in the past have been referred to as *diastases* but this term is now generally restricted to mixtures of enzymes. Thus *malt diastase,* which is prepared from sprouting barley (malt) and *taka-diastase,* which is prepared from the fungus *Aspergillus oryzae,* are mixtures of amylases and *maltase.* When starch is treated with malt- or taka-diastase, the end product is glucose. Pure amylases do not carry the reaction beyond maltose and dextrin. Alpha-amylase is present in germinating seeds of cereals, whereas beta-amylase is found in leaves, shoots, and in seeds but only during their development.

Glycosidases act on carbohydrates which contain glycoside linkages. This would include some of the sugars, such as maltose and cellobiose, as well as the compound carbohydrates which we term *glycosides.* (See the chapter on Carbohydrates.) *Emulsin* or *amygdalase* is a *beta-glycosidase* which, as its name implies, acts on all beta-glycosides. The following is an example of a reaction which is accelerated by a glucosidase.



Esterases

It will be recalled that several types of compounds exist in plants in the form of esters. These compounds are digested by *esterases. Lipases* convert fats to fatty acids and glycerol. Chlorophyll, which was shown to consist of a methyl-phytyl ester of the acid chlorophyllin, is acted on by the enzyme *chlorophyllase.* It is possible that the de-esterification of chlorophyll represents the initial stage of the decomposition of this green pigment as leaves turn yellow or as certain fruits mature and ripen.

Pectin methyl esterases are responsible for splitting off the methyl radical from pectin, the end products being pectic acid and methyl alcohol. Complete de-esterification of pectin destroys its ability to form gels.

Enzymes Hydrolyzing Nitrogen Compounds

Proteins and other nitrogen compounds are reduced to transportable and assimilable forms by enzymes, just as fats and carbohydrates are. As a rule these enzymes are easily demonstrated in germinating seeds because reserve proteins are being rapidly digested at this time. Enzymes which bring about the cleavage of proteins to polypeptides are called *proteases;* those that reduce polypeptides to amino acids are termed *peptidases.*

Two proteases easily demonstrated in plants are *papain* from the papaya and *bromelin* from the pineapple. Plants doubtless contain any

number of proteases and peptidases, however, since they are constantly synthesizing proteins to be used as storage foods and hydrolyzing them again for use during metabolism.

Other enzymes that hydrolyze nitrogen compounds are *urease*, which hydrolyzes urea, *arginase*, which acts on arginine, and *asparaginase* which breaks down asparagine.

Conditions under which Enzymatic Reactions Occur

In the plant cell a coordinated system exists, the like of which cannot be duplicated in the test tube. Nevertheless most of the information which we have obtained concerning enzymic reactions has been obtained from reactions *in vitro*. Thus the student who is being introduced to this phase of physiology will doubtless perform his experiments with isolated enzymes or enzyme extracts. We shall therefore discuss briefly some of the factors which affect enzymatic reactions and attempt to explain how these must be controlled in experimental work.

Hydrogen Ion Concentration. Careful control of the pH of the medium is essential for reproducible results in studying enzymes. Where the optimum pH for a specific enzyme is known, a buffer is employed in both the extracting and the reacting solutions.

The optimum pH varies with different enzymes and may even show considerable variation in the same kind of enzyme when it comes from different sources. Enzymes in the gastric juice tolerate a fairly high hydrogen ion concentration. The optimum pH for pepsin ranges from 1.5 to 2.5. The proteolytic enzyme, bromelin (from pineapples) reacts optimally at a pH of 4.6. Amylase from malt and from *Aspergillus oryzae* both have an optimum pH between 4 and 5, whereas pancreatic amylase reacts best at pH 7. Examples of enzymes which react optimally in media of relatively high pH are tyrosinase (pH 6 to 8) and arginase (pH 9.8).

Temperature. Enzymes are inactivated when exposed to temperatures much above 50°C. Prolonged heating at 100°C. completely inactivates these catalysts. This characteristic is utilized in obtaining controls in enzyme studies by boiling a portion of the enzyme extract. The effect of temperature has been considered in more detail in a preceding paragraph.

Concentration of Enzyme. No very accurate method of expressing enzyme concentration has been developed except that of expressing it in terms of activity. When a plant extract is diluted one, two, or more times the enzyme activity is correspondingly reduced. Based on these results, a high rate of enzymatic activity in the original extract is interpreted to indicate a greater concentration of enzymes. Tests are usually set up under standard conditions, including specifications as to temperature, pH, and quantity of substrate, so that the results are considered to be reproducible. In many instances, however, analyses have been conducted on

fresh plant tissues, which may vary in moisture content. It would seem preferable, therefore, either to analyze dry material or to express results on a dry-weight basis.

Other Factors. A number of other factors have been shown to affect the rate of enzyme activity, some accelerating, some retarding the reactions. Among these might be mentioned the concentration of substrate, accumulation of end products, and the amount of water, salts, and certain metals present, and radiant energy.

Reversibility of Enzymes

It has been assumed that enzymes are reversible in their action largely because the reactions which they catalyze are known to be reversible. Thus in the plant cell sugar is condensed to starch and starch is hydrolyzed to sugar, fats are both hydrolyzed and synthesized, and proteins likewise undergo the same changes. Furthermore, claims have been made that some of these compounds (sucrose, fats, glycosides) have been synthesized *in vitro*. Satisfactory explanation of the manner in which an enzyme catalyzes a reaction in both directions has not been forthcoming. One explanation which has been offered relates to water content. It has been pointed out that enzyme reactions in the living cell differ from those in the flask or test tube because the water supply in the cell may be constantly diminishing during the process. The amount of water may eventually become so low as to reverse the direction of the reaction.

It was once believed that the pH of the medium determined the course of the reaction. We have cited the case of the guard cells of Zebrina leaves in which starch is changed to sugar in the pH range of 6.0 to 7.4, and sugar is changed to starch at pH 5 or less.

The discovery of the phosphorylated hexoses has thrown new light on some of these reactions. It is possible that synthesis of starch is brought about by the action of phosphorylase on glucose-1-phosphate, rather than by the reversible action of amylase. In other words, starch may be synthesized by phosphorylase and hydrolyzed by the amylases.

chapter 16

Translocation

The term *translocation* is employed to designate the process whereby materials are transported from one part of the plant to another. Photosynthate is manufactured only in the organs which contain chlorophyll, yet energy-giving materials are required in all living cells and foods must be moved to these points. The interior of woody stems, and storage organs like roots, tubers, and corms must all be supplied with food for utilization or for storage. In the spring when growth begins, foods again move from storage organs into the actively growing parts.

Since the movement of water in the plant has been discussed under the topic of *transpiration*, we shall limit ourselves in this chapter to a consideration of the transport of inorganic and organic compounds.

A little knowledge of plant anatomy is essential to an understanding of translocation. It will be recalled that in young saplings the bark may be removed from the wood with no great difficulty. This is particularly true in the spring when it is often said that "the bark is slipping." Between the bark and the wood is a single layer of cells called the *cambium*. These are the cells that initiate growth of the trunk. Just outside of the cambium will be found the *phloem*. Phloem tissue contains five principal types of cells: (1) sieve tubes, (2) companion cells, (3) phloem fibers, (4) phloem parenchyma, and (5) phloem ray cells.

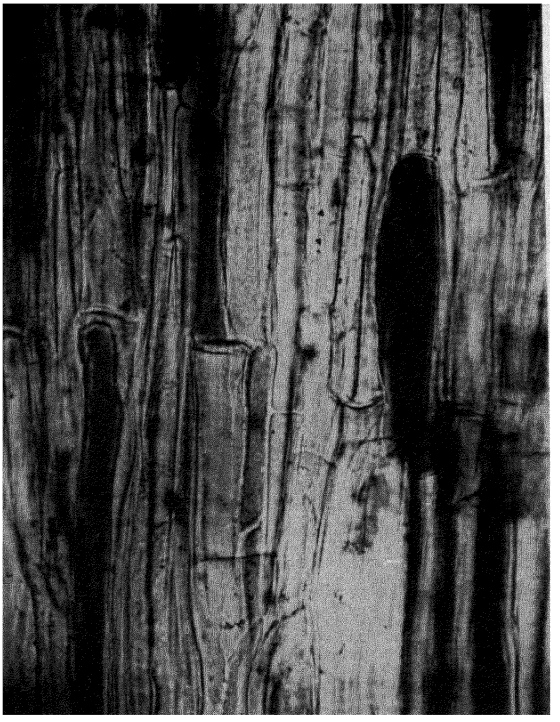
The sieve tubes are so called because of the presence of pores in the end walls. Sieve tube cells are elongated and contain only a thin layer of protoplasm around the walls. These tubes, with porous cross-walls at each end, are the food-conducting elements in the plant.

Fibers are composed of long, thick-walled cells. They lend support to the stem and, as would be expected, are usually lignified. The more woody the tissue the more abundant the fibers.

Vascular rays originate in the cambium and extend in both directions, i.e., into the phloem and into the xylem. Correspondingly, they are called phloem rays or xylem rays. As will be mentioned later, the ray cells function in the cross-transfer of materials.

Within the wood of the stem is found the xylem (from the Greek word *xylon*, wood). There are four general types of cells in this tissue: (1) tracheae, tracheids, (2) xylem parenchyma, (3) fibers, and (4) xylem rays.

The anatomy of the xylem was discussed to some extent under the topic of transpiration. It will be recalled that the tracheae or vessels are continuous tubes, lacking the cross-walls found in the sieve tubes. Furthermore, vessels are composed of dead cells and therefore contain no protoplasm. Xylem fibers and xylem rays perform the same functions as the corresponding tissues in the phloem. Tracheids constitute the only



Longitudinal section of potato stolon, showing sieve tube, companion cell, and phloem parenchyma in living condition after having accumulated neutral red for a short period of time (×325). (Courtesy, Crafts: "Sieve-tube Structure and Translocation in the Potato," *Plant Physiol.* **8**:81–104, 1933.)

organs of water transport in gymnosperms. They are spindle-shaped tubes and are neither as long nor as large in diameter as the vessels or tracheae.

Phloem and xylem, taken together, make up what is called a vascular bundle. A microscopic examination of a cross-section of a young stem would reveal that in monocotyledonous plants (grasses, sedges, lilies, etc.) the bundles are scattered. In the dicotyledons (usually plants with net-veined leaves) the bundles are present in the form of a circle. Furthermore, the phloem and xylem in the dicots are separated by a single layer of meristematic cells, the cambium. The cells of the cambium divide outwardly to form phloem tissue and inwardly to produce xylem tissue.

The vascular bundles are continuous from just behind the root tips, up through the stems, and terminate in the mesophyll of the leaves where they appear as the veins.

Movement of Food Materials

The subject of the movement of food materials in plants has intrigued both scientist and layman for a long time, because, as we shall soon learn, the topic has both scientific and practical application. One of the first experiments in this field was performed in 1671 by Marcello Malpighi, an Italian physician. The experiment consisted of removing a narrow ring of bark from a woody stem. This is called *girdling*. All of the tissue external to the xylem is removed in a girdled twig or stem. In Malpighi's experiment the region above the wound remained alive and continued to grow, whereas the region below the wound failed to develop further. It was concluded from this experiment that water moves upward through the wood (xylem) while the organic substances which are manufactured in the leaves move downward through the cortex.

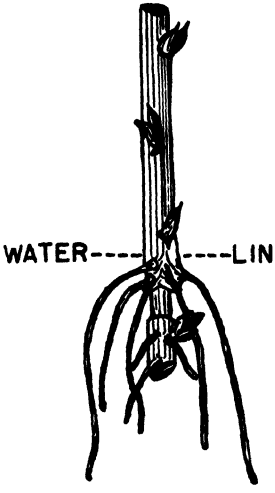
Thus began the practice of girdling, which is still in use today. Large trees are often killed by stripping off a ring of bark about a foot wide when it is desired to remove the trees from an area. When a new grove of orange trees is being planted in the hammock lands of Florida, the large oak trees are girdled and left standing. They are not removed because the dead trees no longer cast shade nor compete for the moisture in the soil. The fact that girdled trees usually live until the next growing season is additional proof that girdling interrupts the flow of food but permits the passage of water to continue. When a single branch and not the main trunk of a tree is girdled, the downward passage of food is stopped only in that branch.

Girdling of individual branches is sometimes practiced when it is desired to increase the size of fruits or to hasten maturity. Such treatment serves to prevent food materials from moving from girdled branches to other parts of the tree. In instances of this kind only a small segment of bark is removed so that callus tissue is subsequently formed and the branch is not killed by the treatment.

A rather striking experiment can be performed with several twigs, using a type like the willow which readily forms roots from cuttings. A girdle, one or two inches wide, is made within an inch of the base. The twig is then kept in water until roots are produced below the water level. Most if not all of the roots will be formed *above* the girdle, a fact indicating that movement of foods and natural plant hormones to the region below the girdle has been interrupted.

All of the evidence thus far cited points only to the fact that food materials move downward in tissues external to the xylem. Attempts to single out the phloem, or more particularly the sieve tubes, as the path of

ransport, was first based upon evidence of another kind. For example, hemical analyses indicated that cells of the phloem contained large quanities of carbohydrates and proteins. And it had been shown that the parasitic plant, dodder (*Cuscuta europa*) sends its roots into both the sieve tubes and the tracheae of its host. It was argued that if the tracheae contain food materials then it would not be necessary for the parasite to tap the phloem too.



Girdling experiment. Girdle near base of twig interfered with translocation of food and growth substances, with the result that longer roots were formed on upper part. (Redrawn after Palladin: *Plant Physiology*, Philadelphia, Blakiston's Son and Co., 1918.)

More specific evidence was obtained by O. Schneider-Orelli in 1909. This investigator made his observations on leaves in which "mining caterpillars" had destroyed portions of vascular bundles. When xylem only was destroyed, starch disappeared from the leaves but remained when the sieve tubes only were destroyed. This was offered as proof that organic solutes move in the phloem, and that they move in a downward direction.

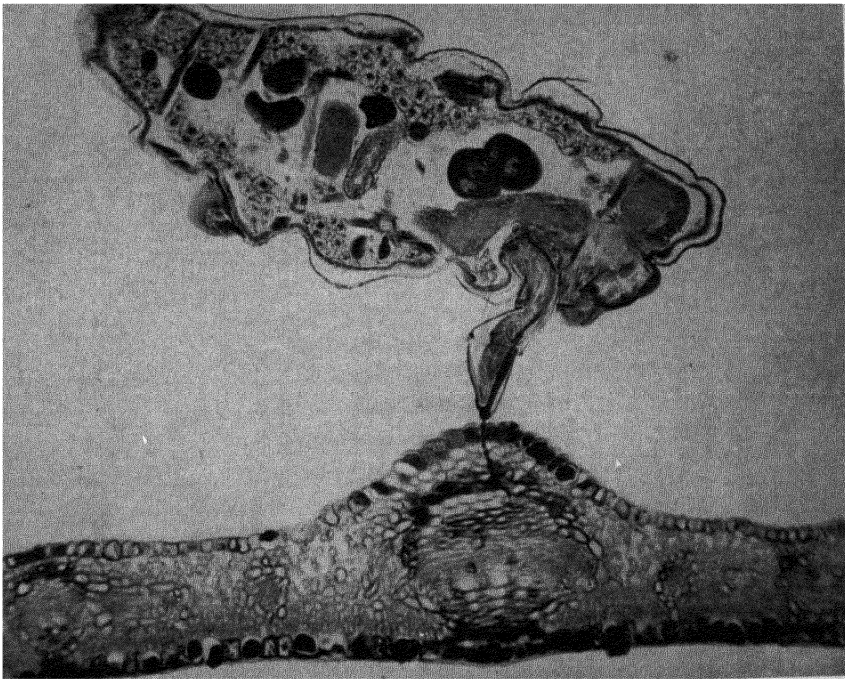
Not long ago a prize-winning photomicrograph showed an aphid (a sucking insect) with its proboscis injected into the sieve tubes of a leaf. It might appear from this that certain insects have long been aware of the abundance of foods in phloem tissue and one wonders whether this information was obtained by trial and error or as the result of instinct.

T. Hartig, as early as 1859, suspected that organic solutes move in sieve tubes because of the relatively large quantities of sucrose in these cells. It was not until 1930, however, that more or less conclusive evidence was presented in support of this claim. At this time W. Schumacher performed a rather delicate operation in which he removed a circle of collateral vascular bundles from a leaf and retained one large bundle. He found that nitrogenous materials continued to be removed from the leaf at 75% of the original rate. When the xylem was cut out of the central bundle, the rate of transfer was 68% of the original. When phloem was removed (as much as was possible) the rate of loss of nitrogenous materials dropped to 5%.

Still more conclusive proof was obtained when Schumacher caused the dye, eosin, to be drawn up by the sieve tubes. The phloem parenchyma remained colorless in this experiment.

To demonstrate the movement of water through the xylem is a much

simpler matter than that of indicating the pathway for translocation of organic materials. Tracheae (vessels) are large, nonliving, continuous tubes. Sieve tubes are considerably smaller; they have perforated cross-walls, and they contain a certain amount of protoplasm. These characteristics of sieve tubes have not only hindered investigations of this type,



Photomicrograph of an aphid (sucking insect) puncturing epidermis of leaf. The proboscis has penetrated the sieve tubes (food-conducting organs). (Courtesy, V. P. Hollis, Univ. of Minn. Photo Lab., *Sci. Monthly,* and Photography-in-Science Salon.)

but they also add the difficulty of explaining *how* the materials move after we have proved that they *do* move in the phloem.

Movement of Organic Solutes

Prior to 1920 the majority of physiologists held that solutions moved upward in the xylem and downward in the phloem. It was once expressed as "up the xylem and down the bast," *bast* being an older term for phloem. Beginning with 1920 many significant contributions to this topic were made by a number of investigators. The extensive work of O. F. Curtis helped to clarify the issue and for this reason a summary of his work will now be presented. The following experiment by Curtis in 1920 illustrated

the mechanism of upward translocation of food materials. Samples of attached woody shoots were treated as follows: (1) control (not ringed, leaves present), (2) ringed with leaves present, (3) not ringed at the middle, leaves removed from upper half, (4) ringed, leaves removed from upper half. Three species of plants were tested, including a number of replications. At the end of a certain number of days the growth of the shoots was recorded. Results for one test, which are typical of others,

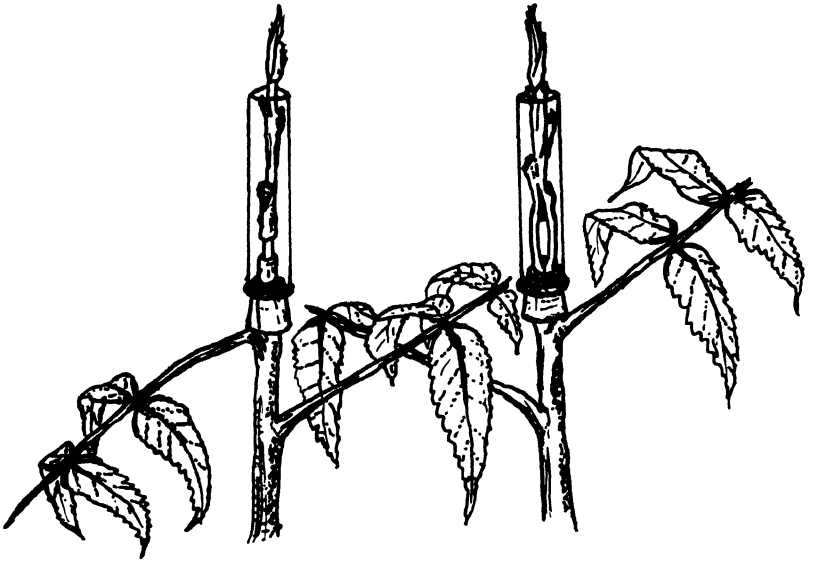


Illustration of Curtis' translocation experiment with defoliated twigs. Left: phloem removed; right: wood has been removed, leaving phloem tissues intact. Glass collar was kept filled with water to prevent drying out of tissues. (Redrawn by permission from *Translocation in Plants*, by O. F. Curtis. Copyright, 1935. McGraw-Hill Book Co., Inc.)

were as follows. At the end of nine days the control shoots had lengthened by an average of 341 mm.; the ringed shoots with leaves still intact grew 118 mm.; the shoots which were not ringed, but which had had their leaves detached from the upper half, had grown 270 mm. in this time. The fourth group, which were ringed and without leaves above the girdle, grew on an average of only 5 mm. in nine days.

To meet the objection that defoliation may have interfered with the transpiration stream and the movement of solutes in the xylem, Curtis modified the ringing experiment. An area below the terminus of the branch was defoliated. In one lot of branches a ring was made at the top of the defoliated area, on another just below the defoliated area, and in a third lot a girdle was made in both places. At the beginning of the experiment there was no starch in the defoliated area. Above and below

the defoliated areas there was starch in the pith, xylem parenchyma, wood rays, and cortex of stem. During the course of the experiment water moved freely up through the xylem. No detectable carbohydrate moved into the defoliated region. The lower ring prevented upward transport and the upper ring stopped its downward flow. When there was phloem connection with the source of carbohydrates, either above or below, carbohydrates moved into the defoliated region and were deposited in the pith, xylem parenchyma, wood rays, and cortex. Thus the evidence not only points to the phloem as the path of the upward and downward course of organic solutes, but also indicates that these solutes move into the xylem from the phloem.

The real test of the true upward path of food substances would consist of severing either the phloem or the xylem. And this is what Curtis did. Using several different species and working with hundreds of shoots Curtis removed the phloem in some twigs and the xylem in others. In both cases the wound was enclosed in a glass sleeve which was filled with water. It was consistently found that growth of shoots with xylem removed was nearly as great as that in controls, and about five times that of the shoots with phloem removed.

Upward Transport of Minerals

For many years most physiologists held the view that inorganic solutes are transported upward in the xylem. This conclusion was based on the fact that soil water, which in reality consists of solutions of minerals, moves upward in the vessels. At the present time, however, it appears that upward movement of inorganic compounds in plants is much more complex than was at first realized. Curtis was one of the earlier opponents of the theory that minerals are transported mainly in xylem tissues. Support of this claim was offered by presenting results of chemical analyses of twigs employed in some of the experiments which have been described previously. It was shown that a larger proportion of minerals, including nitrogenous materials, were transported into the upper part of woody shoots when xylem was removed than when phloem was cut out.

A number of investigators have presented evidence that minerals move upward in xylem tissues. T. G. Mason, E. J. Maskell, and E. Phillis in 1936 reported that nitrogen is translocated upward in cotton plants mainly in the xylem. Somewhat later (1938) H. F. Clements and C. J. Engard stated that this was true also in woody plants, at least for a portion of the minerals. That minerals move upward in the xylem was reported again in 1940 by Phillis and Mason, although these investigators showed also that nitrogenous compounds can move upward in the phloem of cotton plants.

It is apparent from what has been said that minerals can be shown experimentally to move upward through either the phloem or xylem, de-

pending upon which avenues of transport have been left open, but that experiments of this kind fail to indicate the predominant pathway under natural conditions.

A somewhat clearer picture of the means of mineral transport has been obtained as a result of the use of radioactive isotopes. As early as 1937 F. G. Gustafson and M. Darken made a study of translocation of radioactive phosphorus on rooted cuttings of several species of plants. Results of this work indicated that xylem and phloem were equally effective as conducting tissues, at least in so far as phosphorus is concerned. In 1939, however, P. R. Stout and D. R. Hoagland published results of a little different kind. It was reported by these investigators that radioactive phosphorus was transported upward in the xylem, but that lateral movement across the cambium was so rapid as to render results misleading. That is, analyses of wood and bark, which were separated from each other after phosphorus had moved up, gave positive tests for both tissues. By separating bark from xylem by means of oiled paper at the beginning of the experiment, it was shown that phosphorus moved up only in the xylem. Similar results were reported by O. Biddulph in 1940.

Downward Movement of Inorganic Salts

That minerals move downward in plants has been reported by a number of workers. In the cotton plant, as the boll develops, minerals are absorbed from the leaves. T. G. Mason and E. J. Maskell found that during this translocation of materials calcium moved in the xylem, and phosphorus and potassium were carried in the phloem. It was suggested by them that calcium moves in the transpiration stream and is immobilized as soon as it enters the living cell. This view, however, fails to take cognizance of the fact that calcium has to pass through living cells in the root before entering the xylem.

It is known too that in the fall just before the death of deciduous leaves, a large proportion of mineral elements (except calcium) moves back into the stems and possibly to the roots of the plant. Nitrogen, phosphorus, potassium, sulfur, magnesium, and chlorine are all transported from the leaves at this time of the year. It is the consensus of most investigators that the above-mentioned elements travel through the phloem under these circumstances.

O. Biddulph, using radioactive iron and phosphorus, showed that both elements were exported from mature leaves. Phosphorus moved in the phloem. Most of the phosphate after passing to the roots was recirculated in the xylem. Under certain conditions calcium was freely mobile in the phloem.

Recent evidence therefore points to the phloem as the route for most, if not all, of the downward movement of minerals in plants, but the

mechanism of the movements of these constituents in the phloem is not thoroughly understood.

Translocation of Viruses, Growth Substances, and Vitamins

There was a time when the topic of translocation could be closed following a discussion of the movement of water, minerals, and foods. This is no longer true. It seems desirable at this time to trace the course of a few other substances which may be important both in the physiology of plants and in the economy of man. Substances like viruses, growth substances, and vitamins must now be included in the discussion. Crafts (1951) has presented a rather complete summary of what is known in regard to the movement of these substances in plants, and the following has been drawn from his publications.

Four distinct modes of movement have been described for viruses: (1) phloem-limited viruses which are injected into the sieve tubes by insects, (2) parenchyma-limited viruses which move from cell to cell through the plasmodesms, (3) viruses, like tobacco mosaic, which thrive and multiply in the parenchyma but which also invade the phloem, and (4) a type which spreads through the xylem.

As a rule virus proteins are transported with food materials and only a limited number move in the xylem. Phony peach virus is an example of the latter type. In producing artificial infections of this disease it is necessary to make root grafts from diseased to normal trees. Apparently this is the most convenient method of making contact with the transpiration stream.

According to Crafts there is evidence that auxin (growth regulator or growth-modifying substance, see chapter 19) may be distributed in the plant as a result of three mechanisms, acting either separately or in combination. (1) Under natural conditions there is a polar cell-to-cell movement from apex to base, such as that which occurs in stem tips, young oat seedlings, etc. (2) If the auxin is taken up by the plant through the roots then the pathway is through the xylem, i.e. in the transpiration stream. (3) When auxin is taken up by leaves or when it accumulates in the transpiration stream (as the result of no. 2) then the movement is downward and in the phloem. (The mechanisms described under (2) and (3) occur when auxin in large concentration is applied to the plant.)

C. L. Hamner and H. B. Tukey reported in 1944 that spraying the synthetic growth modifying substance 2,4-D on the leaves of the wild morning-glory brought about death of the roots. This announcement stimulated considerable interest in weed killers and was followed by considerable research on translocation of 2,4-D and related compounds. J. W. Mitchell and J. W. Brown (1946), working with bean plants, showed that movement of the stimulus which results from 2,4-D treat-

ment is correlated with the movement of organic foods. Translocation did not occur from leaves with a low sugar content. When the leaves were subjected to conditions which resulted in active photosynthesis, such as exposure to intense light and adequate concentrations of CO_2, then the stimulus began to move through the plant. When 2,4-D is applied to the roots of a plant, the resulting stimulus is translocated through the transpiration stream.

The results of a number of investigators indicate that the flower-inducing substance in plants is translocated in the phloem along with food substances.

Most of the water-soluble vitamins have been found to be transported along with foods. This has been reported for thiamin, pyridoxine, pantothenic acid, and riboflavin.

chapter 17

Respiration

After Joseph Priestley's discovery of photosynthesis in 1772 it was generally assumed that plants purify the air and that animals add "noxious substances." That plants too may add noxious substances (CO_2) was not realized for some time. The physician Jan Ingenhousz (1779) discovered that light and green pigments are necessary for the production of oxygen by plants. Subsequently, as the result of experiments by Ingenhousz and by Senebier, it became apparent that plants under illumination produce oxygen, whereas in darkness they produce carbon dioxide.

The idea that every living cell respires and releases CO_2 does not appear unusual to the physiologist today. This was at first overlooked in the case of green plants because while the plants are carrying on photosynthesis, they are also consuming the CO_2 they respire.

Many years passed, however, before it was realized that this gaseous exchange in living organisms was merely the outward manifestation of a very important process within the cell. Today plant physiologists define respiration as the cellular oxidation of foods with the release of energy. Water and carbon dioxide are evolved as by-products. Some of this energy is utilized in building new materials, in the uptake and accumulation of salts, in processes accompanying growth, and some is released as heat. The last-mentioned form of energy is of little use to plants and can be considered as pure waste.

Respiration studies with leaves, flowers, and with seeds containing mainly carbohydrates, have indicated that the quantity of CO_2 released is approximately equal to that of the O_2 absorbed. That is, the respiratory ratio (CO_2/O_2) is roughly equal to one. This is taken to indicate that in instances of this kind a hexose is the principal substrate because this same ratio is obtained in the complete oxidation of a six-carbon sugar. The equation for the reaction may be written as follows:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 673 \text{ Cal.}$$

It will be noted that this reaction is apparently the reverse of that which occurs during photosynthesis. It must be emphasized here that the above equation represents a simplified summary of the respiratory process. A more detailed discussion of the physiology of the process appears later.

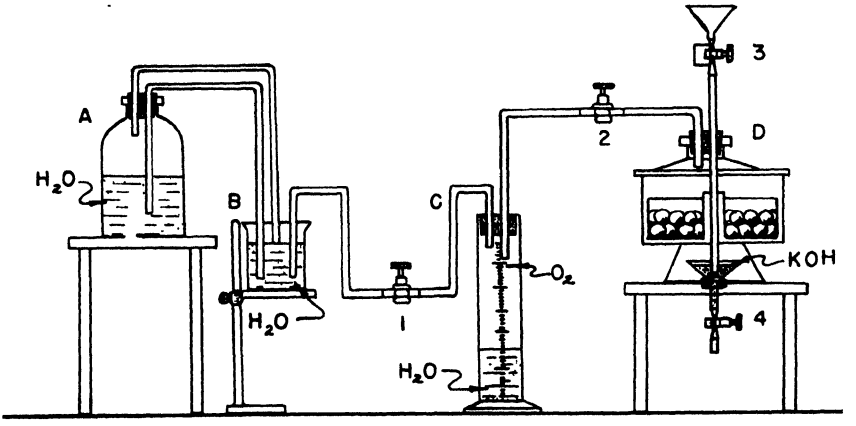
Although carbohydrates appear to be the principal compounds which are oxidized in respiration, it has been shown that other foods may be consumed too. After the supply of carbohydrates has been exhausted the plant will draw upon stored fats and when the latter have been consumed, proteins will be utilized. Both fats and proteins are probably broken down into simpler substances before being oxidized. In foods of this kind (fats and proteins) the proportion of oxygen to carbon is much lower than it is in the case of carbohydrates. The respiratory ratio therefore will be less than unity.

The reverse is true of the organic acids in plants which may be consumed in respiration under certain circumstances. Since the proportion of oxygen to carbon is much higher, the respiratory ratio will likewise be greater than unity in the respiration of these acids.

Two types of respiration are recognized by most plant physiologists: aerobic and anaerobic. The latter is similar to, if not identical with, alcoholic fermentation. These processes will be discussed later.

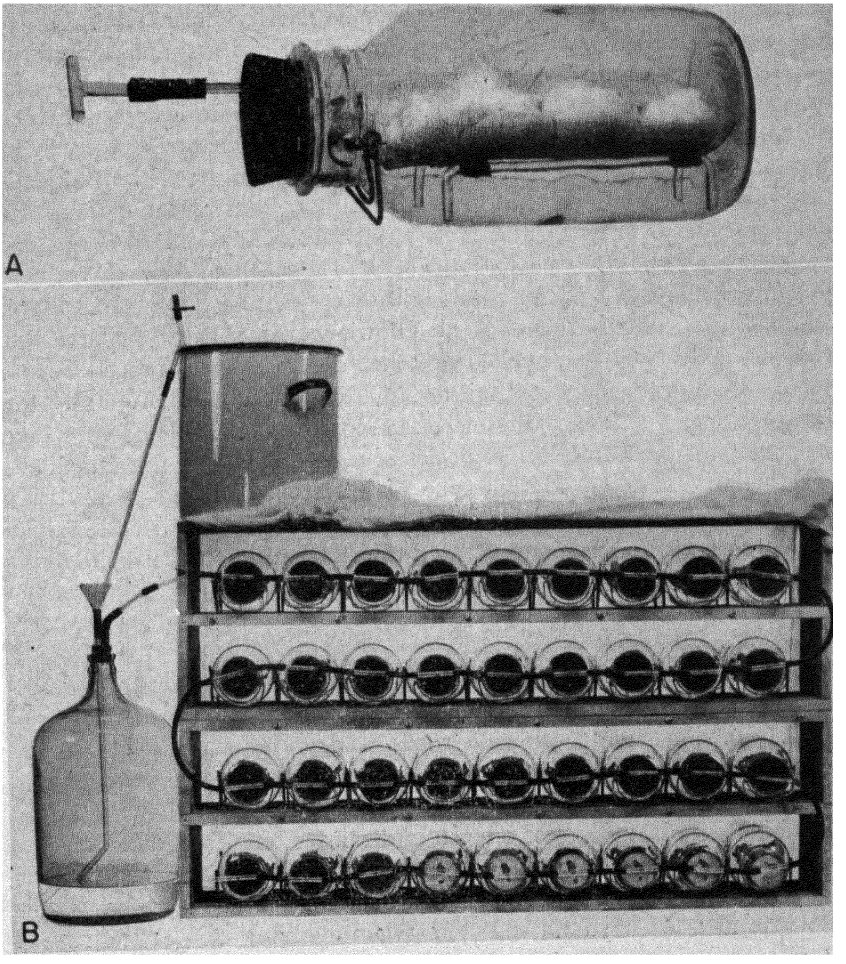
Measuring Respiration

Respiration rates may be measured either by determining the amount of oxygen consumed or the amount of CO_2 released. It is usually more



Apparatus for determining respiration rate of plant materials. Oxygen (from cylinder C) is supplied to respiration chamber (D) at atmospheric pressure as water is siphoned from the reservoir (A) into the constant-level beaker (B). (Redrawn after Haller and Rose: "Apparatus for Determination of CO_2 and O_2 of Respiration," *Science* **75**:439–440, 1932.)

convenient to determine the CO_2 released. Older methods consisted of enclosing the plant in a chamber and passing a stream of air free of CO_2 over the plant. The air, as it leaves the plant chamber, is passed through a suitable absorbent for CO_2. If $Ba(OH)_2$ is used, the CO_2 forms $BaCO_3$ which is insoluble and which is precipitated. Either unaltered $Ba(OH)_2$



Denny's method for conducting respiration studies of a large number of samples simultaneously. (A) Individual unit. (B) The assembled apparatus. Plant material is held above standard NaOH by means of a shelf. The 19-liter bottle supplies oxygen which passes into each bottle through the t-tube, circulating throughout the entire system. (Courtesy, Boyce Thompson Institute for Plant Research, Yonkers, N.Y.)

or precipitated $BaCO_3$ may be determined by chemical analysis. In more recent years physiologists have been inclined to employ either NaOH or KOH for the absorbent. Carbonates of sodium and potassium are soluble in water, but they may be estimated by first precipitating them with barium chloride. Another modern improvement consists of introducing oxygen to the system under atmospheric pressure. It is now considered

that the older system of forcing air or oxygen over the plant creates an abnormal condition which might seriously modify normal respiratory rates. M. H. Haller and D. H. Rose have described a simple apparatus for determining respiration in which test materials are enclosed in a desiccator. As CO_2 is absorbed in the KOH a slight vacuum is created which in turn causes oxygen to flow into the desiccator from a reservoir. F. E. Denny developed a rather simple system which permitted simultaneous study of a large number of individual samples. Test material (fruits or seeds) are placed in horizontal fruit jars containing standard alkali. By means of a T-tube each jar is connected to a source of oxygen or, in a still simpler manner, they may make direct contact with the atmosphere.

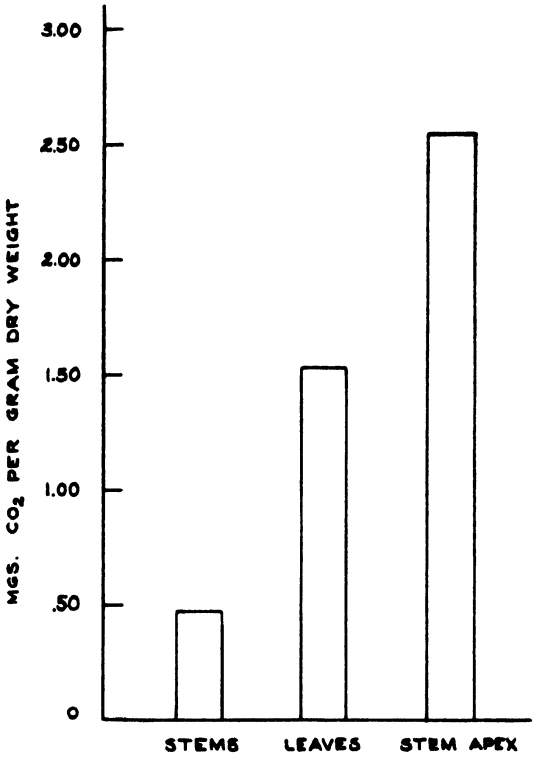
Significant contributions to the knowledge of respiration have followed the realization by physiologists that the respiratory process occurs in every living cell of the organism. Thus it is practicable to study the process in thin sections of living tissues or in single-celled plants like yeast, bacteria, and unicellular species of algae. The Warburg apparatus is particularly adapted to this type of investigation. Our present-day knowledge of the fundamentals of cellular respiration has been obtained by research workers who concentrated their attention on the single living cell or on isolated tissue.

Relative Rates of Respiration

Actively growing parts of the plant show the highest rate of respiration. This is not surprising since respiration represents a release of energy. Growing shoots, growing roots, and germinating seeds respire very rapidly. The rate is much lower in mature tissues and lower still in dormant cells. When resting seeds begin to germinate there occurs a marked increase in uptake of oxygen and release of carbon dioxide.

F. Kidd, C. West, and G. E. Briggs studied the respiratory rates of different organs of sunflower seedlings over an extended period. When the plants were 50 days old the stems were evolving CO_2 at the rate of 0.46 mg. per Gm. of dry weight per hour, the leaves at the rate of 1.52 mg., and the stem apex at 2.56 mg.

Many harvested fruits have been found to experience a "post-harvest climacteric" in respiratory rate. Kidd and West first employed this term in connection with respiration of apples. After setting of the fruit (i.e., after the ovary of the flower has begun to enlarge) the respiration rate of various apple varieties is very high. The rate declines to a minimum at about the time most varieties are harvested, and then begins to rise again to a maximum. Kidd and West called the post-harvest peak in respiration the *climacteric*. During its peak in respiratory rate the fruit has its best flavor and aroma. Following the climacteric the rate declines and the fruits are said to be in senescence.



Relative rates of respiration of different parts of the sunflower plant. (Plotted from some of the data of Kidd, West, and Briggs: *Proc. Roy. Soc.* (London) B. **92**:368–384, 1921 British Crown Copyright.)

Compensation Point

It has been pointed out that when illuminated, plants carry on photosynthesis and respiration at the same time. In the dark they respire only. It follows then that as the light is gradually diminished there will eventually appear an intensity at which the plant consumes in respiration all of the food manufactured in photosynthesis. This is termed the *compensation point.* It is obvious that a plant cannot live long at the compensation point. In the first place, if a plant were to consume all of its respirable material during the day, it would have nothing to live on during the night. Also there would be no surplus food for growth or for storage in the seeds or other storage organs, such as roots and tubers.

Factors Affecting Rate of Respiration

A number of factors directly affect the rate of respiration in plants. They are as follows:

Temperature. In general the respiratory rate of plant tissues increases with a rise in temperature. This was observed by H. C. Gore in his studies with a large number of different fruits. Within certain limits the rate increases two or three times for each 10°C. rise in temperature.

A distinction must be made between initial and continuing rates of respiration, however, as was pointed out with reference to the effects of temperature on photosynthesis. Studies with pea seedlings have indicated that there is a gradual increase in initial rate with increase in temperature between 0°C. and 45°C., but that at temperatures higher than 30°C. the initial rate is not maintained. In this instance 30°C. is apparently the optimum temperature. It should be borne in mind that different plants or plant parts may show considerable variation in regard to optimal temperatures for respiration.

As a rule, lowering the temperature decreases respiratory rates of plants. This is a principle which has found extensive application in commercial refrigeration of horticultural crops. More detailed discussion of this topic is presented later in this chapter. Several modifications of the above statement will be presented here because of rather interesting physiological reactions which are apparently involved.

E. F. Hopkins has reported that the rate of respiration of white potatoes falls off as the temperature is lowered to just above freezing. At this point a rise in the rate of respiration occurs. It is believed that such an increase in the respiratory rate is the result of an increase in the quantity of respirable materials such as soluble carbohydrates, which, as has been mentioned previously, tend to accumulate in Irish potatoes when they are stored at temperatures slightly above 0°C.

Respiration in tropical fruits may be retarded by lowering the temperature, but if these fruits are held too long between 0°C. and 10°C., the effect on respiratory rate appears to be more or less permanent. Winston W. Jones (1942) suggested that the basic metabolism of the fruit is upset by such treatment and that some physical phase of respiration has become a limiting factor.

Protoplasm in the Cell. We have stated that meristematic cells have a relatively high rate of respiration and have suggested that the reason for this is that cells of this type are characterized by considerable metabolic activity. This in turn may be traceable to the large quantity of protoplasm present in meristematic cells. As cells become more mature, vacuoles are enlarged, cell walls are thickened, and respiratory rate diminishes. In like manner the respiratory rate of leaves is higher than that of storage organs (roots and tubers); that of cambium is higher than that of phloem; and sapwood is higher in respiratory rate than is heartwood. All of this suggests a relationship between protoplasmic content of the cell and rate of respiration.

Respirable Material. Other conditions being favorable, the rate of respiration will increase with an increase of sugar content. For instance, the respiratory rate of etiolated leaves is not high, but if these same leaves are floated on a solution of sucrose, the respiration rate will show a considerable increase. It has been shown also that plants respire more rapidly after having been exposed to conditions favorable for photosynthesis. In like manner the respiratory rate of resting seeds and tubers rises as sprouting occurs, because hydrolysis of insoluble foods accompanies resumption of growth.

Oxygen Content of the Atmosphere. Reduction of the oxygen content of the surrounding atmosphere causes no significant lowering in the respiratory rate of most plants until the percentage drops to about 10%. At 5% oxygen definite retardation of respiration occurs. The response of plants or plant parts depends upon several factors. Tissues which normally have a low rate of respiration are not as seriously affected by lower concentrations of O_2 as those which ordinarily respire rather rapidly. Also in the case of plants which possess an effective anaerobic system (like rice, which germinates under water) the effect of lowered concentration of oxygen is not as pronounced as it is with plants which require a normal supply of O_2 for germination.

Increasing the oxygen content of the atmosphere does not appear to have as accelerating an effect as might be expected. Steward and his associates reported only slight increases in the respiration of isolated plant tissues when exposed to concentrations of oxygen above that of normal atmosphere (21% O_2); and even at the highest concentration, which was 100%, the respiratory rate was not markedly increased.

Carbon Dioxide Content of Atmosphere. As a rule the respiratory rate of plants is not affected by increases of CO_2 up to 10% of the surrounding atmosphere. But a number of investigators have reported that as the concentration is increased from 10% to 80%, a progressive decrease in respiration occurs. The results just cited were obtained with germinating seedlings. Specific response to higher concentrations of CO_2 may vary with the particular type of plant or tissue. N. C. Thornton found that concentrations of CO_2 above 20% depressed respiration in strawberries, asparagus, lima beans, and bananas. Similar concentrations of CO_2 showed practically no effects on carrots but increased the respiratory rate of potato tubers and tulip and onion bulbs. (Concentrations below 20% reduced the respiration of potatoes, tulips, and onions.) It will be recalled that potato tubers constituted an exception in regard to respiratory response to low temperatures, probably as a result of accumulation of sugars at temperatures near 0°C. Sugars have been shown to accumulate in potato tubers when stored in higher concentrations of CO_2, and this doubtless accounts for the reported increases in respiration.

Additional investigations will be required, however, to explain similar responses of tulip and onion bulbs.

Despite the exceptions which have been cited, the general statement can be made that high concentrations of CO_2 produce a depressing effect upon respiration of plants, especially if temperature and oxygen supply are low. Plants differ from animals in being able to tolerate concentrations of CO_2 which would produce death in animals in a relatively short time. We shall show in the following pages how CO_2 storage can be employed to prolong the life of horticultural products. There are, however, limits to the endurance of plants to relatively high concentrations of CO_2, and there is danger of damaging tissues or producing death in plants as well as in animals if treatment is too severe. It has been shown that when germinating seedlings are exposed to progressively higher concentrations of CO_2 (10% to 80%), a correspondingly progressive decrease in the respiratory ratio occurs. Results with fruits and vegetables stored under similar conditions (carbon dioxide storage) indicate that the course of respiration must have been changed in these products also.

Other Factors. Not all factors which are known to affect the rate of respiration in plants have been presented here. The degree of hydration of the protoplasm in the cell naturally affects the rate, and mechanical injury to plant tissues will accelerate respiration.

The plant physiologist may employ chemical compounds for retarding respiration, and it is possible to inhibit specific enzymes at various stages of the rather complex process. Cyanides, azides, and fluorides have been employed in these investigations, and much of our present-day knowledge of cellular respiration has been developed as a result of the use of these inhibitors.

Respiratory rate may likewise be accelerated by treatment with very low concentrations of compounds like ethylene, carbon monoxide, chloroform, and ether. Various physiological processes may be stimulated by treatment with ethylene, and it is interesting to note that when this gas is used to accelerate coloring or ripening of fruits and vegetables, the first indications of stimulatory effects appear in increased rate of respiration.

Retardation of Metabolism

For many years man has attempted to prolong the life of horticultural crops by holding them in cold storage. The low temperatures customarily employed in storage houses tend to retard physiological processes which bring about senesence in the commodity. The first noticeable effect of the low temperature is a reduction in the respiratory rate. It is quite obvious that if the life of the commodity is to be extended, the rate of loss of respirable materials (food) must be retarded.

One type of horticultural crop, including seeds, tubers, roots, corms, and bulbs may be stored between growing seasons and subsequently used

for propagating material. Other commodities, like fruits and vegetables, are preserved for use as food. Whatever the case, the purpose is to conserve the food reserves in the stored product.

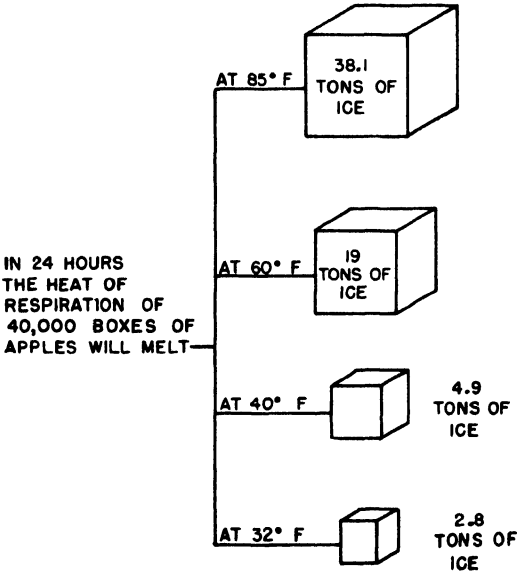
Table 25

EFFECT OF TEMPERATURE ON RESPIRATION RATE OF APPLES*

Storage Temperature (Degrees Fahrenheit)	*B.T.U.'s per Ton per 24 Hours*
32	660–880
40	1110–1760
60	4400–6600
85	6600–15,400

* Rose, Dean H., Wright, R. C., and T. M. Whiteman: *The Commercial Storage of Fruits, Vegetables and Florists' Stocks*, U.S. Department of Agriculture Circular #278, 1949.

The effect of temperature upon the respiratory rate of a commodity like stored apples is shown in Table 25. The rate of respiration is expressed in the number of British Thermal Units (B.T.U.) evolved by a



Effect of temperature on respiration of stored apples. (Drawn from data of Rose, Wright, and Whiteman: "The Commercial Storage of Fruits, Vegetables, and Florists' Stocks," U.S. Dept. of Agriculture Circular #278, 1941.)

ton of fruit in 24 hours. A B.T.U. is the number of calories required to raise one pound of water one degree Fahrenheit. It requires 144 B.T.U. to melt a ton of ice. A ton of apples at 32°F. (0°C.) will release from 660 to 880 B.T.U. in 24 hours, depending upon the variety of apple. At 40°F. (4.4°C.) the rate is almost twice as much, and at 60°F. (15.5°C.) it is

between six and seven times the rate of 32°F. At 85°F. (29.4°C.) the same ton of apples would release between 6600 and 15,400 B.T.U. and the rate of respiration is 13 times that at 32°F. Expressed in another way, if a ton of apples were being held in storage at 32°F., it would require 5.7 tons of ice per day just to prevent the heat of respiration from raising the temperature of the apples above 32°F. It has been said that apples respire as much in one day at 70°F. (21.1°C.) as they do in ten days at 32°F. It follows then that if three days elapse between packing and storing, when the air temperature is 70°F., a whole month of storage at 32°F. has been lost.

Different commodities vary in the length of time that they may be held in storage, and there may be considerable variation in the same species. Final breakdown in fleshy fruits appears to occur when respirable material (carbohydrates) is reduced to a definite minimum. It would seem therefore that a commodity with a high carbohydrate content and a low respiratory rate would lend itself to a long period of cold storage. This is true to a certain extent although a direct correlation cannot always be shown. If such were the case one might be able to definitely predict the potential storage life of each crop of fruits. Whatever the case, a commodity like the mushroom, with little if any carbohydrate content but with a high respiratory rate, certainly does not last long after harvest. Table 26 shows the carbohydrate content, respiratory rate, and minimum storage life of a few horticultural products.

Table 26

CARBOHYDRATE CONTENT, RESPIRATORY RATE (B.T.U.'S), AND STORAGE LIFE OF SEVERAL HORTICULTURAL PRODUCTS*

Commodity	*Carbohydrate* (%)	*Respiration at 32°F. (B.T.U.'s per Ton per 24 Hours)*	*Approx. Storage Life*
Apples	14.9	660–880	3–8 months
White potatoes	19.1	440–880	3–5 "
Lemons	8 7	580	1–4 "
Oranges	11 2	690–900	8–10 weeks
Grapefruit	10 1	460	6 "
Peaches	12 0	850–1,370	10 days to 4 weeks
Strawberries	8 1	2,730–3,800	10 days
Tomatoes	4 0	1,020	7–10 days
Mushrooms	0 0	6,160	2–3 days

* Rose, D. H., Wright, R. C., and T. M. Whiteman: U.S. Department of Agriculture Circular #278, 1949, and Sherman, Henry C.: *Chemistry of Food and Nutrition.* 6th ed. New York, The Macmillan Co., 1941.

Modified Atmosphere Storage

It has been mentioned previously that respiration rate of plants may be retarded either by increasing the concentration of CO_2 or by decreasing the concentration of O_2 in the storage atmosphere. Both principles have

been utilized in recent years in developing what is known as modified atmosphere storage. Physiologists have found for example that the storage life of apples may be significantly prolonged by holding the fruits in a mixture of 5% CO_2 and 2.5% O_2. R. M. Smock and F. W. Southwick have reported that when apples are stored at 40°F. (4.4°C.) in the above concentrations of O_2 and CO_2, the fruits will respire less than when held at 32°F. (0°C.) in ordinary air. Commercial storage houses in England have almost universally adopted this method of modified-atmosphere storage for apples. This is true to a lesser extent in apple storage houses in the United States.

Anaerobic Respiration

When plants are deprived of atmospheric oxygen they continue to respire for a while. The respiratory rate is not only reduced, however, but the course of respiration appears to be changed. Studies made with fruits and vegetables stored in the absence of atmospheric oxygen indicate that after a while relatively large percentages of ethyl alcohol and acetaldehyde are to be found in the tissues. It has been found, in fact, that end products in this case are not water and carbon dioxide. This type of respiration has been called *anaerobic respiration* by many plant physiologists. The equation for this reaction is usually written as follows:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 21 \text{ Cal.}$$

It is apparent from this equation that oxygen consumed in the process must have originated in the molecule of sugar itself. It is for this reason that the process is sometimes designated as *intramolecular respiration.*

Occasionally anaerobic respiration may be observed to occur in the presence of atmospheric oxygen. Rice grains have been reported to carry on anaerobic respiration even in the presence of 8% oxygen.

In instances in which seed coats (of pea seeds) and fruit hulls (of grape berries) are impervious to oxygen, intramolecular respiration may be observed to occur at times.

Alcoholic Fermentation

The term fermentation is generally applied to oxidation processes carried on by species of bacteria and fungi, and in which the end products are alcohol or various organic acids (lactic, acetic, citric, and others). This discussion is limited to alcoholic fermentation.

Under proper conditions a solution of sugar may be made to ferment, producing ethyl alcohol and carbon dioxide. It appears from historical records that man has been acquainted with the process of fermentation for a long time. It has been only in relatively recent years that the physiology of fermentation has been understood.

In 1837 C. Cagniard de LaTour observed for the first time that organisms are involved in fermentation. The fact that microscopic spores of yeasts are always present in the air and that these organisms cause fermentation, had escaped the attention of workers previous to the above date. Theodore Schwann (1810 to 1882) proved this point more strictly by demonstrating that no fermentation ensued once the organisms were destroyed. It was Pasteur who really clarified this subject and who succeeded in getting the view accepted by the scientific world.

Alcoholic fermentation, like anaerobic respiration, differs from aerobic respiration in several respects. The end products are different and the efficiency is lower in the case of fermentation. This will be clear from the following equation, which is used to summarize the process of fermentation.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 21 \text{ Cal.}$$

Alcohol and CO_2 are formed instead of the H_2O and CO_2 of aerobic respiration, and the energy released is only 21 Cal. instead of 673 Cal. But in spite of the reduced efficiency of this process it is still the means by which the yeasts obtain their energy necessary for metabolism.

Relationship between Anaerobic Respiration and Alcoholic Fermentation and between Aerobic and Anaerobic Respirations

Wilhelm Pfeffer in 1878 first proposed the theory that in higher plants anaerobic respiration constitutes the first step in aerobic respiration. This theory is now an accepted fact. As is shown in the discussion of cellular respiration, the first stage of the process consists of the oxidation of carbohydrates to pyruvic acid. This part of the process is not dependent upon atmospheric oxygen. If respiration is arrested at this stage, it is called *incomplete respiration.*

The course then followed depends upon whether or not the organism has access to atmospheric oxygen. In the presence of atmospheric oxygen respiration proceeds aerobically, the final products are water and carbon dioxide, and maximum efficiency is obtained. In the absence of atmospheric oxygen pyruvic acid is reduced rather than oxidized, the end products are alcohol and carbon dioxide, and the efficiency is greatly reduced. D. R. Goddard suggests that the term *alcoholic fermentation* be substituted for *anaerobic respiration* in plants.

* * * * *

An attempt has been made in the remaining part of this chapter to summarize what is known today regarding fundamentals of cellular respiration. The student who plans advanced work in plant physiology may find it useful, and it is hoped that it may serve as reference material for others.

Physiology of Cellular Respiration

With present-day equipment it is not difficult to demonstrate that during respiration of a plant or animal a hexose is oxidized to CO_2 and H_2O with the release of a certain amount of energy. It has long been realized, however, that the equation usually employed to illustrate this reaction represents a very brief summary of all of the reactions involved.

When it was realized that each living cell in a plant or animal is capable of carrying on respiration, a new era in physiology had begun. A number of physiologists focused their attention on the respiration of unicellular plants or animals or on isolated tissues of multicellular organisms. In this way a considerable quantity of fundamental information has been obtained. A complete discussion of cellular respiration is beyond the scope of this book and belongs in a more advanced course; an attempt will be made here, however, to summarize and to simplify present-day knowledge of the subject.

We have begun this discussion with the statement that respiration is a function of the individual living cell. We must constantly bear in mind two additional premises, both of which have been briefly stated previously. These are: (1) alcoholic fermentation and anaerobic respiration are identical, and (2) aerobic "respiration encompasses most of the chemical steps which also occur in alcoholic fermentation."

A little of the historical background will be presented because it illustrates how discoveries are made. Also chemical formulas are included occasionally for the purpose of clarification.

Goddard and Meeuse* offer the following succinct definition and elaboration of respiration:

> Respiration is the oxidation of organic compounds with molecular oxygen serving as the ultimate electron acceptor. The oxidation may be complete with water and carbon dioxide as the final products, or it may be incomplete, with organic acids as the end products. In contrast, the degradation of carbohydrates into two or more simpler compounds by an oxidation and reduction occurring within the original molecules or its products will be considered as fermentation or glycolysis.
>
> Respiration involves the oxidation of cellular metabolites, with the transfer of the electrons through a series of cellular enzymes (and coenzymes or biocatalysts) to molecular oxygen, resulting in the oxidation of the hydrogen to water and the production of α-keto acids. These acids undergo decarboxylation with the release of carbon dioxide. Thus the energy of the metabolite is released in a stepwise manner from a consecutive series of reactions.
>
> The actual respiratory metabolites are probably rarely hexose sugars, but usually phosphorylated compounds, such as phosphoglyceraldehyde, hydroxy acids, or aldehydes. This involves a common pathway of carbohydrate degradation in fermentation and respiration; in this sense respiration encompasses most of the chemical steps which also occur in fermentation.

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* *Annual Review of Plant Physiology*, Vol. 1, Stanford, Calif., Annual Reviews, Inc., 1950.

The various steps in respiration may be conveniently separated into two parts: (1) the conversion of carbohydrate to pyruvate, and (2) the oxidation of pyruvate to CO_2 and H_2O. For the sake of simplicity the first of these steps will be subdivided into two additional steps: (a) conversion of hexose to phosphoglyceraldehyde, and (b) oxidation of phosphoglyceraldehyde to pyruvate.

Phosphorylation (Phosphorolysis)

Since the beginning of the 20th century it has been known that fermentation of glucose is accelerated by the presence of inorganic phosphorus. In 1900 E. Buchner, M. Hahn, and others demonstrated that "press-juice" from yeast would ferment glucose more strongly if sodium phosphate were added. In 1905 A. Harden and W. J. Young each reported that phosphorus formed an ester with fructose which was identified as fructofuranose-1-6-diphosphate. This was subsequently called the *Harden and Young ester.*

In 1936 G. T. Cori and C. F. Cori showed that glucose-1-phosphate is the first product in the phosphorolysis of glycogen in frog muscle. The next year they reported that the enzyme phosphorylase is responsible for the reaction.

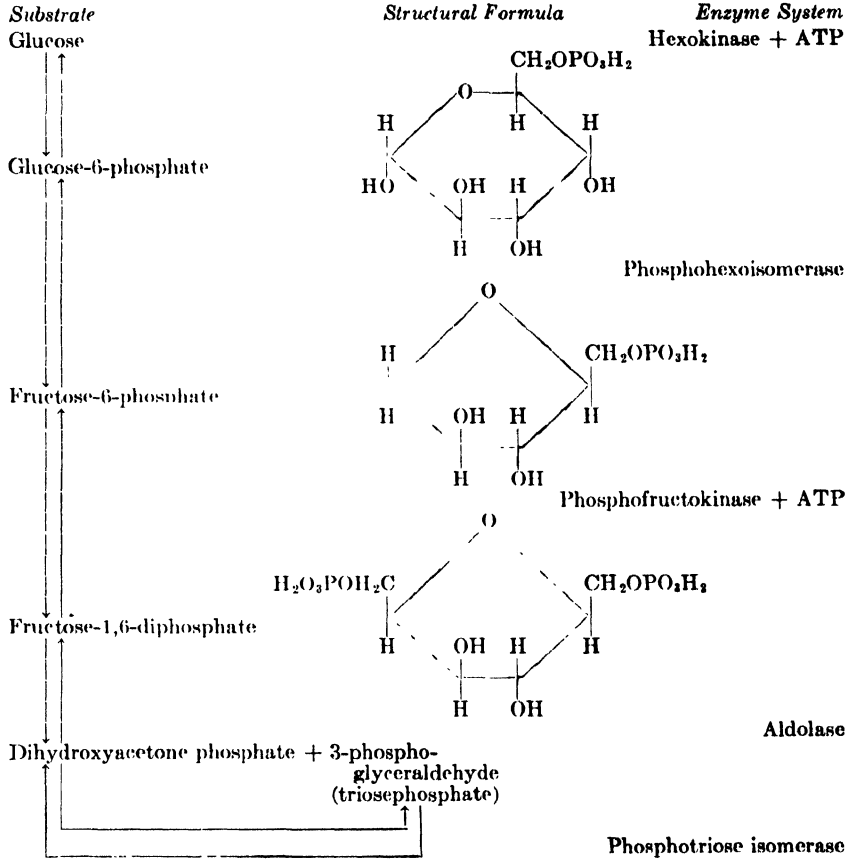
A considerable amount of research was stimulated by the results of these early investigations, so that now it is well established that phosphorolytic reactions play an important role in the breakdown of starch and glycogen and in the oxidation of sugars during respiration. The conversion of a hexose sugar to carbon dioxide and water takes place not in one, but in a series of integrated reactions. Enzymes participating in these reactions are termed *phosphorylases* or *transphosphorylases.* Although phosphorolytic enzymes were first prepared from microorganisms and animal tissues, a large number have now been identified in tissues of higher plants. Thus it is very probable that the basic respiratory mechanisms are quite similar in lower and higher plants and in animals. C. S. Hanes, W. O. James, and B. Tanko were pioneer investigators in the field of transphosphorylases in higher plants, and a large number of other investigators have since made valuable contributions to the subject.

An attempt will now be made to trace as simply as possible the sequence of chemical reactions occurring during the respiratory process. (See Table 27.) Phosphorylation occurs as the result of enzymatic transfer of a phosphate group from a complex substance called adenosinetriphosphate (ATP). This consists of adenine (a purine base), ribose, and three phosphate groups. Energy from oxidation is stored in ATP at a high level and two of the phosphate groups are held by what is known as high-energy bonds. During the transfer of one phosphate group ATP is converted to adenosinediphosphate (ADP). If the reaction is to continue indefinitely ADP must be reconverted to ATP.

If reserve carbohydrates are utilized in respiration, starch or glycogen are converted to glucose-1-phosphate by phosphorylase (starch or glycogen phosphorylase). This may be converted to glucose-6-phosphate or, if glucose is the starting point, then glucose-6-phosphate is formed directly

Table 27

TRANSFORMATION OF GLUCOSE TO 3-PHOSPHOGLYCERALDEHYDE AND DIHYDROXYACETONE PHOSPHATE



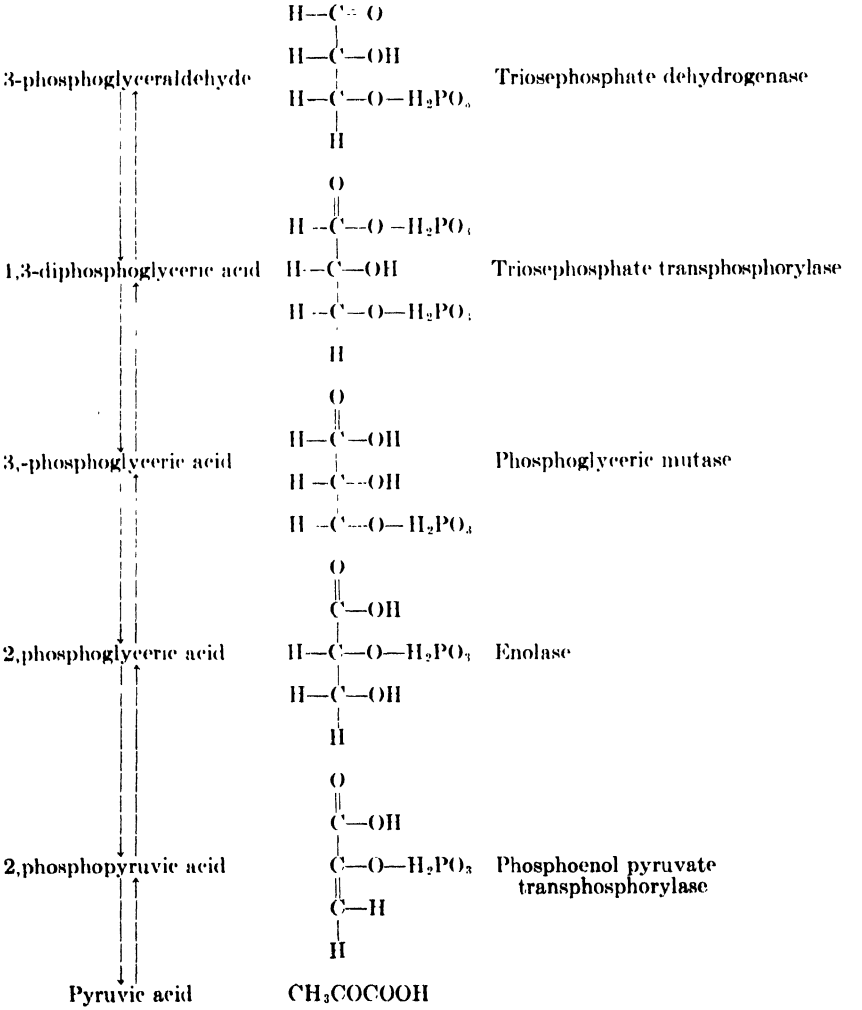
by the action of the enzyme *hexokinase*. Glucose-6-phosphate is isomerized to fructose-6-phosphate by *phosphohexoisomerase*. The next step is the formation of fructose-1,6-diphosphate by action of *phosphofructokinase*. There now occurs a breaking of six-carbon compounds into three-carbon compounds. *Aldolase* is the enzyme which participates in this process, forming 3-phosphoglyceraldehyde (triosephosphate) and dihydroxyacetone phosphate.

Conversion of Triosephosphate to Pyruvic Acid

We have shown that during phosphorolytic reactions ATP and ADP serve as phosphate donors. In the next reaction and several subsequent ones we shall be concerned with hydrogen donors. Enzymes which catalyze transfer of hydrogen are designated *dehydrogenases*. Two hydrogen

Table 28

CONVERSION OF TRIOSEPHOSPHATE TO PYRUVIC ACID*



* Adapted from Bonner, James: *Plant Biochemistry*, New York, Academic Press Inc., 1950, and from Stumpf, P. K.: "Glycolytic Enzymes in Higher Plants," *Annual Review of Plant Physiology*, Vol. 3, Stanford, Calif., Annual Reviews, Inc., 1952.

donors known to participate in reactions of this kind are diphosphopyridine nucleotide (cozymase or coenzyme I) and triphosphopyridine nucleotide (coenzyme II). These two donors are usually abbreviated as DPN and TPN. The molecule consists of nicotinamide (a vitamin), ribose, adenosine, and the phosphate groups. The hydrogen- or electron-carrying group in both nucleotides is nicotinamide. It is reversibly oxidized and reduced. Dehydrogenases act on two substrates at the same time. One substrate is oxidized (dehydrogenated) while the other is reduced (hydrogenated).

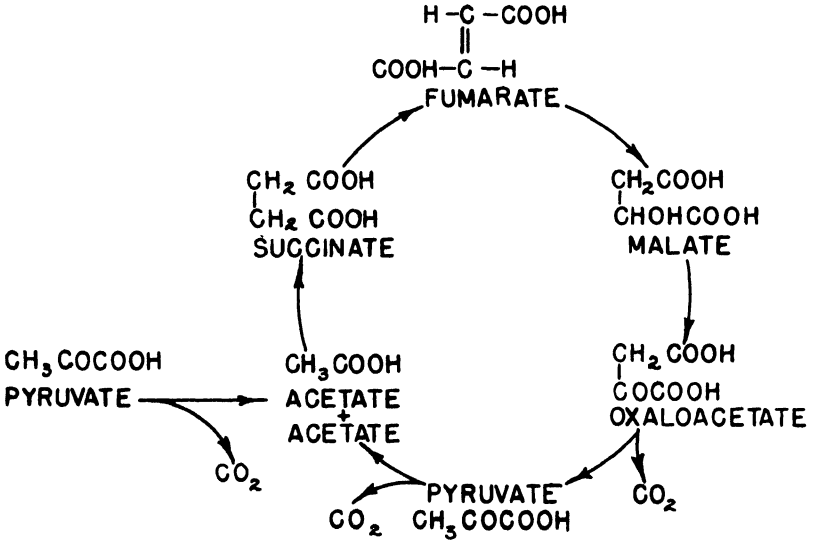
In the next step in this chain of reactions (see Table 28) 3-phosphoglyceraldehyde is converted to diphosphoglyceric acid (or the glycerate) by *triosephosphate dehydrogenase* and then to 3-phosphoglyceric acid by the enzyme *triosephosphate transphosphorylase*. Coenzyme I functions as the hydrogen donor. Dihydroxyacetone phosphate does not take part in respiration except to be reconverted to 3-phosphoglyceraldehyde. *Phosphoglyceric mutase* transforms 3-phosphoglyceric acid to 2-phosphoglyceric acid, which in turn becomes converted to 2-phosphopyruvic acid, the reaction being catalyzed by *enolase*. Final reaction in this portion of the process consists of the formation of pyruvate by action of the enzyme *phosphoenol pyruvate transphosphorylase*. The phosphate group which is removed from 2-phosphopyruvic acid is combined to ADP to form ATP, thus supplying high-energy compounds for a continuation of the process, as was indicated previously.

Oxidation of Pyruvates to Carbon Dioxide and Water

The final step—oxidation of pyruvic acid to H_2O and CO_2—is not well understood. The idea that organic acids participate in the respiratory process was suggested by T. Thunberg (1920) and F. Knoop (1923) and it was A. Szent-Györgyi who first demonstrated that organic acids do function in respiration. It has been proposed that the reactions proceed in the form of a cycle to which various names have been given, such as the *tricarboxylic acid cycle*, *citric acid cycle*, or *Krebs cycle*. No experimental evidence has been obtained which proves that these cycles exist. A summary of one of the simpler forms is presented, however, so that some idea of these theories may be grasped. Krebs has formulated a cycle from the theories of Thunberg (1920) and of Knoop (1923). (See accompanying diagram.) In this scheme two molecules of pyruvic acid are converted to two molecules of acetic acid with loss of CO_2. Progressive changes from here on are acetic → succinic → fumaric → malic → oxaloacetic → pyruvic, and back to acetic acid. Carbon dioxide is also evolved when oxaloacetic acid is converted to pyruvic acid and when acetic acid is again formed from pyruvic acid.

Enzyme systems have not been included in the diagram but it should

be realized that hydrogen atoms are transferred from one acid to another in the process with the aid of dehydrogenases, and that in the end hydrogen is combined with oxygen to form the water of respiration.



Oxidation of pyruvate to CO_2 and H_2O by way of succinic acid cycle as proposed by Thunberg and Knoop. (Redrawn after Bonner: *Plant Biochemistry*, New York, Academic Press Inc., 1950.)

Other and more complex cycles have been proposed, but since we are dealing primarily with theories there is no point in making an elementary textbook more complex by including any more of these systems.

Terminal Oxidases

Final stages in respiration consist of the activation of molecular oxygen by enzymes which are called *oxidases*. Oxidizable substrates lose hydrogen, which combines with activated oxygen to form water. Thus it is only in this final stage that atmospheric oxygen participates in the process. The enzymes *ascorbic acid oxidase*, *cytochrome oxidase*, and *tyrosinase* are the principal oxidases which are found in plants and they are often referred to as *terminal oxidases* because they function in the final step of the whole respiratory process.

chapter 18

Growth and Development

Growth and development are oft-observed phenomena in the biological world but it is doubtful if many of us are aware that many of the mechanisms involved in these processes are still not completely understood. Ordinarily, we think of growth as having ceased when animals attain adulthood in their life cycles. This is also true for some, but not all plants. Under favorable conditions perennial plants may continue to grow indefinitely. Giant redwood trees of California, for example, have been growing for 4,000 years and are continuing to grow. The characteristic of continuous growth has sometimes been cited as a distinguishing feature between the plant and animal kingdom. But this distinction is not entirely accurate, because it is not true of all plants, and in addition, certain marine animals are also capable of continual growth. Whatever the case, the topic of growth should serve to supply the student with ample food for thought.

The term *growth* does not lend itself to a simple definition. It is often defined as an increase in bulk or volume, but such a definition is also applicable to nonliving phenomena, such as the swelling of colloids or the enlargement of crystals. In this discussion we are concerned only with living organisms and in living organisms true growth involves the production of new living matter or protoplasm. In fact, growth is one of the fundamental properties of life.

The phenomenon of growth is in general dependent upon the presence of tissues in which cell division can occur. Tissue of this kind is called *meristem* (from the Greek word *meristos*, divided). Meristematic tissue will be found in several distinct regions of a growing plant—in the tip of the stem as it extends upward, in root tips as they push their way through the soil, and in the narrow band of cambial tissue which causes the thickening of the dicot stem.

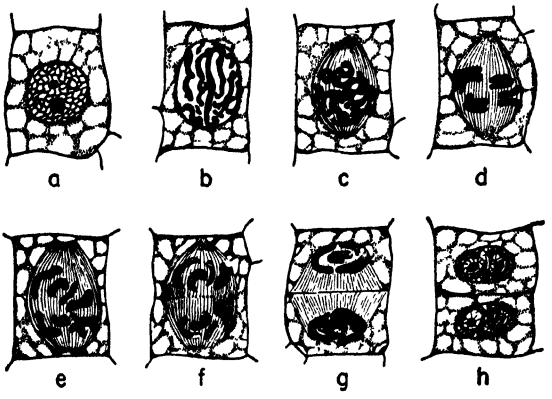
Growth processes include cell enlargement as well as cell division. This is discussed in detail later.

Cell Division

Two types of cell division may be observed in living organisms: *mitosis* (ordinary cell division) and *meiosis* (reduction division). We are con-

cerned at the moment with mitosis because meiosis constitutes an essential phase of the processes of sexual reproduction.

The *nucleus* of the cell plays a prominent part in mitosis. At the beginning *chromatin* appears as an irregular network but soon arranges itself into rod-shaped, compact bodies which are called *chromosomes*. These are transparent and hardly visible under the microscope unless they are stained. Soon the chromosomes are split longitudinally. Each half becomes a new chromosome with all of the hereditary characters of the original cell. A spindle of protoplasmic strands is formed and the two



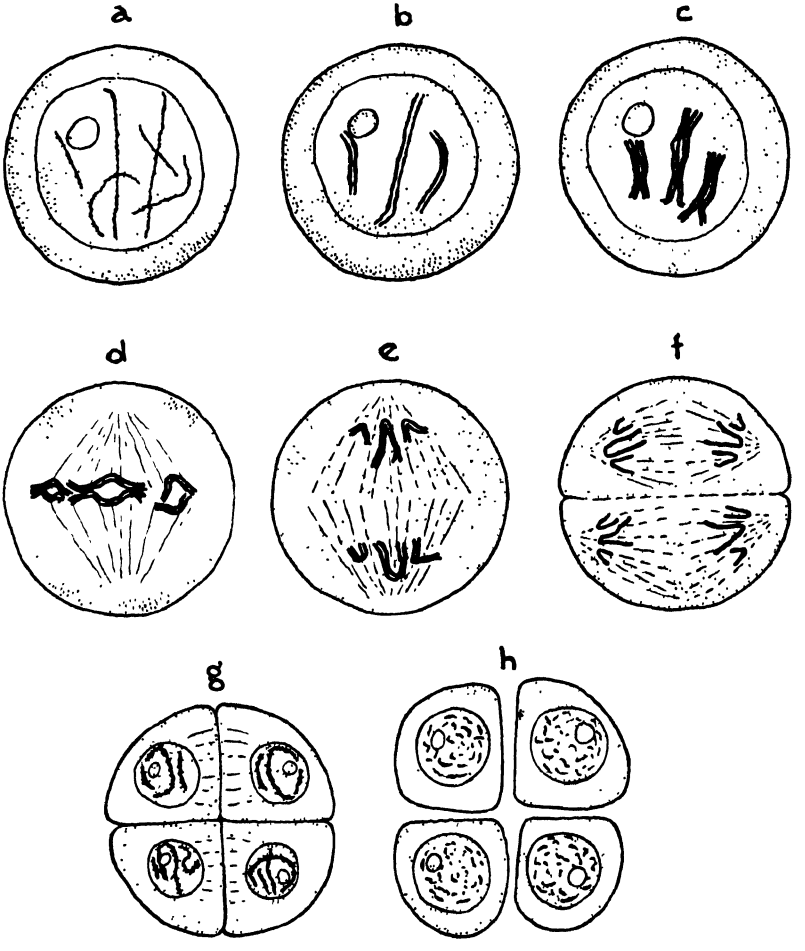
Drawing of cell in successive stages of mitosis. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

sets of chromosomes migrate to opposite poles of the nucleus. A new nucleus is formed as each group of chromosomes becomes organized into an irregular network of chromatin material once more. In the meantime a new, thin cell wall has been forming, separating the mother cell into two daughter cells.

In meiosis the chromosomes, instead of splitting in two, separate into two groups so that each new daughter cell, for the time at least, contains only half the original number. This is true of both male and female cells. At the time of fertilization, when male cells unite with female cells, the original number of chromosomes is restored, the difference from mitosis being that half have come from each parent. A more complete discussion of cell division may be obtained by reference to textbooks on cytology.

Sometimes meristematic cells originate from other living cells, but in general they have their origin in embryonic cells which have existed before them. It will be apparent from this statement that all living organisms that are in our midst at present must represent an unbroken line of descent from their progenitors in ages past. In a somewhat similar manner, the horticultural practice of budding or grafting, in which meristematic tissue is transferred from one tree to another, may serve to extend

the life of a particular tree indefinitely. Thus all navel orange trees throughout the world today are actually a part of one tree in Brazil which was the original source of propagating material.



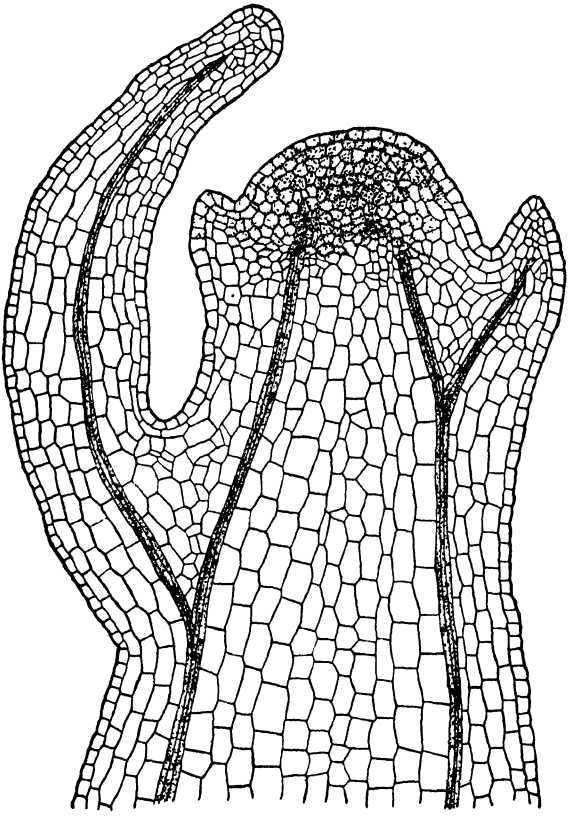
Successive stages in meiosis (reduction division). (Courtesy, Gibbs: *Botany*, New York, The Blakiston Co., Inc., 1950.)

Primary Growth

Primary growth is a term applied to growth which originates in the tip of the root or in the apex of the stem. When a seed germinates, both shoots and roots soon emerge and they continue to extend themselves, causing elongation of the plant's axis. Primary growth also includes branching of the roots and stems and the formation of flower clusters.

In other words, primary growth is to be distinguished from increases in diameter, which result from cambial activity.

Microscopical sections of the apex of a young shoot would reveal three zones of activity: (1) cell division, (2) cell enlargement, and (3) cell maturation. The zone of cell division constitutes the meristem which has

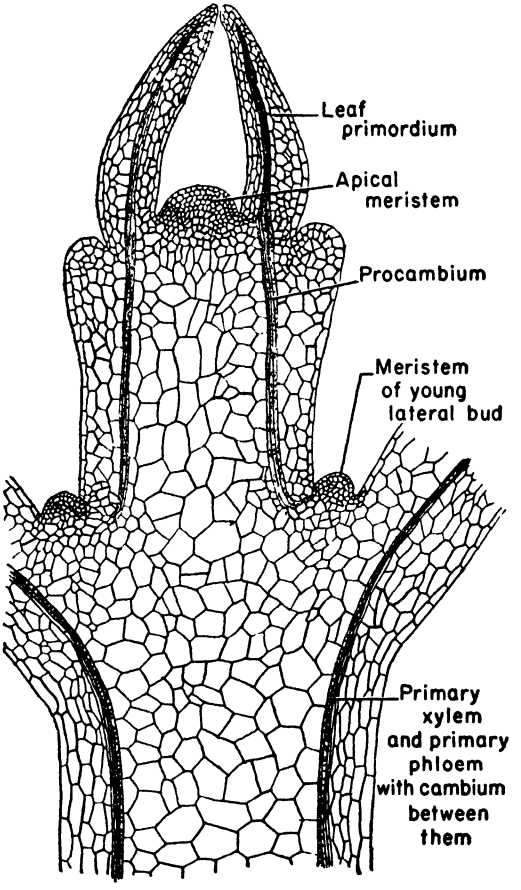


Apical region of stem. Note meristems which are more compact. Projections are beginnings of leaves. Early stages of conducting tissues may also be observed. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

been described. The more primitive cells of the meristem are called *promeristem*. These are truly embryonic cells. They are characterized by thin, delicate cell walls and appear to contain large quantities of protoplasm and only minute vacuoles. Nuclei are prominent. Intercellular spaces are lacking in this tissue, thus giving it a compact appearance. Older cells of the meristem are larger, the walls are thicker, and vacuoles more prominent. Intercellular spaces containing sap are present.

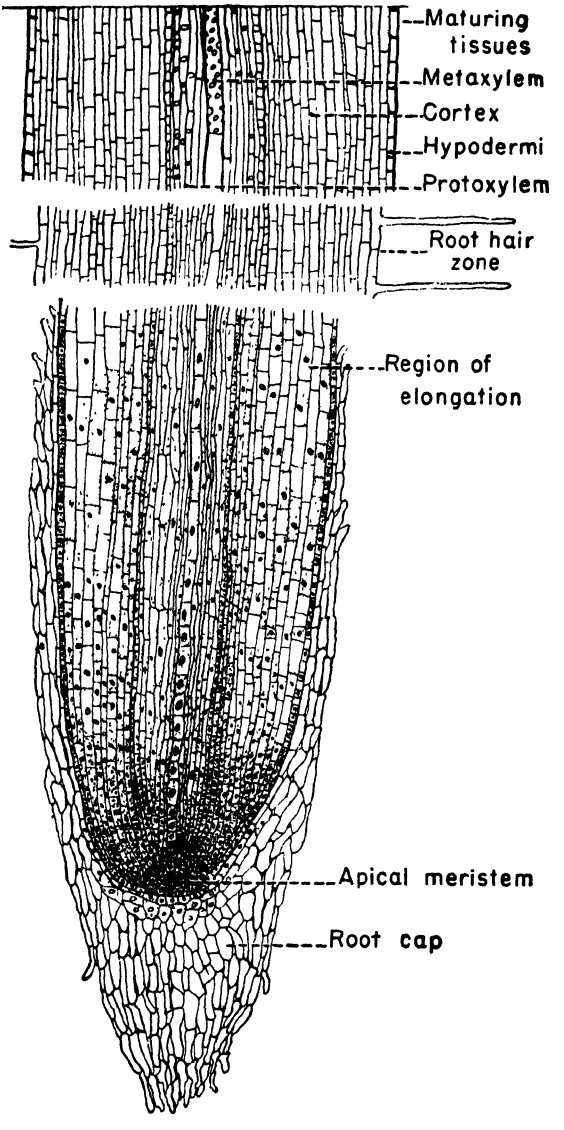
In chapter 17 on Respiration, we stated that the respiratory rate was highest in cells of the growing regions of stems and roots. The reason for

this statement should now be apparent. The zone of cell division is the center of intensive metabolic activity. Carbohydrates are required both for respiration and for construction of new cell walls. Amino acids are necessary for the synthesis of proteins. Large quantities of water are absorbed. A number of minerals including trace elements are assimilated.



Longitudinal section of apical region of stem. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

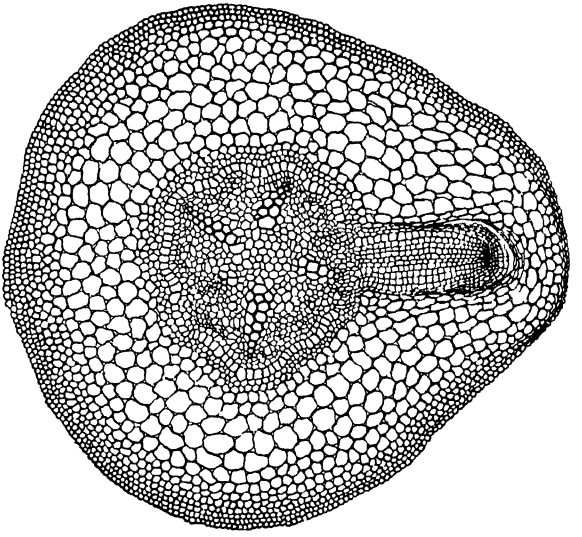
Below the zone of cell division is found the region of *cell enlargement*. It should be realized that some enlargement may occur in meristems too, but that enlargement predominates in this second zone. Since many of these cells enlarge in the direction of the axis of the plant, this phase of growth is often called the *zone of elongation*. The stem moves forward as a result of both cell division and cell enlargement. The zone of elongation may be as much as ten centimeters in length and even longer in some instances.



Longitudinal section of root tip showing various zones. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

The most obvious characteristics of this phase of growth are the formation of a large central vacuole and an increase in the area of the cell wall. Large quantities of water are taken up as the vacuoles enlarge and the cytoplasm is reduced to a thin layer pressed against the cell wall. Carbohydrates are assimilated as cellulose and pectic compounds are syn-

thesized in the cell wall. A. Frey-Wyssling points out that amino acids also are utilized during this stage of growth because cell elongation, more than being merely an enlargement of vacuole and cell wall, consists of an increase in amount of cytoplasm as well. Amino acids which are taken up at this time are utilized in the production of essential nitrogenous constituents of cytoplasm such as proteins, etc. What was formerly overlooked is that cytoplasm in the elongating cell, though present as a thin



Transverse section through root of the bean, showing origin of lateral root (×55). (Courtesy, Gibbs: *Botany*, New York, The Blakiston Co., Inc., 1950.)

layer, increases in quantity because the cell wall elongates more than 1000 per cent during the process. Respiration is relatively high during enlargement of cells and water disappears from the intercellular spaces.

Below the zone of enlargement is found the region of maturation, although the two may overlap. Maturing of the cell wall occurs after enlargement ceases. The walls of parenchymatous cells are thickened as the result of additional layers of cellulosic materials. In the tracheids, tracheae, and fibers, lignin is added to the walls. Thus both physical and chemical changes may occur in the cells during maturation. Some mature cells, like those of the cortex, retain their protoplasm when mature. Others, like the tracheae, lose their protoplasm during maturation. The respiratory rate during this phase of growth is not as high as was true of the other two phases.

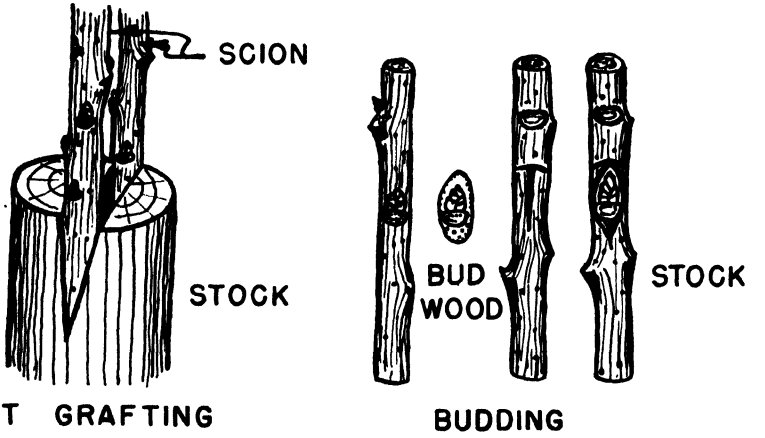
Root tips are characterized by the same three regions as described for stems, although there exist certain differences. The meristem in the stem is terminal, but in the roots this region occurs just behind the cap. The

root is provided with a cap which protects the tender embryonic tissue as it is pushed through the soil during the process of growth. The region of elongation in the roots is much shorter than in the stems (about 1 mm. compared to 10 cm.).

Other differences exist between the growing regions of the stems and roots, especially with reference to the origin and location of lateral branches. Lateral organs in stems originate in the cambium which is just beneath the surface, whereas branch roots originate from meristematic cells in the interior and force their way outward. Lateral organs of the stem originate in the zone of cell division; in roots they appear in the zone of maturation.

Secondary Growth

A cross-section of a dicot stem will reveal a uniseriate layer of cells between the phloem and the xylem. This is the *cambium*. This too is meri-



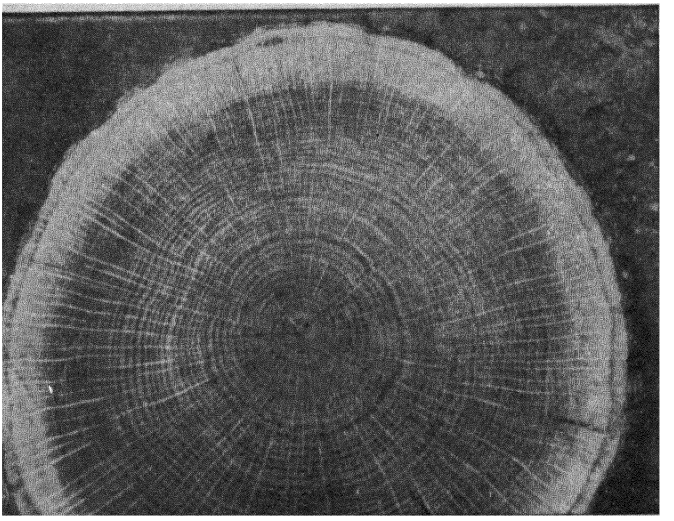
Success in grafting and budding depends upon the union of cambial tissue of scion (or the bud) with the stock. (Redrawn after Fuller and Tippo: *College Botany*, New York, Henry Holt and Co., 1949.)

stematic tissue. In most plants the cambium is a kind of cylinder extending from just below the stem tip to just behind the root tip, with the exception of gaps formed by the origin of leaves and branches.

The cambium gives rise to two types of cells: isodiametric ray cells, and the elongated elements of the phloem and xylem.

If the bark is removed from a sapling, the fresh, slippery tissue which is exposed is cambial tissue. When horticulturists bud or graft one variety of tree on another, the cambial tissues of the two plants must be brought into direct contact. It is only in this manner that a union will be made. The graft is made by the growing together of cambial tissues of scion and stock.

It is the cambial growth which produces increases in the diameter of dicot stems. As growth is renewed each spring in perennials, new xylem cells are formed on the interior of the cambium. New phloem tissue is formed on the outside. In this way all cells exterior to the cambium are moved outward. As the phloem is pushed outward, the older tissues are transformed to bark and are eventually sloughed off. Thus in examining the stump of a freshly cut tree, one finds mainly wood (xylem) since most of the active phloem is removed with the bark.



Cross-section of oak log. Note sapwood (light) and heartwood (dark). (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

The age of a tree can usually be told by counting the so-called annual rings in the stump. The rings are the result of a line of demarcation between one season's growth and the next. Wood formed in the spring of the year contains more and larger vessels and the cells are thin-walled. As summer progresses the cells become gradually denser and more thick-walled. The line of demarcation between cessation of growth and the renewal in the spring is quite pronounced and the resulting concentric layers constitute the annual ring.

Not all of the wood of a mature tree contains physiologically active cells. Ordinarily two types of wood may be discerned—*sapwood* and *heartwood*. The two layers can be distinguished as follows. The sapwood is nearer the bark and contains active xylem tissue. Beyond the sapwood is the dry heartwood. In many trees the heartwood is darker in color. It consists of dead cells and serves only to give strength to the trunk. The heartwood of old trees frequently dies and decays, leaving the bark and

sapwood in the main trunk. Nevertheless a tree in this state may live for years and continue to bear fruit.

Cambial tissue which has thus far been mentioned is often called *vascular cambium*, because it occurs in vascular bundles. Another type of meristematic tissue, *cork cambium* or *phellogen*, produces layers of cork in



The heartwood of a tree serves only for support and has no physiological function. The apple tree in the picture has been hollow for 20 years but still continues to live and to bear fruit.

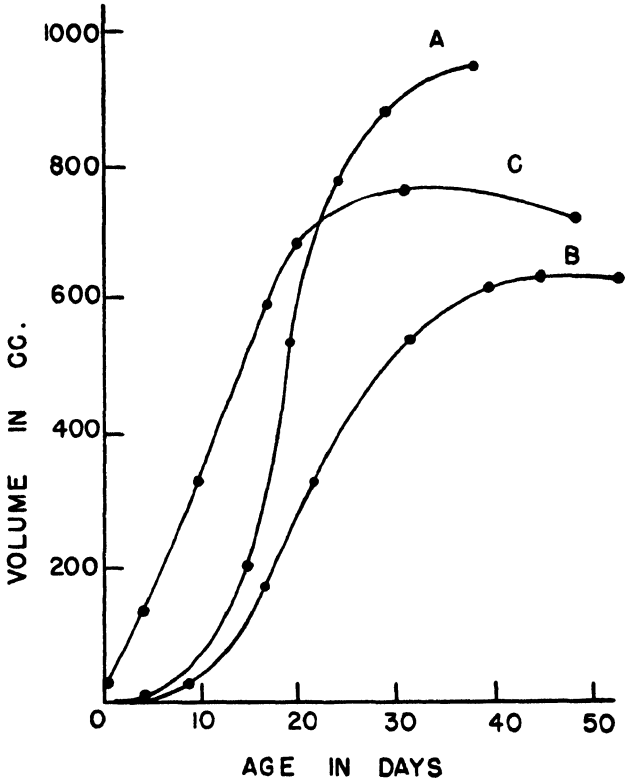
stems and in most perennial roots as these organs grow older. Cork cambium originates in the bark of stems and in the pericycle of roots.

Growth Curves

Physiologists have many ways of expressing growth quantitatively. Records may be made of increases in length, area, diameter, weight, and volume. When recording increases in weight the results may be expressed on the basis of either fresh or dry weights.

When the total increment is plotted against time, a very characteristic curve is obtained which is called the *growth curve*. It is sigmoid or

S-shaped. This means that growth at first is relatively slow. There follows a period of acceleration and finally the curve flattens out. The curve can be developed by recording growth of plants or plant parts. In fact a curve of this type depicts growth in a very general way. It is applicable to measurement of the growth of colonies of microorganisms, of virus in-



Growth curves of fruits. (A) Muskmellon. (B) Cucumber. (C) Scalloped summer squash. (Redrawn after Gustafson, "Growth Studies on Fruits," *Plant Physiol.* **1**:265–272, 1926.)

fections in plants, as well as the growth of animals, including man, and the growth of populations of countries.

Initiation of Growth

We have discussed the mechanics of growth without explaining what *initiates* the process. We now know that there are growth substances or hormones which must be present before growth is inaugurated. We shall discuss these interesting substances more fully in chapter 19.

Tissue Culture

A discussion of growth and development would not be complete without brief mention of *tissue culture*. This relatively new technique (as far as plant tissues are concerned) has resulted in valuable contributions to the subject of plant physiology. According to Philip R. White (1951) tissue culture is broadly defined as cultivation of isolated plant tissues and organs *in vitro*. Studies of this type have been made with (1) root cultures, (2) embryo cultures, (3) cultures of other members such as stem tips, and (4) cultures of undifferentiated masses.

The exact origin of a new idea is difficult to trace, and so it is with the idea of tissue culture. Schwann in 1839 promulgated the theory that all cells are essentially elementary organisms, and that they are capable of autonomous existence. G. Haberlandt in 1902, in a classic paper, set forth the principle of tissue culture although he never succeeded in making individual cells or groups of cells grow in artificial culture.

For a while physiologists began wondering whether or not the individual plant cell were capable of autonomic existence. All attempts to cultivate plant tissues *in vitro* had thus far proved unsuccessful.

Failure to grow segregated plant tissues may be traceable to three inherent difficulties. First, most plant cells are not bathed in a free, complete nutrient medium. Plant cells thus differ from animal cells, which are constantly supplied with blood plasma. Hence there is no natural nutrient which might be extracted from a plant and used as a medium for growing segregated tissues or cells. Second, most plant cells are enclosed in a rigid wall which prevents them from engulfing their food. This restricts the form of food which can be used. Furthermore, excising the tissue, preparatory to culturing, ruptures the cell wall and causes a certain amount of shock to the enclosed protoplasm. Third, normally only a few specialized cells (meristems) in the plant are capable of growth. Mature cells in a plant are practically inert from the standpoint of cell multiplication.

Success in plant-tissue culture was first attained in 1922 when W. Kotte, one of Haberlandt's students, worked with excised root tips. Colorless meristems proved much more satisfactory than the green, mature cells which had been used previously.

Between 1922 and 1936 most of the studies were devoted to perfection of techniques. Investigations yielded considerable information, not only in regard to the techniques of tissue culture, but they also contributed to a better understanding of organic and inorganic nutrition of plants. For example, the culture medium, in addition to supplying necessary minerals, must furnish cells with an effective source of energy, such as a carbohydrate. There soon ensued a controversy in regard to whether sucrose or dextrose was the most satisfactory source of energy. It now appears

that only sucrose is a truly effective source of energy for dicotyledonous plants, whereas monocots (wheat, corn, barley) prefer dextrose or its equivalent. Utilization of sucrose is dependent upon the concentration of inorganic phosphorus. This suggests that enzymatic phosphorylation is involved at the surface of the absorbing cells. It has been claimed that the material actually absorbed is a freshly formed (nascent) hexose-phosphate. Neither hexose and phosphate in uncombined form nor preformed hexose-phosphate appear to be absorbed.

Contributions to the field of inorganic nutrition of plants have also been made as the result of studies using tissue culture. P. R. White is of the opinion that excised tomato roots (and probably other tissues) require iron, copper, molybdenum, and probably zinc, manganese, boron, and iodine. All of these trace elements but iodine have now been accepted as essential to plants. One wonders why this element is required in tissue culture and not in solution cultures of the whole plant.

It has been found that most tissues in artificial culture require other substances besides minerals and carbohydrates. Usually vitamins and certain amino acids must be added unless it is known that the tissues synthesize these compounds. Thiamine must be added to the medium of all cultures except corn roots and carrot tissue grown in the light. Niacin, ascorbic acid, and indoleacetic acid have been found to be necessary for growth in certain instances.

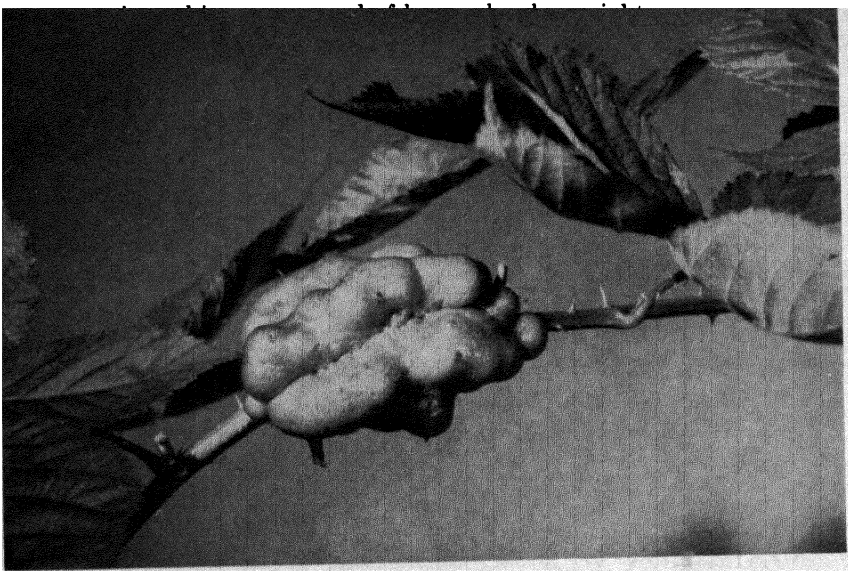
A Growth Factor in Coconut Milk and Other Similar Substances

Although the culture of excised plant embryos is not tissue culture in a true sense, it is usually included in a discussion of the subject. This seems advisable because techniques are similar and also because both types of study have made valuable contributions toward a better understanding of growth.

J. Van Overbeek, M. E. Conklin, and A. F. Blakeslee (1941, 1942) were attempting to grow excised embryos of jimsonweed (*Datura*) hybrids and found that the plants failed to produce endosperms. It was apparent that the hybrids would have perished in nature. The technique was then modified by fortifying the nutrient medium with coconut milk which is itself a "liquid endosperm." Coconut milk apparently supplied the necessary growth factor because the embryos then continued to grow.

Somewhat later this line of investigation took a rather unique turn. When S. M. Caplin and F. C. Steward were unable to grow noncambial carrot tissue in culture, coconut milk was again added to the culture medium. The result was a very rapid growth of the tissue. It was found that the milk was necessary only for the initiation of growth. Here then is an instance in which mature cells can be made to begin dividing and growing again, a power normally possessed only by meristematic cells.

Similarly effective material has been found in malt, and in wheat and corn ovules at the milk stage. The factor will withstand prolonged boiling in a neutral or slightly acid medium and is dialyzable. It is apparently



Insect gall on cane of wild blackberry.

Plant Galls

Plant galls are tumorlike growths which develop as the result of certain bacteria, viruses, insect stings, and wounds. Galls have often been compared to cancer in animals because they represent the proliferation or renewal of growth in what were apparently mature cells.

A number of investigations have been conducted with galls which have been produced by the crown-gall organism, *Phytomonas tumefaciens*.

Anatomical studies have indicated that tissues of the epidermis, cortex, cambium, and pith may be stimulated to abnormal growth by the crown-gall organism. Once a crown gall has become established, it is possible for it to continue growing without direct stimulus of the causal organism. For instance, secondary galls, which are free from bacteria, frequently appear on plants. Likewise primary galls may be rendered bacteria-free by treatment with heat. These sterile galls may be grafted to normal tissue or they may be transplanted into artificial media where they will continue to grow.

It can be readily seen that artificial culture of materials of this type affords ample opportunity to study both normal and abnormal growth, free from the influence of the host plant.

It appears (R. S. de Ropp, 1948) that crown galls give rise to growth-promoting substances which are capable of causing epinasty of petioles and initiation of roots. This growth factor is capable of moving from tumor to normal tissue, providing the two are grafted together, but does not appear to diffuse into agar. The factor is not present in active form in extracts prepared from tumor tissue.

Thus it appears that galls grow as the result of the presence of some growth-promoting substance. But what it is that initiates growth in these galls in the first place is still unknown.

chapter 19

Growth-modifying Substances

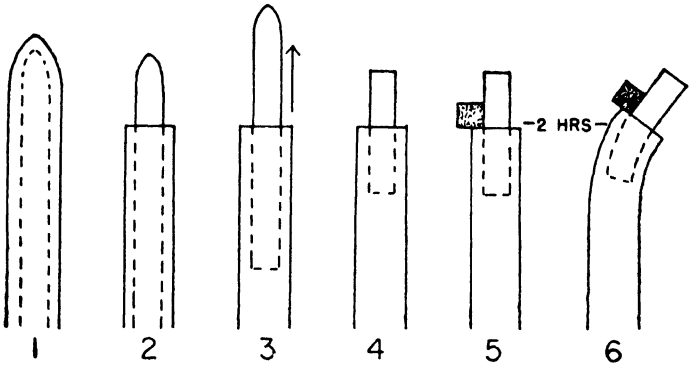
In animal physiology a *hormone* is a substance which is produced in or applied to one part of an organism and is transported to another part where it produces a physiological effect. Cortin, epinephrine, estrone, insulin, and thyroxine are typical examples of animal hormones. These substances are secreted by ductless glands and are transported in the blood stream to other parts of the body. More progress has been made in isolating and identifying hormones in animals than has been made with plants, as evidenced by the more than 30 of these substances which are now listed in textbooks on animal physiology as compared to the three which have been identified in plants.

The expression *growth-modifying substances* has been chosen as the title of this chapter for several reasons. It is felt that the word hormone should be restricted to a naturally occurring substance to distinguish it from compounds which have been prepared in the laboratory, even though the latter may have many of the characteristics of plant hormones. The terms *auxin* and *phytohormone* are often employed to indicate a hormone of plant origin. The term *growth-promoting substance* is not particularly applicable because many of these compounds are now employed either to promote growth or to inhibit it, depending upon the concentration applied.

Plant physiologists had long suspected that plants as well as animals contain hormones. Certain phenomena had been observed which indicated that a stimulus applied to one part of a plant was subsequently transmitted to another part, thus fulfilling the requirements of a hormone. A pioneer in this field of study was Charles Darwin. This original investigator studied the very common phenomenon of plants growing toward light. In unilateral illumination (light coming from one side) seedlings can be shown to bend toward the source of the light. This is often observed with young plants in a dimly lighted room.

In 1881 Darwin discovered that if he covered the tips of grass seedlings, they no longer bent toward the source of light. It was well-known at the time that the zone of cell enlargement is well below the tip of a grass seedling. It was therefore evident that, although light acted on the tip of the plant, some substance must be translocated from the tip down to the region of cell enlargement.

In 1907 P. Boysen-Jensen began his classic experiments which led the way to the discovery of hormones in plants. To appreciate the significance of these experiments one must be familiar with the anatomy of a young shoot of grass. The true leaf is at first enclosed in a sheath or *coleoptile*. This can be observed by cutting off the top of the coleoptile or by holding up the shoot in front of a strong light. Boysen-Jensen more or less corroborated Darwin's observations by showing that if the tip of the coleoptile were removed, the shoot would no longer respond to unilateral



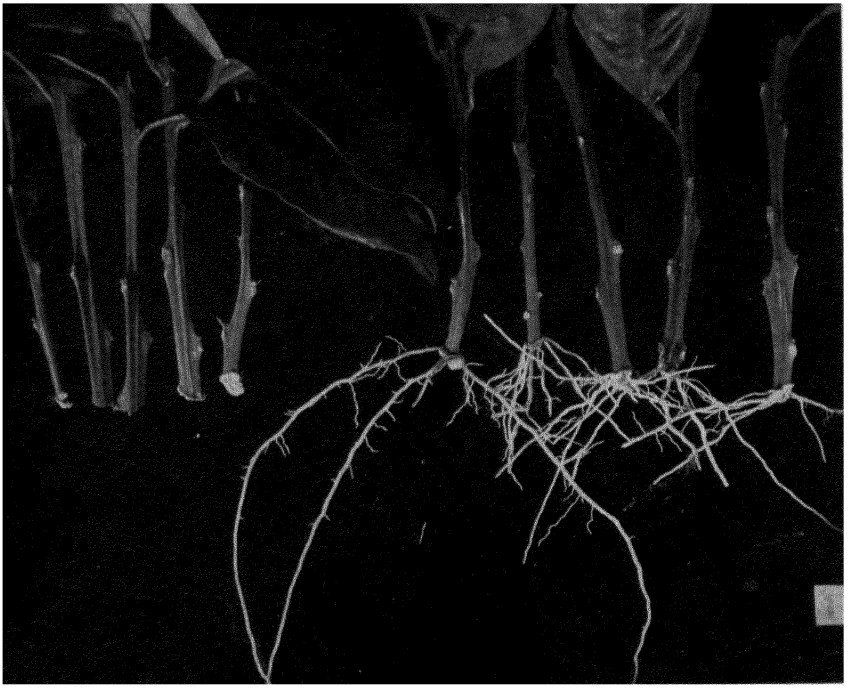
Agar block method for quantitative determination of auxin. The tip of the coleoptile is removed and the primary leaf is pulled loose. Agar block containing auxin is placed on one side of coleoptile, causing it to grow more rapidly on the treated side. (Courtesy, Went: "Auxin, the Plant Growth-hormone," *Botan. Rev.*, **1**:162–182, 1936.)

lighting. But when a cut was made near the tip and a block of agar was placed between the severed tip and the remainder of the shoot, then the plant again bent toward the source of light. This suggested that chemical substances were produced in the tip of the coleoptile, and that they migrated downward. More refined tests by Boysen-Jensen and by F. W. Went in 1928 illustrated rather clearly that illuminating one side of a plant causes growth-modifying substances (hormones or auxins) to migrate from the illuminated side to the shaded side, thus causing the plant to grow toward light.

Soon a technique was developed for assaying tissues or organs for content of growth-modifying substances. One method is called the *oat-coleoptile technique*. Standard oat seedlings are grown in the dark under favorable conditions of temperature and humidity. When the seedlings are about 4 cm. in length the tip of the coleoptile is removed. These operations are conducted in a red or orange light. Substances to be tested for activity are dissolved in agar which, when solid, is cut into blocks of about 2 × 2 × 1 mm. The block of agar is placed on one side of the decapitated coleoptile. As was shown in the instance of unilateral light-

ing, the auxin on one side of the shoot will cause bending in the opposite direction. It has been found that the degree of curvature is proportional to the amount of auxin in the block. The method thus serves as a satisfactory quantitative test for plant hormones.

The next important step in this interesting field of investigation was the isolation and identification of several growth-modifying substances. In 1931 F. Kögl and his co-workers isolated from biological sources three



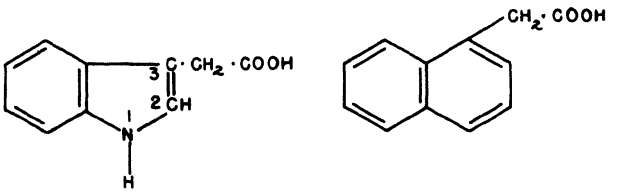
Rooting of lemon cuttings with growth substances. Those on right were treated with 3-indoleacetic acid. (Courtesy, U.S. Dept. of Agriculture.)

chemically pure, crystalline substances which showed all the reactions of auxins when tested by the oat-coleoptile technique. The three compounds were isolated from biological sources but not necessarily from higher plants. The sources were malt, vegetable oils, yeasts and molds, and human urine. The substances were *auxin a* ($C_{18}H_{32}O_5$), *auxin b* ($C_{18}H_{30}O_4$), and *heteroauxin* ($C_{10}H_9O_2N$). The latter is identical with indole acetic acid.

In 1939 O. Fischnich and F. Laibach in Germany independently showed that indoleacetic acid could be used to induce rooting of woody cuttings. In this same year P. W. Zimmerman and F. Wilcoxon, at the Boyce Thompson Institute, discovered several new synthetic growth substances, and their successful use of these in rooting of cuttings introduced

many new techniques in this field of investigation. Subsequently a large number of organic compounds were tested for their ability to cause cuttings to form roots, and the list of effective compounds increased.

Another line of development with growth-modifying substances had its beginning at a much earlier date. H. Fitting (1909, 1910) observed that pollen applied to stigmas of orchid flowers caused the ovaries to swell and stimulated other phenomena which normally occur after the flowers drop off. Similar effects could be produced by nonliving pollen or by aqueous extracts of pollen. But the time was not ripe for complete appreciation of the significance of these observations. It was not until



3 - INDOLE ACETIC ACID ALPHA NAPHTHALENE ACETIC ACID

Typical plant growth-promoting substances. Heteroauxin and 3-indoleacetic acid are identical.

much later that physiologists saw any relationship between pollination of flowers and the action of plant hormones. In 1933 Laibach showed that the active substance in pollen is identical with one of the three auxins previously mentioned.

In 1936 F. G. Gustafson applied growth-modifying substances to unpollinated flowers of the tomato and of several other plants. Compounds tested were phenylacetic acid, indoleacetic acid, indolepropionic acid, and indolebutyric acid. As a result of these treatments fruits enlarged and matured, but produced no seeds. Production of fruits without seeds is termed *parthenocarpy*.

Shortly before 1939 F. E. Gardner and P. C. Marth made an interesting and useful observation while studying the effects of alpha-naphthaleneacetic acid on the production of roots on holly cuttings. It was observed that treated cuttings retained leaves and berries longer than did untreated lots. In other words, abscission of leaves and fruits was apparently retarded by such treatment. These results suggested other possibilities, such as treatment of fruit trees for the purpose of preventing premature dropping of fruits. Additional experiments showed that this reasoning was correct, and it was found that when many varieties of apple trees were sprayed with alpha-naphthaleneacetic acid, the fruits remained on the trees until time for harvesting. In this way the so-called

preharvest dropping of apples could be greatly reduced. Treatment of fruit trees in this manner has now become a widespread commercial practice.

The next great advance in this area was made in 1941 with the discovery of *selective herbicides*. Many investigators had a part in this phase of the subject; it is not possible to name any one person who deserves more credit than another. Suffice it to say that while testing the effect of various synthetic compounds on plant growth it was observed that one compound, 2,4-dichlorophenoxyacetic acid (2,4-D), caused the death of broad-leaved plants without seriously affecting the narrow-leaved ones, like grasses.

When 2,4-D in proper concentration was sprayed on lawns it killed such weeds as dandelions, plantain, and others. The grass showed no ill effects. It was immediately apparent from these tests that 2,4-D might be employed as a selective herbicide, at least in instances in which the weeds were broad-leaved plants. Commercial application of this principle soon followed and treatments of this kind were applied not only on lawns but on other grass crops such as wheat, rice, corn, and sugar cane.

Research workers then began a search for a herbicide which, in contrast to the action of 2,4-D, would destroy narrow-leaved plants without injuring broad-leaved ones. There are instances, for example, in which members of the grass family are classed as weeds because they reduce yields of desirable crops. Johnson grass (*Sorghum halapense*), quackgrass (*Agropyron repens*), Bermuda grass (*Cynodyn dactylon*), crabgrass (*Digitaria sanguinalis*), and foxtail millet (*Setaria italica*) are common examples of often unwanted grasses. Our requirements are often still more specialized in that we may desire to destroy one grass without harming another, such as removing crabgrass from bluegrass lawns. As a rule most hormone-type herbicides are not satisfactory for controlling weedy grasses, but other satisfactory compounds have been developed rather recently. Phenyl mercuric acetate has been reported as a satisfactory agent for control of crabgrass in lawns. Two other compounds, o-isopropyl n-phenyl carbamate (IPC) and sodium trichloracetate (TCA) have shown considerable promise in their selective action on grasses.

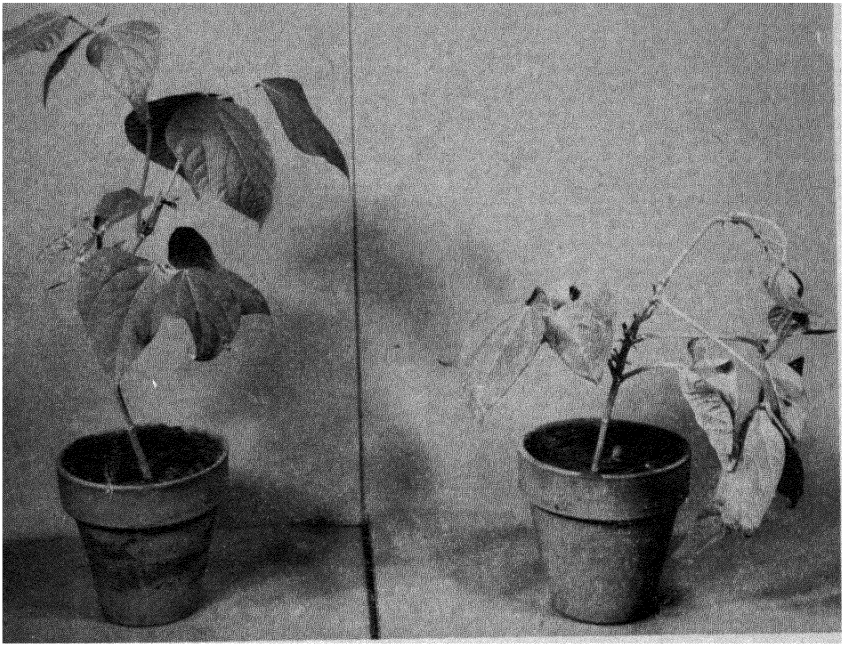
Announcement of the discovery of maleic hydrazide in 1949 served to revive interest in graminicides because of the rather unique qualities of this compound. Maleic hydrazide can be considered a hormone type of substance because its action on established grasses is systemic. When applied to foliage it inhibits not only growth, but also formation of flowers and development of viable seeds.

We have attempted to outline definite steps in the historical development of growth-modifying substances. It may have seemed at times that practical application was being stressed. This was not the intention at the moment. Many of the fundamental discoveries in plant physiology met a few long-felt needs in horticulture with the result that their practi-

cal application was immediately foreseen and appreciated. No attempt has been made to discuss the many types of practical applications. At the end of this chapter we shall present these in outline. We are now ready to inquire into the mode of action of growth-modifying substances.

Physiology

One of the common characteristics of hormones is their effectiveness in relatively low concentrations. By low concentrations is meant 0.001

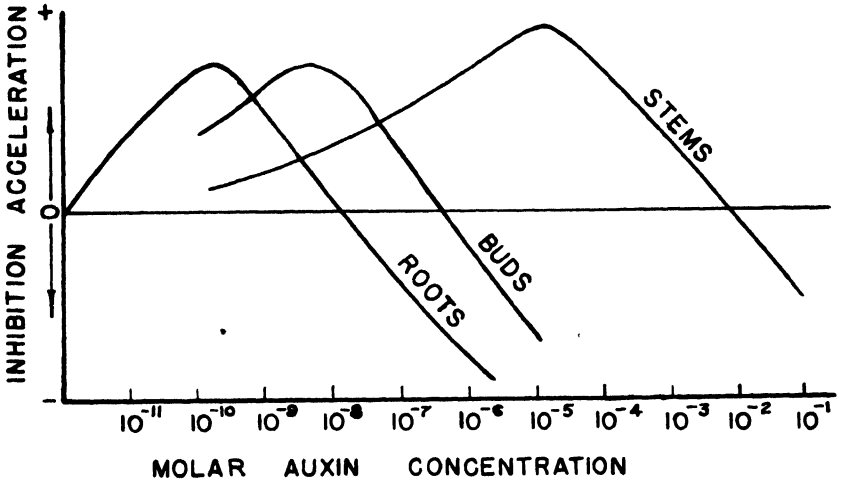


Epinasty produced on bean plant by spraying with 2,4-dichlorophenoxyacetic acid in concentrations normally employed for killing weeds. Left: normal plant; right: plant receiving spray.

molar or less. Phytohormones may be effective in lower concentrations than are many animal hormones. The minimum effective dosage of insulin on the rabbit is 0.25 microgram (microgram = 0.001 mg.) per gram of the animal, and with estrone (a sex hormone) in the mouse the rate is 0.001 microgram per gram of organism. On the other hand, auxin promotes growth of the oat plant in a concentration as low as 0.0001 microgram per gram of tissue. Expressed in another manner, auxin is effective at the rate of one gram per ten billion grams of tissue.

Growth-modifying substances in plants usually accelerate growth in very low concentrations, whereas in higher concentrations they may cause retardation of growth or even death. The compound, 2,4-D, acts as a

weed-killer in concentrations of 1,000 parts per million. In producing parthenocarpic tomato fruits the concentration is reduced to ten parts per million. Being beneficial in light dosages and deleterious in large concentrations is an action somewhat similar to the action of micronutrients (Cu, B) in plants and animals, and to some of the vitamins in animals. Furthermore, one concentration of a phytohormone may stimulate growth in one organ and inhibit it in another. The optimum concentration of auxin required for promoting growth of roots is 10^{-10} molar, which is



Relation of auxin concentration to elongation of roots, buds, and stems. (Courtesy, Thimann: "On the Nature of Inhibitions Caused by Auxin," *Am. J. Botany* **24**:407–412, 1937.)

0.0000000175 Gm. per liter or between one and two parts per billion. The optimum concentration for stimulating growth of buds is 10^{-8} molar, and for growth of stems it is 10^{-5} molar (0.00175 Gm. per liter). The same concentration which produces a stimulatory effect on stems causes inhibition of root growth.

Despite many observations which have been made regarding the effect of auxins on plant growth, very little is known about the exact mode of action of these substances. It was once held that they increase the plasticity of the cell wall, thus permitting elongation to occur as a result of the cell's own osmotic force. This theory is no longer held. It was formulated at the time when it was known that auxins promoted elongation of the cells of stems and coleoptiles and before the discovery that other processes are affected. It is now known that growth-modifying substances may affect the initiation of roots on both stems and mature roots, the division of cells in the cambium, the growth of the ovary of a fruit, which involves cell division as well as enlargement, the production of tumors

from parenchymatous tissues, and the streaming of protoplasm. Participation of auxin in so many vital processes of the plant has been cited by K. V. Thimann to indicate that growth substances act on some metabolic process which is common to most cells. More specifically, Thimann postulates the theory that a substance like indoleacetic acid is capable of protecting enzymes from naturally occurring inhibitors.

That auxins are catalytic in their action is obvious from the minute amounts required for activity. R. H. Burris describes two modes of action of herbicides, each of which is related to enzymatic activity. The immediate effect of some weed-killers is to induce mobilization of carbohydrates with the result that the plant literally "burns itself out." The second mode of action relates to an inhibitory influence on respiration although the evidence is not clear cut.

The list of synthetic compounds which appear to exert hormonal activity in plants is quite long, and they appear to be chemically rather diverse. A number of research workers have endeavored to determine structural requirements for the activity of a molecule. J. B. Koepfli, Thimann, and Went have summarized the results of various workers and have listed the minimum structural requirements of a compound for primary growth activity. (Capacity for inhibiting growth of pea seedlings was interpreted as indicating primary growth activity.) According to Koepfli, Thimann, and Went, the following structures are essential: a ring system in the nucleus with a double bond, a side chain with a carboxyl group, and a particular space relationship between the ring and carboxyl group. Two compounds which were among the first to be synthesized and which have found extensive applications are indoleacetic acid and alpha naphthaleneacetic acid. Reference to the structural formulas of the above two compounds will serve to illustrate what has been said regarding the structural requirements for growth-modifying activity.

Compounds like ethylene and carbon monoxide do not meet the structural requirements listed above although these two gases (and several others) can be employed to initiate physiological processes identical with those induced by growth-modifying substances. It has been suggested that the action of ethylene and carbon monoxide is indirect, in that these gases merely render more active those natural growth substances which are already present in the plant.

Flowering Hormone

A certain amount of evidence exists which indicates that plants contain a substance or substances which induce flowering. In the discussion of photoperiodism which is to follow (chapter 21) it will be shown that a stimulus applied to one part of the plant induces flowering in another part, thus conforming to the requirements of a hormone. M. C. Cajlachjan proposed the name *florigen* for this hypothetical hormone.

In 1948 R. H. Roberts reported the preparation of granular particles from a plant extract, which when redissolved and applied to the leaves of a cocklebur plant, induced blooming under conditions in which blooms would not normally be expected to occur.

Wound Hormone

Injured roots and stems generally develop callus or wound tissue over exposed cells as a result of the division of cells adjacent to the wound. Callus formation has been observed in noncambial tissues also. Haberlandt (1921) observed that callus would not form on freshly cut plant tissues if they were immediately rinsed with water. Haberlandt was of the opinion that injured cells produced a growth substance which he named *wound hormone.*

In 1939 J. English Jr., J. Bonner, and A. J. Haagen-Smit isolated from bean pods a substance which has the characteristic of a wound hormone and which they have called *traumatic acid.*

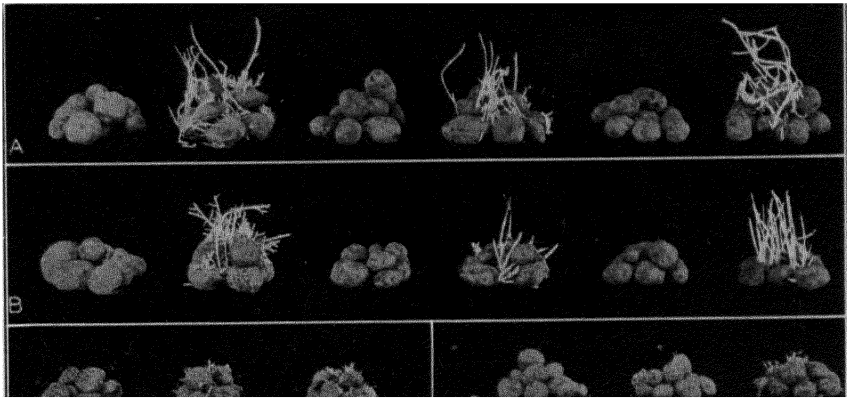
Practical Applications of Growth-modifying Substances

Some of the practical applications of growth-modifying substances were mentioned during the discussion of the historical development of these substances. No doubt other instances of applicability have become apparent during the discussion. We have mentioned the rooting of cuttings, the prevention of preharvest dropping of fruits like apples, pears, oranges, and grapefruit, the production of seedless tomatoes, and the use of some of these compounds as weed-killers. Several more practical applications will now be listed. Instances often exist in which it is desirable to inhibit the growth of buds. It has been found practicable to prevent sprouting of stored potatoes, and to prevent growth of shoots on newly planted nursery stock until the roots have become established. Attempts are being made to retard the opening of buds of fruit trees in the early spring before danger of frost has passed, although this phase is still in the experimental stage.

Under certain circumstances abscission of leaves may be delayed in the same manner that premature dropping of fruits is prevented. On the other hand, occasions arise when just the opposite condition is desired, that is, when it is necessary to defoliate the plant quite rapidly. This is accomplished with the use of unsaturated gases (ethylene) and certain growth substances. Rose bushes are defoliated prior to storing for the winter in order to reduce loss of water through transpiration. Cotton plants must be defoliated before the cotton bolls are harvested by machinery. In like manner "fruit thinning" is sometimes accomplished chemically to insure a smaller crop of fruit with higher quality.

Thus far it might appear as if growth-modifying substances act only on cell division and enlargement. They have been shown to accelerate,

to retard and to inhibit growth, to induce abscission and to prevent it. At least one instance is known in which these compounds are used to cause a shift from the vegetative stage to the reproductive stage of growth or, in other words, to induce blooming. It is now commercial practice to



Inhibiting sprouting of potato tubers by treatment with growth-modifying substances. A and B: tubers after three month's storage at 18°C. Left lot of each pair was treated with vapor of methyl ester of alpha-naphthaleneacetic acid. Lots at the right of each pair were not treated. C and D: tubers after one year at 10°C. Left and center lots received different amounts of the methyl ester of alpha-naphthaleneacetic acid. Right lots are controls. Six different commercial varieties are represented. (Courtesy, Boyce Thompson Institute for Plant Re-. search, Yonkers, N.Y. and F. E. Denny.)

induce blooming in pineapples by spraying with compounds like alpha-naphthaleneacetic acid. This is the only instance in which flower production is induced on a commercial scale by chemical stimulation.

Growth-modifying substances have been found to induce postharvest ripening in certain fruits like bananas, apples, pears, and tomatoes, and to reduce the development of physiological disorders. Examples of these physiological disorders are storage scald of apples and the physiological breakdown of pineapples. Some of these developments are too recent to have received very extensive commercial application.

chapter 20

Radiant Energy and Plant Growth

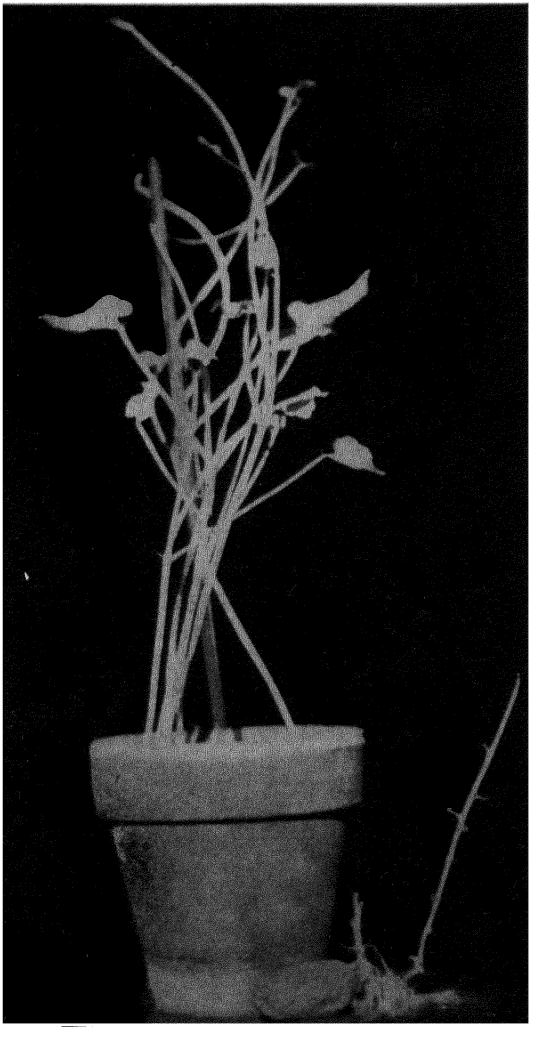
Under the topic of radiant energy we shall discuss the effect of both visible and invisible radiations. But first let us consider only the visible portion of the spectrum. Although light may influence many phases of growth, there are probably only three and possibly four *photoreactions*. These reactions occur in photosynthesis, phototropism, photoperiodism, and probably there is one which affects dormancy (to be discussed later). The first three reactions may occur simultaneously and the specific requirements regarding wavelength of light are not necessarily identical. Light also regulates other processes such as the accumulation of food reserves in bulbs, changes in the acidity of leaves, variation in anthocyanins, and it may affect the internal anatomy of the plant to some extent.

Effect of Visible Radiations

The manner in which a plant responds to light depends upon three factors: quality, intensity, and duration of light. The effect of the last-mentioned factor is discussed under the topic of photoperiodism (chapter 21).

The maximum intensity of sunlight in temperate zones (at noon on a summer day) is equal to about 10,000 foot-candles. Under natural conditions, however, plants which ordinarily grow in full sunlight are rarely exposed to light of maximum intensity for very long periods. Under experimental conditions it has been shown that dry weight, thickness of leaves, and rigidity of stems increase as light intensity is gradually increased up to that of full sunlight. On the other hand, maximum height of plant and maximum area of leaves has been found to occur at light intensities which are well below that normally encountered on bright summer days. It thus appears that intense light tends to reduce elongation of stems and the increase of leaf area, whereas lowered light intensity has the opposite effect. As a result of this characteristic, partly shaded plants tend to extend their trunks and branches into regions where they will receive maximum light. No doubt many amateur gardeners have observed that when plants are grown in light of low intensity the stems become long and spindly, much like those grown in complete darkness.

Elongation is largely the result of cell enlargement although cell division appears to be stimulated too. Intense light, on the other hand, tends to reduce cell enlargement and cell division, and to accelerate maturation.

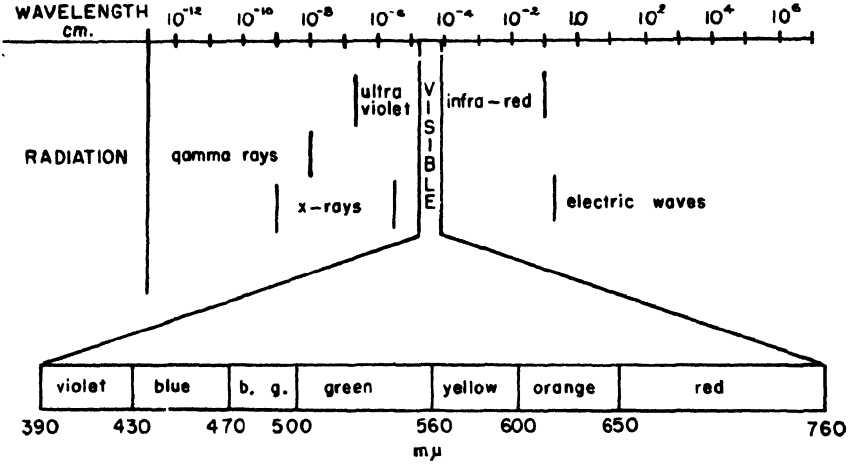


Etiolated bean and potato plants. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

Thus far we have been discussing the so-called "sun-loving" plants. A number of plant species are adapted to light of lower intensity such as those found on the floor of the forest. Ferns, mosses, violets, and azaleas are typical examples of shade-loving plants. Algae and mosses are often found growing in caves under light intensity which is no greater than that

of full moonlight. Some of the conifer seedlings (piñon pine, spruce, redwood) are able to maintain their initial dry weight in light intensities between one and five per cent of full sunlight.

It is to be hoped that some day we may learn more about the physiology of plants which carry on photosynthesis in reduced light and those which are able to manufacture chlorophyll in the absence of light. Investigations of this type might yield information of fundamental value which some day may have practical application.



Wavelengths of visible and invisible radiations. (Redrawn by permission from *Modern Introductory Physics*, by Ira M. Freeman. Copyright, 1949. McGraw-Hill Book Co., Inc.)

Quality of light is usually designated by a specific color or, more properly, by wavelengths which produce certain colors. It will be recalled that white light is composed of all the colors normally seen in the rainbow. Sir Isaac Newton demonstrated this principle while still a student at Cambridge University.

One of the earliest attempts to study the effect of light of different quality was made by Julius Wiesner (1874) who grew plants under double-walled bell jars containing colored solutions between the walls. More modern methods consist of using specially prepared colored plates which transmit known bands of wavelengths. An elaboration of this method was once utilized at the Boyce Thompson Institute by constructing greenhouses with spectral glasses designed to transmit different regions of the solar spectrum. Dispersion of white light by the use of prisms is another means of utilizing light of varying wavelengths. Whatever method is employed, precautions must be taken to make certain that light intensity has not been altered by filtering or dispersion.

In 1936 F. E. Meier observed the growth of a unicellular alga (*Sticho-*

coccus bacillaris) under light of different wavelengths but of equal intensities. The rate of growth was over threefold in blue light, and more than twofold in red and yellow light. The number of cells decreased when cultures were exposed to green light. More recent studies have shown that the rate of growth of higher plants is much less in green light than in blue-violet or orange-red portions of the spectrum. Response to a given wavelength may differ with different organs of a plant.

By this time it must have become apparent to the student that normal growth and development of plants requires the full spectrum of sunlight or its equivalent.

Effect of Invisible Radiations

The biological world may be affected by radiations which are invisible to the human eye. Rays slightly longer than the longest visible red wave. are called *infrared;* those shorter than violet rays are called *ultraviolets* Infrared radiations are often called *heat rays.* No great amount of information is available on the effects of infrared rays on plants and plant growth. In general the longer wavelengths appear detrimental to plants. Johnston reported that infrared radiations caused tomato plants to develop longer internodes, larger leaves, and to contain less chlorophyll than controls in which treatment was similar except that infrared rays were lacking.

Still longer radiations fall into the category of radio or electromagnetic waves. These may extend from 100000 to 10^{12} millicrons (a kilometer) in length. Electromagnetic waves have been employed from time to time to destroy lower plants, such as bacteria and yeast, but their practicability has not yet been established. Radio waves of high frequency have been reported to increase the respiration rate of stored fruits and in some instances to cause injury of the fruit.

High energy radiations are to be found in the region beyond the violet. In addition to the ultraviolet rays (10 to 390 millimicrons) there exist x-rays, gamma rays, and cosmic rays, in the order of diminishing wavelength. Energy value of a radiation increases with decrease of wavelength. X-rays and shorter radiations are spoken of as *ionizing radiations* because they have the capacity of converting elements to ions by removing electrons and leaving positively charged residues. Gases may thus be ionized as the result of the passage of these high-energy waves through them. The effect of ionizing radiations is not confined to gases but may also be produced on solids, including living tissues. Ultraviolet light, though rich in energy when compared to visible light, does not possess the power of removing electrons. The net effect of this type of radiation is to raise an electron to a higher level of energy. The result is called *excitation,* and ultraviolet light is spoken of as *nonionizing radiation.* Let us now consider some of the effects of ultraviolet radiations on plants.

Ultraviolet Radiations

A certain amount of ultraviolet light is present in the sunlight which filters through the earth's atmosphere. It consists of the longer ultraviolet rays—about 300 to 390 millimicrons. Ultraviolet light of shorter wavelength than 300 millimicrons is usually deleterious to plant life. In fact there is some difference of opinion regarding the usefulness of the longer ultraviolet rays to plants.

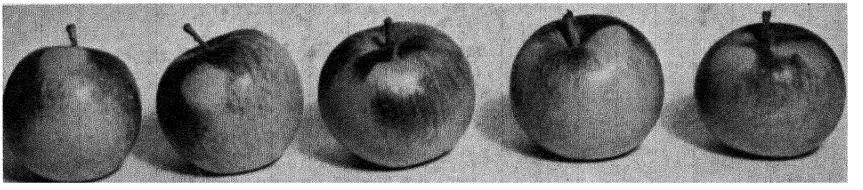


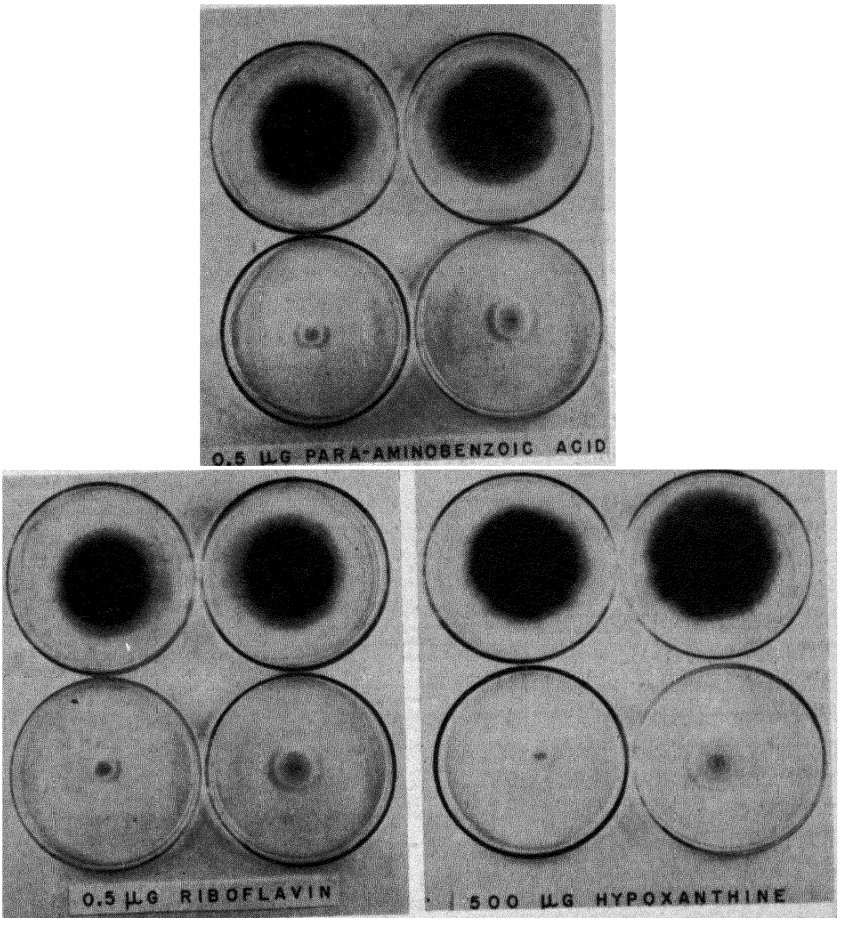
Illustration of the necessity for sunlight in the development of anthocyanin pigments in certain varieties of apple. No red pigment was formed in areas which were continuously shaded by leaves.

Reference has been made to the work of Arthur in which it was reported that the ultraviolet rays of sunlight are necessary for formation of anthocyanin pigments in red varieties of apples. In Arthur's work, shorter ultraviolet rays, that is, those emitted by mercury-vapor lamps, prevented the development of red color in harvested apples. Somewhat similar results have been observed in other fruits. Exposure of green oranges and green tomatoes to radiations from mercury-vapor lamps has been found to "fix" the chlorophyll in these fruits, and to retard or prevent changes in color which are normally associated with ripening.

Other physiological effects of ultraviolet radiations which have been reported consist of retarding enzymatic activity *in vitro*, inactivating viruses in plants, and causing the destruction of bacterial cells.

Ionizing Radiations

X-rays. The discovery that mutations in certain animals could be induced by treatment with x-rays stimulated research along similar lines with plants. As a general rule, however, x-radiation proved deleterious to plants. E. L. Johnson reported that young tomato seedlings receiving x-radiation showed abnormalities in leaves, flowers, and fruits. When irradiation was applied at the time of budding, the buds became yellow and dropped out without blossoming. Other workers have treated seeds, such as those of *Citrus* and *Helianthus annuus* with x-rays and have reported abnormal development of plants which were grown from these seeds.



Effect of x-rays on fungi. Lower two colonies in each set of four represent an atypical strain of *Aspergillus niger* produced by subjecting a culture of this fungus to soft x-rays. Normal colonies are shown in upper pairs. Photo also shows effect of certain vitamins on growth. Left pair of each four were grown on basal media, whereas the two on the right received certain vitamins as indicated. (Courtesy, Diller, Tytell, A. G., Tytell, A. A., and Kersten, "A Soft X-ray Mutant of Aspergillus niger van Tieghem," *Plant Physiol.* **25**:340–353, 1950.)

H. J. Kersten, H. L. Miller, and G. F. Smith state that treatment of corn embryos with x-rays resulted in a statistically significant stimulation of root growth. X-rays have been employed to induce mutations in plant viruses and in bacteria. Likewise, with proper dosage, viruses may be inactivated and bacteria destroyed. X-rays, because of their high energy content, are much more effective than ultraviolet radiations for this purpose.

Radium Rays. A study of the effect of radium rays on plants is complicated by the fact that three types of radiations are involved. However, much of the earlier work with radiation was carried on with radium or its salts because this was the only type of radioactive substance available. Some of the general effects of radium rays on living cells may be summarized as follows. The physiological results may be similar to those produced by x-rays. Younger, and especially embryonic, tissues show more sensitivity than mature tissues. Exposure to radium produces a retarding or often inhibitory effect on all cell activities. The presence of chlorophyll in the cell appears to profoundly modify the effects of radiation. Sublethal doses of radioactivity have been found to produce morphological changes in a plant which have been carried over in successive generations.

Radioactive Isotopes

Interest in radioactivity was revived with the phenomenal advances made in the field of atomic physics. Investigations of plant physiologists in this field followed several lines of endeavor. Elements with artificial radioactivity were prepared by physicists by means of the cyclotron and made available to physiologists. These elements are the so-called *radioactive isotopes*. Both plant and animal physiologists have found them useful in tracer techniques, that is, in tracing the course of the elements through the plant or animal by means of the radioactivity of the element. A compound is prepared in which one of the elemental constituents is radioactive and is referred to as being "tagged." Thus a better understanding of many of the processes in plants has been obtained. Only a few can be mentioned here. Both radioactive carbon and radioactive oxygen have been followed during the course of photosynthesis, and it was by this means that it was learned that oxygen released by the plant comes from water and not from carbon dioxide. Various metals have been traced through the plant, a technique which has contributed much to the knowledge regarding the specific function of both major and minor elements. The use of radioactive isotopes has enabled the soil chemist to learn much about the specific effects of fertilizers which have been applied to the soil. For example, much of the phosphorus added as fertilizer may be retained by the soil in an insoluble form. In fertilizer experiments it is impossible to determine whether the plant has benefited by the phosphorus which has just been added or whether some of the insoluble portion has been made available by the plant. When tagged phosphorus is added to the soil, it can be traced throughout the plant.

Radioactive growth-modifying substances have been employed for the purpose of studying the paths by which these compounds are transported through the plant. It is possible also to learn something of the specific action of these substances by this method of approach.

The application of radioactive isotopes in experimental plant physiology is still in its infancy. Thus far it has been used largely in studying photosynthesis, translocation, and mineral nutrition. Perhaps these techniques may someday help to enlighten us regarding the detailed actions of enzymes and viruses, or assist in determining the specific roles of the many compounds, such as pigments, glycosides, alkaloids, and essential oils, whose specific functions are at present not understood.

The development of the atomic bomb in modern warfare has created new problems for the plant physiologist. What effect will this high concentration of radioactivity have on the plants which survive atomic explosions? How will the minerals in the bombed soil react following the explosion, and how will they subsequently affect the plant which absorbs them? To what extent may plants accumulate and concentrate radioactive substances? Will they be damaged by quantities that are not yet concentrated enough to damage animals, or may the reverse be true? These and many other questions await solution by the student of plant physiology.

Light in General

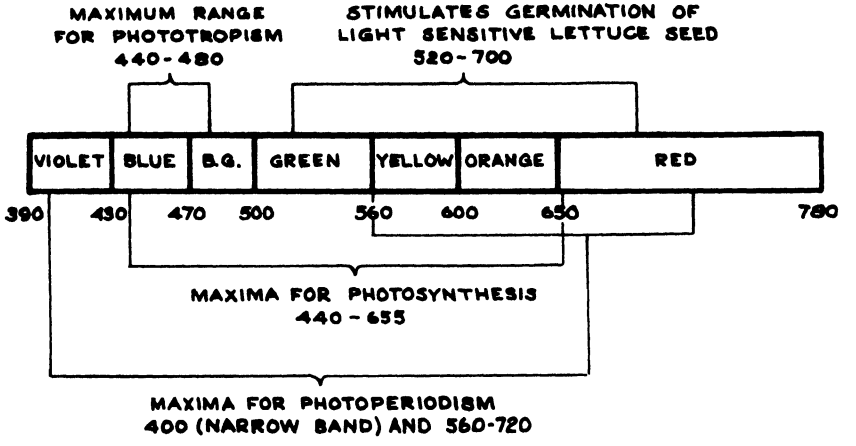
Several interesting observations have been recorded which suggest at least a relationship between light and physiological responses. The red side of an apple generally contains more ascorbic acid (vitamin C) than the green or yellow side. Since exposure to sunlight induces the formation of anthocyanin pigments in red apples, it is probable that the increased amount of ascorbic acid is the result of exposure of the one side of the apple to full sunlight. Additional support of this theory is to be found in the report that citrus fruits on the outer branches of the tree, well exposed to sunlight, contain more ascorbic acid and more soluble solids than the fruits growing on the inside branches. It is possible that production of ascorbic acid in plants may be indirectly dependent upon photosynthesis since it has been suggested that vitamin C may be formed from certain sugars.

It has been observed also that oranges which are borne on the inside branches mature with a much poorer color than fruits on the exposed branches. Shaded fruits retain their green color longer, never completely "degreening," and may ripen with a pale-yellow rather than a deep-orange color. Both the decomposition of chlorophylls and the increase in carotenoid pigments in the rinds of maturing oranges therefore appear to be in some way dependent upon sunlight.

* * * * *

The specific manner in which radiant energy produces physiological changes in plants is not well understood. It is possible that sublethal dosages of x-rays produce ionizing reactions in organic constituents of

the cell, and that in some instances activating effects result from ultraviolet radiations. Changes of these two types doubtless occur when alterations in hereditary characteristics are induced by treatment with ionizing radiations.



Certain portions of the visible spectrum are more effective than others in inducing different responses by plants.

It is more difficult to explain the mechanisms involved in the four photoreactions which have been mentioned at the beginning of this chapter. Effective wavelengths for inducing these reactions are found in the visible portion of the spectrum, and in some instances maximum effects are produced by exposure to the long, or less energetic, end of the spectrum. Furthermore, response to stimulation is much more rapid in photoreactions than is the case when changes in chromosomal structures have been induced by x-ray and ultraviolet radiations. More is known about the mechanics of photosynthesis than about the other reactions. Contributions to the knowledge of the quantum efficiency of the photosynthetic reaction and of the energy relationships involved in phosphorolytic reactions have added appreciably to our store of knowledge. But we are still far from a complete understanding of the exact manner in which radiant energy is transformed to chemical energy in photosynthesis.

Opportunities for fundamental investigations in this field are unlimited and they should appeal to teams of cooperating physicists and physiologists.

Growing Plants under Artificial Light

Both commercial and amateur gardeners frequently find it desirable to grow plants under artificial illumination. In most instances artificial light is supplied as a supplement in the greenhouse during the short days

of the winter. Occasionally attempts are made to supply all of the light from artificial sources. The question then is, "What is the best substitute for sunlight?" We are forced to admit at the beginning that no perfect substitute for solar radiation is known at present. The kind of lighting which we shall describe is the best substitute for sunlight but is certainly not its equal.

For general purposes Arthur recommended that 500- or 1000-watt incandescent lamps be supplemented with mercury arc lamps. Incandescent lamps produce energy which is mainly in the red end of the spectrum. The mercury arc lamps should be applied for only two hours a day.

Tests made by a number of investigators indicate that fluorescent lamps, supplemented with incandescent lamps, produce results similar to those obtained by use of incandescent and mercury arc lamps, but a larger number of fluorescent lamps is necessary to produce equal intensity. This is a disadvantage when space is limited.

More detailed methods have been described for supplying artificial light in special types of research. Parker and Borthwick employed carbon arc lamps in which the carbon cores contained rare earth metals of the cerium group. With these "sunshine" carbons the energy of the visible spectrum was greatly increased, especially in the blue-violet region. Spectral distribution from lamps of this type can be varied by introducing different chemicals in the carbon core, but the radiation does not duplicate solar radiation.

As was mentioned previously, at least three photoreactions are known to occur in plants and each of these reactions is more or less specific in its requirement of solar energy. Bearing this in mind, J. W. M. Roodenburg, in the Netherlands, has proposed different types of illumination for different purposes. Summarized recommendations follow:

1. Photosynthesis: 50 to 100 watts per square meter of neon light during the night to supplement sunlight.

2. Increasing day-length: 5 to 50 watts per square meter of incandescent-filament lamp.

3. Forcing plants with infrared: 100 watts per square meter of radiation with incandescent lamps.

4. Eliminating deficiency of the blue end of spectrum: slight supplementation with mercury-vapor lamps.

chapter 21

Photoperiodism

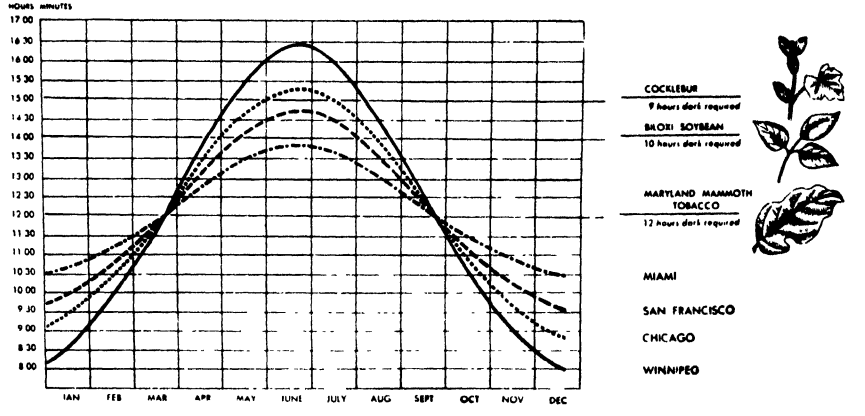
During late winter and early spring, as the days begin to lengthen, most of us take a new lease on life. Cheering notes of song sparrows and cardinals in the twilight of early morning hours seem to reflect our own feelings. Even before all evidences of winter are gone it may be observed that buds of elm and maple trees have begun to swell. We need not be biologists to appreciate that other creatures may be responding to the stimulus of longer daylight hours but it is doubtful if many of us realize that the proportion of light to darkness in a twenty-four-hour cycle may actually control the blooming of many plants. As a matter of fact this was long overlooked or at least was not appreciated by plant physiologists until 1920.

To begin at the beginning we must go back to 1906. At this time an old variety of tobacco, Maryland narrow-leaved, was being grown on the U.S. Department of Agriculture's Experimental Farm at Arlington, Virginia. Several of the plants grew to extraordinary height and produced an abnormally large number of leaves. When cold weather arrived and the plants had not bloomed they were transferred to the greenhouse. During the winter this giant tobacco bloomed and produced seed. The type bred true and gave rise to a new variety which was called *Maryland Mammoth*. It soon became a very valuable type for commercial purposes. The only known means for producing seed of this variety, however, consisted of repeating what has just been described, namely, transferring the plants to the greenhouse at the onset of cold weather.

This phenomenon puzzled research workers for many years. Finally it was observed by a now famous team of research workers, W. W. Garner and H. A. Allard, that these plants always bloomed during the short days of the winter months, and this suggested to them that the length of day might be the critical factor.

Garner and Allard immediately started a series of experiments to test this theory and the results were first published in 1920. When out-of-door work could be resumed in the summer, potted plants of Maryland Mammoth tobacco and Biloxi soybeans were placed on movable platforms which could be rolled into light-tight sheds. In one of the experiments the plants were kept in darkness from 4:00 p.m. until 9:00 a.m. each day.

In this way a "short day" could be maintained during the normally long days of summer. In these experiments the tobacco and soybean plants which had had their length of day (*photoperiod*) shortened, soon produced flowers. Those which had been left out-of-doors continuously failed to bloom. A number of other species of plants were exposed to varying photoperiods in this manner and the effect of day-length on flowering was definitely established. The term *photoperiodism* was applied to this phenomenon. As a result of a large number of experiments by Allard and Garner and subsequently by other investigators throughout the world,



Graph illustrating variations in photoperiod at several different stations. (Courtesy, Naylor: "The Control of Flowering," *Scient. Am.* **186**:49–56, 1952. Drawing by Eric Mose, Scient. Am.)

flowering plants may now be classified according to their photoperiodic requirements for blooming. Those which produced flowers sooner when their days were shortened were called *short-day* plants. Those which bloomed only when their days were lengthened were called *long-day* plants. Some plants were apparently not influenced at all by length of day and these were considered to be the *indeterminate* types.

The critical photoperiod is not the same for all species of plants in the same group. Plants are usually classified as short-day or long-day types depending upon whether the critical period represents the lower or upper limit of day-length which will cause a shift to reproductive growth. In other words a long-day plant is one which can flower only when the daily duration of light exceeds a certain number of hours. A short-day plant is one that flowers only when the days are short and which fails to flower when the day-length exceeds a certain value.

It will be shown later in this chapter that, with short-day plants, the long night which follows a short day is a very important part of the photoperiodic cycle. Long-day plants, on the other hand, will usually bloom under continuous illumination.

A partial list of long-day, short-day, and indeterminate types of plants appears in Table 29. In general plants which have originated in tropical latitudes will require short days for blooming. Plants of more northern latitudes (roughly north of 60°) are long-day plants. All three types of plants occur in temperate zones.

Table 29

CLASSIFICATION OF SEVERAL PLANTS IN REGARD TO PHOTOPERIODIC RESPONSE

Long-day	*Short-day*	*Indeterminate*
Beet	Aster	Buckwheat
Clover	Chrysanthemum	Chickweed
Lettuce	Cocklebur	Dandelion
Radish	Ragweed	Tomato
Spinach	Strawberry	Zinnia
Timothy	Sweet potato	
	Violet	

It will readily be seen that discovery of the principle of photoperiodism was important from at least two angles. It served to explain differences in blooming habits of many flowering plants and it had immediate practical applications. It explained why short-day plants, like the poinsettia, could be made to bloom in the South but failed to produce flowers when grown in the summer in the North. The principle might be applied in experimental seed production as in breeding experiments. It suggested the possibility of shortening the day in the North (in greenhouses) rather than necessitating the removal of short-day plants to the South for seed production. It also suggested the use of light in greenhouses for the production of long-day plants in winter.

Is There a Flowering Hormone?

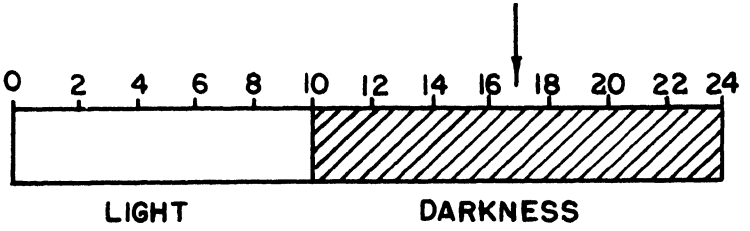
There is ample evidence that flowering in plants is hormonal in nature. In fact, nearly a 100 years ago Julius von Sachs, a German, advanced the idea that flowering substances are produced in leaves and transported to other parts of the plant where they cause blooming to occur. Laboratory evidence for this theory was discovered in 1934 by investigators in America, the Netherlands, and Russia. Other research workers have contributed to the subject and the accumulated evidence may be summarized as follows. A short-day plant, like the cocklebur, may be induced to bear flowers if its leaves are exposed to a diurnal cycle consisting of 10 hours of light and 14 hours of darkness. If the leaves are removed at the close of the dark period, no blooming occurs. If one leaf, or only one-eighth of a leaf is left on the plant after exposure to the short-day and long-night treatment, the plant will bloom. In this manner the effect of the stimulus on a leaf or fraction of a leaf appears to be transported to another part of the plant. The flower-inducing substance is able to move through graft unions. A single leaf from a flowering plant can be grafted to a non-

flowering plant and cause the latter to bloom. The grafted leaf may even be one which was not taken from a flowering plant but which was exposed to the required photoperiod before grafting on the nonflowering plant.

Parker and Borthwick were able to apply the photoperiodic stimulus to a leaf and then to prevent its passage through the petiole. When an ice pack was placed around the petiole of a leaf, photoperiodic stimulation was not transmitted from the leaf to the other parts of the plant. The temperature of the petiole was in the same range which Curtis had shown would retard or inhibit translocation of solutes. All of these results point rather strongly to the possibility that plants possess a flowering hormone.

Importance of the Dark Period

It is now known that the critical factor in inducing blooming is not length of day but rather duration of the dark period. As mentioned previously, the cocklebur, a short-day plant, can be made to bloom by exposure to a photoperiodic cycle consisting of 10 hours of light and 14 hours



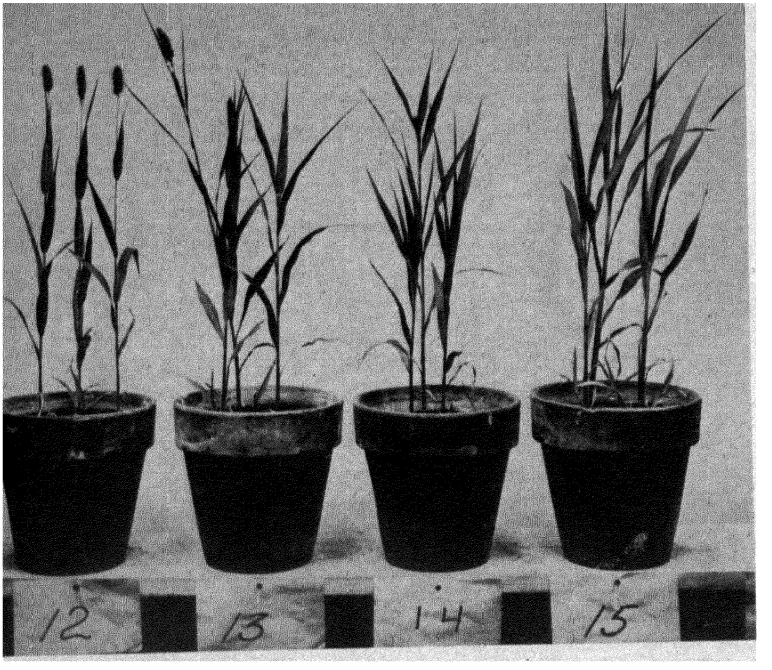
Interruption of the long dark period for as little as one minute with radiation of correct wavelength will prevent blooming of the cocklebur (a short-day plant).

of darkness. K. C. Hamner and J. Bonner (1938) succeeded in preventing the blooming of the cocklebur during a short photoperiod by interrupting the dark period. Hamner and Bonner found that only a minute's exposure to a bright light was necessary to change the flowering habits of this plant. In other instances a 30-minute interruption of the dark period may be required to prevent blooming.

Light Intensity

Artificial light may be used for extending the natural photoperiod and it has been shown that the stimulating effect does not depend upon high intensity. Experiments by R. B. Withrow and H. M. Benedict with stocks (*Matthiola incana*) indicated that the day could be satisfactorily lengthened with light of 10 foot-candles. This is one-thousandth the intensity of full sunlight. China asters showed a complete response to 0.3 foot-candle and a certain amount of response to additional light of only 0.1 foot-candle. The latter intensity is only slightly stronger than full

moonlight. T. von Gaertner and E. Braunroth have shown that moonlight is intense enough to control flowering in a few plants. Perhaps the age-old folklore regarding the effect of the light of the moon on gardening may some day be shown to be rooted in practical wisdom.



Millet, a short-day plant grown with photoperiods varying from 12 to 15 hours as indicated. (Courtesy, Bureau of Plant Industry, U.S. Dept. of Agriculture.)

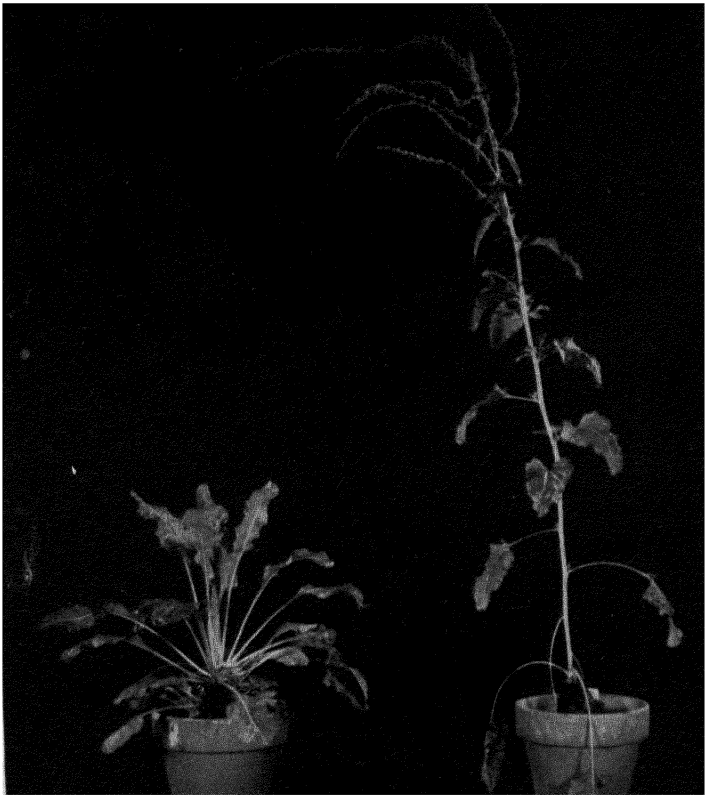
That light of low intensity is sufficient to break the dark period has been impressed upon operators of greenhouses on several occasions. In one instance short-day plants like poinsettias did not bloom on schedule during the short winter days. Investigation showed that light from the night watchman's flashlight was sufficient to change the flowering habits of the poinsettias. On another occasion it was reported that nearby street lights were found to be the cause of breaking the dark period.

The Action Spectrum

By *action spectrum* is meant the minimal energy required at various wave bands to produce a particular plant response. It will be recalled that with regard to photosynthesis all of the visible spectrum is effective to a certain degree with a maximum in the red (6550 Å)* and a secondary

* An ängstrom (Å) is equal to one-tenth of a millimicron.

maximum in the blue (4400 Å). Borthwick, Parker, and Hendricks have reported somewhat similar results while studying the action spectrum of photoperiodism. These workers also found that all regions of the visible spectrum are effective. Furthermore, two maximum regions of effectiveness were observed: a rather broad band in the orange-red (5600 to



Sugar beet, a long-day plant grown with an 8-hour photoperiod (left) and a 16-hour photoperiod (right). (Courtesy, Bureau of Plant Industry, U.S. Dept. of Agriculture.)

7200 Å) and a narrow band in the blue (4000 Å). Radiation at 6400 Å was about 200 times more effective than that at 4800 Å when applied to the cocklebur.

Knowledge of the action spectrum of photoperiodism is of value for two reasons. The most effective wavelength may be employed for interrupting the dark period in experimental work, thus saving both time and money. And information gained from a study of the absorption spectra may help answer the question as to what pigment in the plant is absorb-

ing the energy of the radiation. Since the action spectrum for photoperiodism does not entirely coincide with that for photosynthesis, it is evident that chlorophyll is not necessarily the pigment which is involved. Borthwick, Parker, and Hendricks offer the suggestion that the substance might be a blue pigment. This suggestion is based upon the fact that maximum effectiveness in photoperiodic response occurred in the red end of the spectrum. Much more work will be required before the final answer is obtained.

Induction Period

No mention has thus far been made regarding the *induction period*, that is, the minimal period of exposure to a long day or a short day which is required to induce blooming. This differs in different plants. Induction period for teosinthe (a relative of present-day maize) is 20 days. In other plants the induction period is six to ten days. The Biloxi soybean may be induced to initiate flowering after two to three days' treatment, and the cocklebur will respond after a single long night.

Theory of Photoperiodic Action

Final proof of the existence of a flowering hormone (*florigen*) will never be complete until the active substance is isolated, crystallized, and identified. Circumstantial evidence at least suggests that such a substance exists and most of this evidence has been cited previously. Two questions now arise. Does the same type of compound initiate blooming in both long-day and short-day plants? Is the flowering hormone, if it exists, identical in all plants? We can answer the first question affirmatively with a certain amount of assurance. The wavelength of light which is most effective in inducing flowering in long-day plants is also most effective in preventing initiation of flowers of short-day plants when applied to the middle of the dark period. Furthermore, the same quantity of energy is required for floral initiation in long-day plants as is needed for preventing it in short-day plants.

Evidence also exists which indicates that the flower-promoting stimulus is identical for all plants. Naylor (1952) states that a plant can be made to bloom by grafting on it a leaf from another variety, species, genus, or even from another family. A certain parasitic plant which grows on the roots of red clover is probably never exposed to light and yet it blooms. It is assumed that this parasite obtains its stimulus for blooming from its host.

One theory regarding the action of florigen may be summarized as follows. The active substance or an intermediate is subject to destruction by light. A certain critical concentration of this substance is necessary for induction of flowering but a large quantity prevents blooming. Short-day plants require a long dark period to accumulate sufficient quantities

of the hormone to induce blooming. The theory is based on the premise that the flowering hormone in long-day plants is protected from photosensitized destruction.

Borthwick is of the opinion that the substance which finally controls flowering may be at the end of a chain of reactions, and that the light reaction is possibly the first link in the chain.

chapter 22

Reproduction in Plants

A part of the life cycle of higher plants consists of the production of flowers, which in turn is followed by the development of fruits. Often the flowers are inconspicuous, as with grasses and certain shade trees, with the result that blooming may be overlooked by the average person.

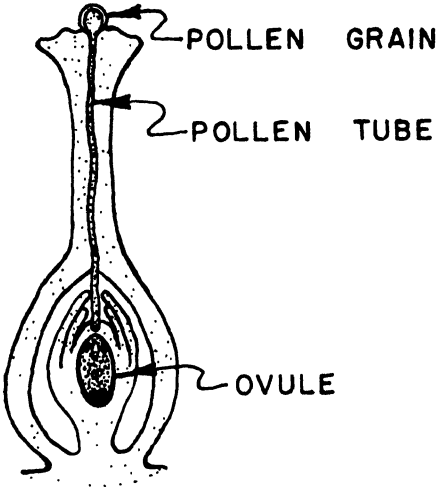


Blossom of the orange (*Citrus sinensis*).

Most flowers contain both male and female organs but this is not always the case. Corn, oaks, conifers, willows, and others bear two distinct kinds of flowers, male and female, on the same plant. Still another type may be found in which male and female flowers are each borne on separate plants. Holly and papaya plants are typical examples of this group.

With few exceptions, under natural conditions production of fruits

must be preceded by fertilization of the ovules by pollen from the anthers. Some plants are self-pollinated; others are cross-pollinated. From a physiological point of view we are interested first in what causes a plant to



Diagrammatic representation of fertilization of ovule of flower by pollen grain.

shift from the vegetative phase of growth and, second, in the nature of the changes that occur following pollination of the flowers.

Induction of Flowering

Photoperiod. One of the most important environmental factors which induce plants to produce flowers is termed the *photoperiod*, or length of day. This has been discussed in chapter 21 on Photoperiodism. It will be recalled that there are short-day and long-day plants and that blooming is dependent upon the period of illumination. There are also intermediate and indeterminate plants. It was brought out that with short-day plants the critical factor is the length of the dark period, that is, short-day plants require long nights. Long-day plants, however, can usually be made to bloom under continuous lighting although the duration of the dark period may become a critical factor under an artificial photoperiodic cycle such as 14 hours of light followed by an equal number of hours of darkness. Such a treatment has been reported to inhibit flowering of long-day plants. Although photoperiodism represents a new concept in physiology, the actual mechanism involved is still not understood. It is apparent that a flowering hormone is involved. H. A. Borthwick, M. W. Parker, and S. B. Hendricks believe that the hormonal substance is produced during photosynthesis because both light and darkness are necessary for flower production.

In 1949 A. C. Leopold pointed out that light is necessary only for the

flowering of green plants. His experiments indicated that truly etiolated plants, that is, those which have never been exposed to light at all, will bloom in complete darkness. Experiments were performed with potatoes, peas, and kidney beans, and it was suggested that this phenomenon probably occurs in certain saprophytes in which flowers are initiated underground. Leopold expressed the opinion that green plants contain a flowering inhibitor which requires the photoperiod for suppression.

Temperature. Temperature may affect reproduction in several different ways. Most plants have an optimum temperature range in which they complete their reproductive cycle. Short-day plants which are adapted to relatively low temperatures will bloom in the spring. Those requiring short days and high temperatures will bloom in the late summer and fall.

Certain types of plants are rather specific in their temperature requirements for blooming. For example, it has been reported that when celery plants were held in a cold frame at 40°F. to 45°F. for 15 to 30 days and then transferred to a greenhouse at about 68°F., the plants all developed seedstalks in six months. Plants held continuously at 68°F. showed no seedstalk development. Those grown in a temperature of 70°F. developed no seedstalks regardless of previous treatment. It would seem from this that a relatively high temperature may counteract the tendency to bloom which was induced by exposure to low temperature.

Cabbage is somewhat like celery in requiring low temperatures to initiate blooming. Since cabbage is a biennial it does not produce seedstalks until the second year. If vegetative plants are placed in a warm greenhouse after the first year's growth, however, they will continue indefinitely in the vegetative phase of growth. J. C. Miller reported an experiment in which a cabbage plant was kept in a warm greenhouse for three years. During this time it grew to a height of eight feet and produced six heads successively. When moved to a lower temperature, the plant flowered in a few months. Lettuce also seems to have specific requirements for flower formation. This plant has been shown to produce seedstalks when grown in a temperature range of from 70°F. to 80°F. If grown continuously at 60°F. to 70°F. the plant remains in the vegetative state. Information of this type is of value to the plant breeder when he wishes to produce seed for propagation. It is implied, of course, that the work be done in the greenhouse where temperatures may be controlled.

Under natural conditions specific temperature requirements for blooming may become a disadvantage. In the commercial production of celery and lettuce for food, the grower does not desire flower formation. Sometimes, in the southern states, winter temperatures may fluctuate sufficiently to change the type of growth of these two vegetables. Flowering or even the initiation of floral organs may render the crop unsuitable for marketing. Several other vegetable crops, like beets and onions, may be

induced to produce seeds by subjecting them to the proper temperature ranges.

Another interesting example of the effect of temperature on blooming is to be found in the winter cereals. Winter wheat and rye are customarily planted in the fall. The seeds germinate and the young plants live throughout the winter. In the spring they make further growth, producing a crop during the summer. If these winter varieties are planted in the spring they make only vegetative growth. It is essential, therefore, for them to be exposed to low temperatures for an interval to insure the subsequent formation of seedstalks. It was reported in 1858 that seeds of this kind might be conditioned and planted in the spring, instead of sowing them in the fall. The conditioning treatment consists of soaking the seeds and holding them near a temperature of 0°C. for several weeks. They may then be planted in the spring and will produce flowers and seeds. This treatment is called *vernalization* or *yarovization*.

Carbohydrate Nitrogen Ratio. During the last decade of the 19th and the first of the 20th century, G. A. Klebs conducted some experiments designed to control the time of fruiting of plants. His work was concerned largely with algae and fungi. Klebs was able to keep plants in the vegetative stage or he could induce sexual reproduction if he wished. He suggested that among other factors, the relation between carbohydrates and nitrogen was of major importance in controlling the stage of growth.

In 1918 E. J. Kraus and H. R. Kraybill formulated rather definitely the hypothesis that the ratio of carbohydrates to nitrogen is an important factor in determining whether that plant will continue in the vegetative stage or whether it will shift to the reproductive phase of growth. These investigators, in a series of studies with tomatoes, established four different groups, depending upon the ratio of carbohydrates to nitrogen.

Briefly, the two extremes in this group represented either a deficiency of carbohydrates with respect to nitrogen or a deficiency of nitrogen with respect to carbohydrates. Plants in these two groups did not produce satisfactory growth either from the vegetative or reproductive standpoint. In the intermediate groups a moderate carbohydrate and high nitrogen ratio represented a strong vegetative growth. If the carbohydrate content were high and the nitrogen low, then the plants would show abundant flowering and would be highly fruitful.

The investigations of Kraus and Kraybill stimulated others in a study of the C/N ratio and the literature soon became replete with reports on this topic. Although the subsequent research tended to support their hypothesis, it never found practical application in horticulture because there appears to be no definite, or approximately definite, ratio at which fruiting in plants takes place.

Chemical Treatment. It will be apparent that the two methods known to induce flowering of certain plants (control of photoperiod and/or temperature) are not practicable on a very large commercial scale in which the plants are grown out-of-doors. Some simple method for inducing bloom, such as a chemical spray, would find ready acceptance among horticulturists. But there are relatively few plants which have



The appearance of red color in the crown of a pineapple plant is the first evidence that the plant has changed from vegetative to reproductive stage of growth.

been stimulated to flowering by chemical treatment. One which has is the pineapple (*Ananas comosus*). Blooming in this plant can be accelerated by exposure to the hydrocarbon gases, ethylene and acetylene, or by spraying with alpha naphthaleneacetic acid or dichlorophenoxyacetic acid. Some of these methods are now used commercially. Why this plant, above all others, can be made to bloom in this manner is not known, nor is it clear as to what especial reaction is produced by the treatment. One explanation which has been offered is that the pineapple originated in the tropics where there are no great seasonal fluctuations in either temperature or photoperiod. The pineapple plant, according to this theory, has never developed a sensitivity either to temperature or length of day in regard to flowering.

The unsaturated hydrocarbon gases (ethylene, acetylene, etc.) are capable of initiating a number of other physiological processes in plants,

as is true of the synthetic growth-modifying substances previously mentioned. Neither the hydrocarbon gases nor the synthetic growth substances are considered to be true hormones. Instead, they appear to act as triggers which set off hormonal mechanisms. It may be significant that in the pineapple plant the first indication that a flower-inducing treatment has been successful is the appearance of anthocyanin pigments in the crown of the plant. It is customary for commercial growers of pineapples to look for this proof of effectiveness of the treatment.

Formation of Fruits

The first observable effect of successful pollination of the flower is enlargement of the ovary. The enlarged ovary (and sometimes related parts) subsequently becomes the fruit. Development of the fruit follows the normal growth curve which has been described previously. Just what physiological effect does pollination have on the ovary? It is evident that pollen grains must transfer auxin to the ovules. At any rate, fruit-set may be obtained in many plants by treating unpollinated blossoms with growth-modifying substances. Fruits which are produced in this manner are seedless. This is because treating the flowers with growth-modifying substances initiates growth of the ovary but does not take the place of fertilization and subsequent union of male and female cells in so far as development of the embryo is concerned.

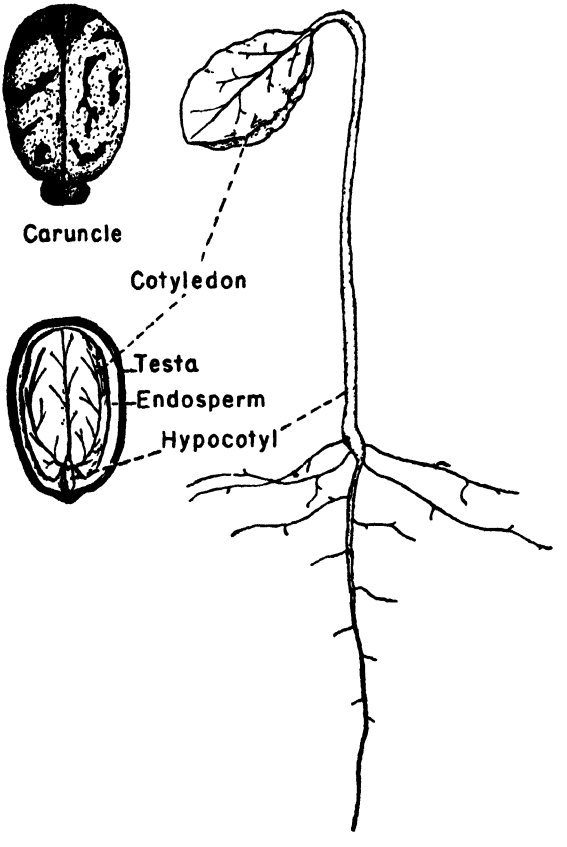
Hsiang (1951) made a study of the physiological and biochemical changes accompanying pollination in orchid flowers. The immediate effect of pollination in a species of *Cymbidium* was as follows. The lip of the perianth as well as the inner or ventral side of the column turned from white to red. The pigment was proved to be an anthocyanin although the appearance of the color was not associated with changes in the pH of the sap. Other changes observed, which had also been reported by earlier workers, were wilting of the perianth, closing of the stigma, enlargement of the column, and some swelling of the ovary. Hsiang found that wilting of the ovary was the result of the increased rate of transpiration of this organ. The column increased in both fresh and dry weight, and the perianth lost both water and dry matter following pollination. Cut discs of columns from pollinated flowers took up more water on a percentage basis than discs from columns of unpollinated flowers. The difference in osmotic pressure was not sufficient to account for the difference in amount of water absorbed. When this experiment was conducted in nitrogen rather than in air, the difference in water uptake between the two types disappeared. The author concluded that the stimulation of water uptake by pollen (or auxin) is related to aerobic processes. It was also found that columns from pollinated flowers had greater water-holding capacity than those from unpollinated flowers. This suggested that pollination causes an increase in the hydrophilic colloids in the column tissue.

A number of other changes in the physiology of plants following pollination have been reported. J. White observed not only a rapid rise in respiration but also a markedly increased respiratory quotient. E. E. De Turk, and his co-workers reported that the synthesis of phytin began immediately following pollination.

It is quite evident therefore that reproductive processes in plants are associated with pronounced changes in the physiology of the whole plant. They are not unlike animals in this respect.

Germination of Seeds

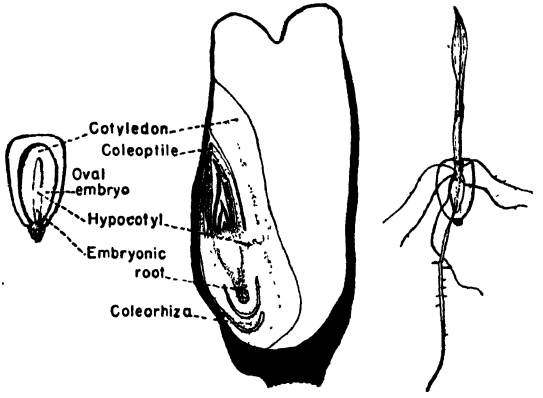
The dominant plants of the earth today are Spermatophytes, or seed-bearers. Although the production of seeds and the subsequent germination of them to produce new plants appears to be a simple process, many physiological reactions are involved.



Castor bean seed and seedling. Note large cotyledon. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

The mature seed of the plant contains an embryo or rudimentary plant which possesses the ability to grow under suitable conditions and to develop into a mature plant. In addition to the embryo, there is a compact supply of food which is usually enough to last the young seedling until it can manufacture its own food and thus become self-sustaining. The seed is enclosed in one or more seed coats, and these coats are often hard and durable and even water-resistant.

The embryo consists of a plumule (the first true leaves), a hypocotyl (rudimentary root), and one or more cotyledons. The latter organs are



Corn grain and seedling. Seeds of this type contain stored food in an endosperm. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

called the seed leaves. Dicots have two cotyledons; monocots have only one. In seeds like peas, beans, and other legumes, the cotyledons serve as organs for storage of food. In types like cereals, food is stored in the endosperm ("*outside* the sperm or germ").

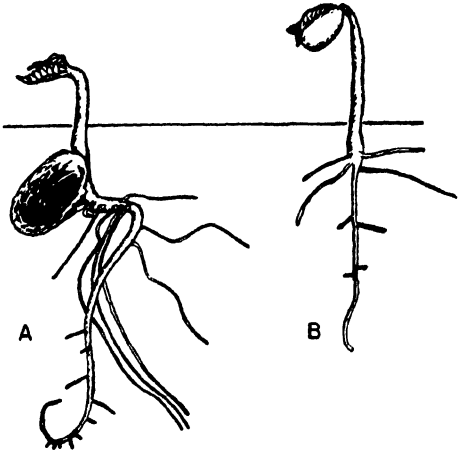
Three and sometimes four factors affect the germination of seeds. These are: moisture, temperature, oxygen, and light.

Moisture. It is common knowledge that moisture is essential for the germination of seeds. When garden seeds are planted it is customary to pack down the soil over the seeds so that moist particles of earth are in direct contact with the seeds. In the laboratory when seeds are grown in jars or petri dishes, they are placed on moist blotters or filter papers. Actual contact with liquid water is not necessary because seeds may start germinating in an atmosphere which is saturated with water.

Temperature. Suitable temperature is another factor necessary for the germination of seeds. Early in the spring in temperate zones the soil is usually saturated with moisture but the temperatures are too low to permit germination of most seeds. As the season advances, and the air and soil temperatures gradually rise, more and more seedlings emerge. Those requiring higher temperatures do not appear until later in the

season. Temperate zone species will germinate in a range from just above 0°C. to around 35°C. Tropical and subtropical seeds have a minimum temperature for germination of 5°C. to 10°C. and a maximum of 45°C.

Oxygen. For most species of plants the oxygen requirement during germination is relatively high. Energy for growth is obtained from respiration and this process requires oxygen. Garden seeds planted in low areas with water-logged soils may fail to germinate entirely because of deficiency of oxygen. This may be easily demonstrated in the laboratory.



Types of germination. (A) Scarlet runner bean germinates with cotyledon below the ground. (B) Garden bean represents type in which cotyledon emerges with seedling. (Courtesy, Emerson: *Basic Botany*, New York, The Blakiston Co., Inc., 1949.)

Wheat seeds placed in a wide-mouth bottle and covered with water will swell but will not germinate. If, on the other hand, a stream of air is constantly bubbled through the water the seeds will germinate, producing green leaves (in light) and will appear normal in every way.

There are certain seeds that germinate under water, like the cattail (*Typha latifolia*). Oxygen requirements for this type are considerably lower than for other plants. It has been shown experimentally, however, that seeds of this species will not germinate in the complete absence of oxygen.

Light. Light is a factor in the germination of only certain types of seeds. Certain plants like mistletoe and strangling fig will not germinate unless exposed to light. In others, light acts as a stimulant and thus increases the rate of germination. In this group are the grasses, tobacco, evening primrose, mullein, and others. The seeds of still other types will be retarded in their germination if exposed to light. Thorn apple (*Crataegus*) and pigweed (*Amaranthus*) must be germinated in the dark.

Physiology of Germination

Germination of seeds begins with the imbibition of water and the consequent swelling of the hydrophilic colloids. The respiration rate then rises and this increases the oxygen requirements. Hydrolytic enzymes become active and insoluble foods in the cotyledons (starch, proteins, and fats) are all hydrolyzed to simpler components (sugar, amino acids, and fatty acids).

Table 30

CHANGES IN THE RESERVE FOODS OF SUNFLOWER SEEDS DURING GERMINATION*

Constituents	*Seed*		*5 Days*		*10 Days*	
	Cotyledon	*Hypocotyl and Roots*	*Cotyledon*	*Hypocotyl and Roots*	*Cotyledon*	*Hypocotyl and Roots*
	Grams of constituents per 100 seeds and seedlings					
Ether extract	3 786	0 219	2 53	0.123	0.501	0 273
Total sugar	0 279	0 019	0 09	0 298	0 090	0 351
Reducing sugar	trace	trace	none	0 271	0 090	0 351
Protein	1 66	0 117	1 03	0.125	0.630	0 195

* Miller, Edwin C.: *Plant Physiology*, 2nd ed. New York, McGraw-Hill Book Co., 1938.

In Table 30 will be found some of the changes occurring during germination of sunflower seeds. It will be observed that there is a loss of reserve foods in the cotyledons and a gradual gain of these constituents in the hypocotyl and roots. At this time reserve foods are being used both as a source of energy (respiration) and in the synthesis of new tissues (protoplasm and cell-wall materials).

Still other changes have been found to occur in seeds during germination. Carotene (provitamin A), ascorbic acid, and many vitamins of the B complex have been shown to increase in seeds following germination. It is apparent from this that a diet consisting mainly of dry seeds, like cereals and legumes, may be considerably supplemented by first sprouting the seeds. The wide use of sprouted mung bean seeds in the Orient has a certain amount of scientific justification, although the custom antedated the discovery of vitamins by hundreds of years.

The use of malted barley by the brewing industry represents another practical application of sprouted seeds, since the term "malted" means sprouted. The purpose of sprouting is to increase the content of amylolytic enzymes. Malt diastase in the sprouted barley grains consists of a mixture of amylase and maltase, which catalyze the conversion of starch to glucose.

chapter 23

Dormancy

At a certain time of the year seeds, bulbs, tubers, corms, and the buds of woody or herbaceous plants may fail to grow even though the environmental conditions are suitable for growth. When such a condition occurs these organs (or the whole plant) are said to be in a *dormant condition*. Seeds of some plants are capable of germinating in the same season in which they are produced, while others may remain dormant for a period of weeks, months, or even years. Likewise, newly formed potato tubers, lily bulbs, and gladiolus corms will undergo a period of rest or dormancy before they will start growing under natural conditions. New potatoes will not sprout for 75 to 90 days after they are mature, whereas old potatoes may be made to sprout in 10 days.

Dormancy in the buds of woody and herbaceous plants may be observed during the fall and early winter. If twigs of many temperate zone species are cut and brought into the home or into warm greenhouses in the fall, they will in all probability make no growth at all. If the whole plant is brought into a warmer environment at that time, it may remain dormant for a year. It is apparent, therefore, that these plants or plant parts may require certain conditions and sometimes drastic treatments in order to *break dormancy*. The causes of dormancy in seeds, and the means of breaking dormancy in both seeds and buds will now be considered.

Seeds

Some of the factors which cause dormancy in seeds are as follows.

1. *Impermeability of seed coats to water*. In seeds like those of legumes (*Leguminosæ*) and members of the mallow family (*Malvaceæ*) the coats are extremely hard and are water resistant. Hard seed coats maintain the low constant moisture content of the embryos by sealing them hermetically. Although it is probable that in some instances hard coats may cause dormancy merely by offering mechanical resistance, it is more often the water-repellent nature of the coats which delays germination. A hard-coated seed may be considered as a natural example of highly effective water-proofing in thin layers.

Dormancy of this type has been successfully broken by treatment of the seeds with fat solvents, such as ether, acetone, or xylene. Universal

use of ether cannot be recommended because it has been known to kill the embryo. Other methods for breaking dormancy in hard-coated seeds consist of treating first with alcohol and then with water, moderate heating, shaking in a bottle, mechanically rupturing the seed coat, and scarification of the seeds with sulfuric acid.

2. *Seed coats impermeable to oxygen.* Dormancy in some types of seeds has been found to be caused by inability of the embryo to receive sufficient oxygen for growth. The classic example of this type of seed is that of the cocklebur (*Xanthium* sp.). The cocklebur contains two seeds, one extending nearer to the base than the other, usually referred to as the *lower seed.* Farmers once claimed that one of these two seeds germinates the first season after maturity, and the other in the second season. J. M. Arthur investigated this claim and in the main confirmed it. In 1909 W. Crocker showed that delayed germination of the upper seed was the result of the impermeability of the seed coat to oxygen. The upper seed of the cocklebur could be made to germinate by growing it in pure oxygen. Germination was also obtained in ordinary air either by pricking the seed coat with a pin or by removing the coat entirely.

Dormancy in the seeds of grasses and many composites appears to be due to the impermeability of seed coats to oxygen.

3. *Immature embryos.* Seeds of European ash, ginkgo, holly, etc., remain dormant because the embryo is rudimentary or incompletely developed. These embryos mature during the period of dormancy. The exposure to moderately high temperature or the supplying of sugar and minerals may hasten their development. Several investigators have reported accelerating development of the embryos of jimsonweed (*Datura* sp.) by removing them from the ovary and growing them under specialized methods of tissue culture.

4. *Dormant embryos.* Another type of dormancy is to be found in seeds in which the embryo itself is dormant. Common examples are seeds of apple, peach, basswood, tulip tree, dogwood, pine, hemlock, iris, and lily-of-the-valley. Under natural conditions seeds of these species of plants fall to the ground in the fall and are partly or wholly covered by cold soil during the winter. Dormancy is broken as a result of exposure to low temperatures. One winter may suffice or it may require several years. Changes which occur in the seeds under these conditions are referred to as *afterripening.* It was once assumed that dormancy was broken as a result of alternate freezing and thawing, but it is now known that this is not true. Exposure to temperatures between 1°C. and 6°C. for from two to three months is all that is necessary in most cases. Breaking dormancy in this manner has been practiced for centuries, by use of the method of *stratification.* This term stems from the practice of placing in a soil first a layer of seeds and then one of sand. It is now customary to mix the seeds with moist sand, granulated peat, or other media. The purpose of the

strata is to insure optimum conditions involving several factors such as temperature, moisture, and oxygen.

H. B. Tukey and R. F. Carlson have reported breaking the dormancy of peach seeds (dormant embryos) by treating the embryos with thiourea. However, even though this treatment induced growth of the embryos, the seedlings which subsequently developed did not show normal characteristics. It seemed that the embryos were not physiologically mature even though they germinated. Obviously all of the physiological changes involved in the afterripening processes are not completely understood.

Light

The relation of light to dormancy is rather involved. First it should be stated that this factor is necessary for the germination of some seeds, while it inhibits germination of others. Many seeds (corn, beans, clover, and others) germinate equally well in light or darkness.

Some seeds, like those of Indian tobacco (*Lobelia inflata*) will not germinate in the absence of light. Germination of seeds of many grasses such as Kentucky bluegrass is improved by exposure to light. Germination of tobacco seed is not stimulated by light unless the seed is first soaked in water. Some of the mistletoes not only require light for germination but have been reported to die when held continuously in darkness.

It has been reported that with several light-sensitive seeds, other treatments may be substituted for the exposure to light. Some of these treatments involve exposure to alternating temperatures (5°C. and 23°C.), removal of seed coats, hot-water treatments, and soaking in hydrogen peroxide, sodium sulfocyanate, or hydrochloric acid. Crocker reported that treatment with Knop's solution may be substituted for light for a number of light-favored seeds, the nitrate in the solution being the specifically active agent.

L. H. Flint and E. D. McAlister, working with light-sensitive lettuce seed, reported that violet, blue, and green light rays inhibit germination while the orange and red rays favor it.

Germination of Orchid Seeds

Because of many misconceptions that seem to prevail on the subject of germination of orchid seeds, brief mention will be made here regarding some of the physiological principles involved. For many years orchid growers experienced considerable difficulty in growing the plants from seeds, although orchids normally produce thousands of seeds. A yield of one or two plants per thousand seeds was all that could be expected. (Orchid seeds are quite small, each weighing 0.3 gamma or three ten-millionths of a gram.) It was customary to plant the seeds on substrates containing leaf mold, peat, or rotting wood in order to maintain a high percentage of organic matter. Since many of the orchid roots in nature

are infected with fungi it was believed that perhaps a symbiotic relationship existed in which certain fungi were necessary for germination of the seeds. One of the early practices consisted of growing seeds on sterile artificial media containing pulverized tubers of an orchid. When the seeds in this medium were inoculated with fungi from the orchid roots, some seeds germinated but many of them died. Sometimes, when a higher concentration of the powdered orchid bulb was added to the medium, the seeds germinated under sterile conditions. L. Knudson was the first person to offer an explanation of what was occurring under these unusual conditions. Pulverized tubers of the orchid contained, among other substances, large quantities of compound polysaccharides like starch and pentosans. Knudson believed that the fungi served only to digest these polysaccharides, making them readily available to the seedlings. His conclusion proved to be correct, for subsequently he was able to grow orchid seeds on sterile media, provided that he supplied them with ample quantities of either glucose, fructose, or sucrose. Without sugar the seeds would swell and often turn green in light but growth would not continue. The embryos might remain alive for some time, two years in some instances. It appeared from these results either that orchid seeds contain insufficient food to maintain continuous growth, or that an inhibiting substance is produced when photosynthesis begins. In his earlier work Knudson found that some of the fungi used for inoculating the seed exerted another indirect effect. This consisted of producing a more favorable pH for growth (4.7 to 5).

Buds

Potato Tubers. Extensive studies on breaking dormancy in potato tubers have been made at the Boyce Thompson Institute for Plant Research. The investigations were begun following a request for information from potato growers in Bermuda. Seed potatoes in the northern United States are normally dug in September. Buds on these potato tubers remain dormant for from 9 to 12 weeks, depending upon the variety. Planting time in Bermuda, however, is early October and the growers on this island desired a method which would induce immediate growth in freshly dug potatoes.

Denny headed the project at the Boyce Thompson Institute, and tested a total of 224 chemicals for their effect on breaking dormancy of potato tubers. Two types of compounds proved effective. They were ethylene chlorhydrin and potassium, and sodium or ammonium thiocyanates. The first of these chemicals was especially desirable because of the wide margin between effective dosage and toxicity. Treatments of the tubers with these compounds completely eliminated the need for a rest period. Furthermore, treatments of this type do not appear to affect the yield or to produce any formative modifications of the developing plants.

Gladiolus Corms. Attempts to break the dormant period in gladiolus corms have been successful to a certain extent, but the results are not quite as satisfactory as they were with potatoes. Dormancy in certain varieties of gladiolus may be broken by treatment with ethylene chlorhydrin applied in closed containers. Ethylene and ethyl ether are not effective.

Because of varietal variation in regard to dormancy, however, it is desirable to study each variety separately. Chemical treatment is effective in some varieties soon after harvest, and in others only after the corms have been held for a period at proper storage temperatures. In still other varieties, only holding the corms in storage will suffice to break dormancy.

Deciduous Trees and Shrubs

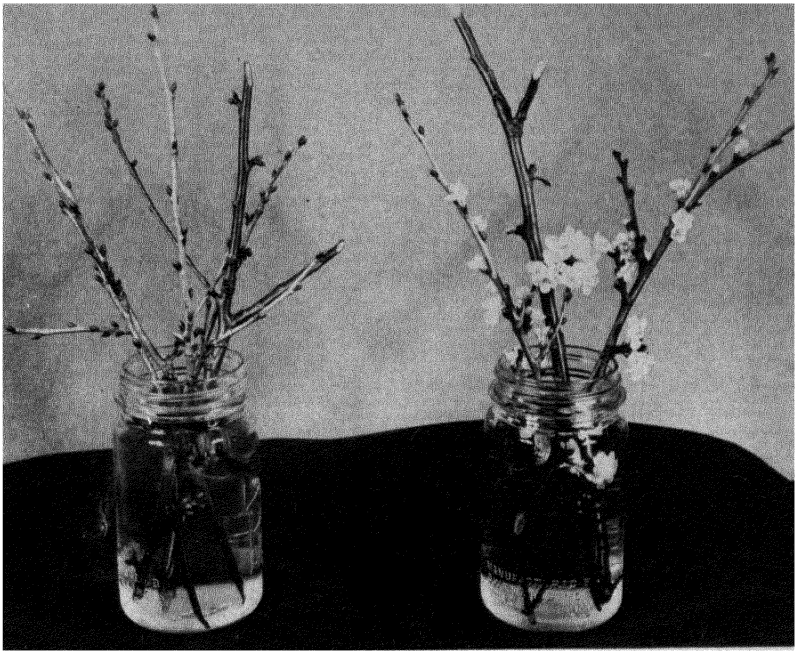
Mention has been made of the fact that buds on woody plants in temperate zones enter a rest or dormant period in late summer or early fall. This state of dormancy is broken by a period of low temperatures which prevails during the ensuing winter. Different species require different periods of low temperatures for breaking dormancy. W. L. Howard made a rather extensive study of dormancy in buds of woody plants by transferring cuttings to the greenhouse during the winter months. He investigated 234 species. More than half of them began to grow when brought into the greenhouse about November 1st. A larger number had broken dormancy by the first part of January. By the latter part of February all but five species began to grow when removed to the greenhouse.

That exposure to low temperatures serves to break dormancy may be demonstrated with simple experiments. F. V. Coville performed an experiment with a blueberry plant in which one branch was extended through an opening in the glass of the greenhouse, exposing it to winter temperatures, while the main plant was kept at warm temperatures inside. The treated branch (outside) resumed growth upon the arrival of spring (April 18) while the parts of the plant which were kept within the greenhouse remained dormant.

One year an exceptionally warm winter prevailed in the commercial peach-growing districts of central Georgia. When spring came and the peach trees failed to bloom, local plant physiologists realized that the winter had not been long enough or cold enough to break dormancy in these plants. This was demonstrated by cutting off some of the twigs and holding them in cold storage. After a definite period of time the twigs were brought out of storage and they immediately bloomed. Hutchins found that, in general, peaches require exposure of 1,000 hours to temperatures of 45°F. (7.2°C.) or lower to break dormancy, although each variety has its own specific requirement regarding number of hours. It is not necessary for the plants to have continuous exposure because the effect appears to be cumulative. It is for this reason that temperate-zone

fruits, like apples and peaches, cannot be grown successfully in the tropics. However, plant breeders have developed varieties of peaches which require only moderately low winter temperatures and which are being planted in subtropical regions.

Other methods have been found effective for breaking dormancy in woody plants. For example, Molisch reported that immersing branches of dormant trees in a water bath at 30°C. to 35°C. for 9 to 12 hours was an



Cherry blossoms in December. Twigs of dormant cherry trees were cut in December and stored at room temperature for two weeks. Those on right have had dormancy broken by exposure to vapor of ethylene chlorhydrin.

effective treatment. W. Johannsen found that many dormant plants could be made to resume growth after being exposed to ether or chloroform for a day or two. The interval between treatment and resumption of growth, however, varied with the time of year. In late summer the response was not evident for several weeks, while in late winter it was observable within a day or two.

It will be apparent, however, that none of the artificial methods reported for breaking dormancy in woody plants would be practicable for extensive commercial use, as in fruit production. They may have some application in potted plants, which can be confined for treatment, but they would be difficult to apply to large plantings out-of-doors.

chapter 24

Tropisms or Tropic Movements

Tropisms are growth movements which occur as the result of environmental factors (stimuli) acting with a greater intensity from one direction than from another. The first observable result is a curvature of the plant or organ. Response is usually directional, that is, curving either toward or away from the source of the stimulus. Curving toward the stimulus is said to be a *positive tropism;* it is said to be *negative* when growth is away from the source of the stimulus. A distinct difference exists between tropisms and other types of movements, such as *nastic movements,* in which the plant or plant part reacts without reference to the source of the stimulus. Thus in *phototropism* the plants grow either toward or away from the light, and the direction of the source of the stimulus is important. In the case of *epinasty,* the leaves and stems of the plants curve downward and the growth reaction or curvature is always in the same direction, no matter how the stimulus is introduced into the environment.

Phototropism

The specific reaction of a plant or plant part to unequal illumination on opposite sides is termed *phototropism.* Positive phototropism may be demonstrated as follows. A flower pot containing seedlings may be placed under a box, and if light is admitted through a small aperture on one side, the plants will grow toward the source of the light. Sometimes the response may be observed after only a few hours. Anyone who has grown potted plants on the window sill of a room has doubtless observed this tendency of most plants to grow toward the light. The sunflower is so named because, in the growing plant the heads are turned toward the east in the morning and toward the west in the evening, following the course of the sun.

The explanation of phototropic responses has interested physiologists for many years. Prior to 1928 many different hypotheses were advanced by students of this phase of plant physiology. It was held by some that a quantity of potential energy is released in the plant by action of the stimulus, just as pulling the trigger of a gun causes a sudden release of energy. Other suggestions, based on results obtained with a fungus, *Phyco-*

myces nitens, were that the sporangiophore constituted a transparent cylinder in which "the rays are concentrated at the back," causing the organism to grow toward the light. That the phototropic effect was primarily a growth movement was not realized until about 1914 when Arpad Paal suggested such a relationship. His explanation was that certain substances are produced in the unstimulated tip of the coleoptile of the oat (*Avena sativa*) and distributed evenly down the sides to the zone of elongation. After unilateral (one-sided) illumination, the substance is translocated to the shaded side, causing more rapid growth. Presence of a plant



Phototropism in squash seedlings. Plants have bent toward light source. (Courtesy, Gibbs: *Botany*, New York, The Blakiston Co., Inc., 1950.)

hormone was indicated by the work of Charles Darwin as early as 1881. It will be recalled from the discussion of hormones that Darwin demonstrated that activation occurred only in the tip of the shoot and that the phototropic response could be inhibited by shielding the tip from light. It remained for Boysen-Jensen (1910 1913) and F. W. Went (1926) to prove the direct relationship between auxins and tropisms. This is just another instance in which a fundamental discovery (isolation and identification of auxins) served to clarify an issue and to validate a hypothesis which had been advanced long before experimental evidence was available.

The chain of events leading up to a better understanding of phototropism was discussed in the chapter 19 on Growth-modifying Substances. It will be recalled that Boysen-Jensen demonstrated that the growth substance could be made to pass from the decapitated tip of the coleoptile through a block of agar to the zone of elongation. Went permitted auxin

to diffuse from a decapitated coleoptile into an agar block. This "activated" block, when placed on one side of a decapitated coleoptile, caused the plant to bend in the opposite direction in the dark.

This does not mean that the principle of phototropism is entirely understood. According to A. R. Schrank (1950) this differential growth, resulting from unilateral illumination, may be achieved in one of several ways or in a combination of them. These are as follows:

(1) A translocation of the auxin from the illuminated side to the shaded side.

(2) Photo-inactivation of auxin on the illuminated side.

(3) Increased auxin production by light. (This would serve to explain negative phototropism such as that exhibited by certain fungi and by the roots of higher plants.)

Although any one of the above-mentioned mechanisms may constitute an essential part of the reaction, *the lateral transport of auxin still remains as one of the more important links in the chain of events.*

Effective Wavelengths. Phototropic responses are not equal in all parts of the visible spectrum. Johnston found that the most effective wavelengths ranged from 440 to 480 millimicrons, which is the blue and blue-green part of the spectrum. No phototropic effect is produced by the red end of the spectrum. For this reason quantitative determinations of auxin (using oat coleoptile method) are made in the red light of the photographic dark room.

In the green region the phototropic effect is very slight. The sensitivity of ultraviolet light is only 25% of that shown by the optimum wavelength.

Light Intensity. Phototropisms may be initiated by light of very low intensity. A. H. Blaauw found that etiolated oat seedlings reacted phototropically to light having intensity of 1.58×10^{-5} (0.0000158) foot-candles. This is equivalent to about $\frac{1}{1000}$ of the intensity of full moonlight. It should be recalled that the photochemical effect of light on plants equals the product of time and intensity. With light of the low intensity just mentioned, Blaauw found that exposure of 43 hours was required.

Geotropism

When a viable seed is dropped into the soil, and conditions are favorable for growth, the shoot eventually emerges above ground and the roots grow downward. The shoot is said to be *negatively geotropic* and the roots, *positively geotropic.* The term *positive geotropism* is used to indicate that the organ grows toward the center of the earth. The stimulus in this instance is believed to be gravity. This may be demonstrated in another way. A small seedling may be placed in a horizontal position. It will not be long before the stem turns upward and the roots curve downward toward the center of the earth. Amateur gardeners sometimes worry lest

they have planted corms and bulbs upside down. The principle of geotropism, however, indicates that nature has taken care of such things, and that although planting a bulb upside down might delay emergence, it does not inhibit it entirely.

The reason for the geotropic responses of roots and shoots has been only partially explained. Charles Darwin in 1881 observed that this "perception of gravity was seated in the root tip." From then until 1929 experiments were conducted in which the effect of the gravity factor was studied. Plants were placed in a horizontal plane and centrifuged. It was



Branches grow upward (negatively geotropic) irrespective of position of main axis. Trunk of young locust tree was broken during a storm. (Sketched from author's photo.)

found that when the centrifugal force was sufficient to overcome the force of gravity, roots grew outward, whereas shoots grew toward the center of the described circle. Investigators of that period labored under the illusion that gravity was instrumental in influencing the production of auxins. An important contribution was made by H. E. Dolk in 1929 when he showed that the total amount of auxin produced by the oat coleoptile was not affected by gravity. This investigator showed, however, that when a young seedling is placed in a horizontal position, more auxin accumulates in the underside than in the upper side. Dolk proved this by allowing auxin from the upper and lower halves of the stem to diffuse into different blocks of agar. It thus appears that auxin molecules tend to settle to the bottom, like heavier particles in a suspension.

Growth of the seedling from a horizontal position is explained as follows. A large concentration of auxin accumulates on the lower side of the stem. The same concentration of auxin that accelerates shoot growth will at the same time inhibit root growth. The heavy concentration of auxin on the lower side of the stem has an accelerating effect so that the underside grows more rapidly than the upper, and the direction of the shoot is

away from the center of the earth. In like manner, relatively high concentrations of auxin on the underside of the root act as inhibitors, with the result that the upper side grows more rapidly than the underside, and the net direction of root growth is downward.

This does not explain why, if the plant is kept in an upright position, the auxins do not accumulate in the roots to such an extent that all growth of these organs is inhibited. It is possible that under normal conditions the auxins are consumed by the meristematic cells in the roots as fast as they are transported to this region, or that certain auxin inhibitors are present.



Aerial roots of the banyan tree evidencing positive geotropism.

Electropism

Physiologists have given a certain amount of attention to polarity in plants and to the possibility that differences in electric potential may play an important part in metabolism. The apices of stems, hypocotyls, and coleoptiles of a number of species of plants have been shown to be electronegative while the basal portions are electropositive. Furthermore, tests have shown that when an Avena coleoptile is placed in a horizontal position, the underside becomes positive to the upper side within one minute. Since this apparently occurs in a shorter time than that estimated for the translocation of auxin, it is assumed that changes in polarity naturally precede the transport of auxins.

So much has been said regarding the possible relationship between electrical polarity and auxin transport that it now seems logical to discuss electropism. That electrical currents exert definite effects on the growth of plants has been amply demonstrated, the specific effect depending upon the voltage and duration. Reports have also appeared which indicate that electric currents may produce oriented responses, i.e., *electropisms*. Germinating pollen tubes have been observed to grow predominantly toward the cathode in an electric field. Onion roots have been found to orient themselves toward the anode in a transversely applied electrical stimulus. Schrank has demonstrated that a direct current of 5 to 20 microamperes, applied transversely for two minutes to the 5 mm. segment of the Avena coleoptile, induces a bending toward the positive pole of the current-applying circuit. Inherently the Avena coleoptile has no transverse electrical polarity. Although the curvature produced by the electric current was the result of growth processes, the direction of initial curvature was always toward the electropositive side. In previous reports

curvature has always been directed toward the electropositive side. Up to the present time no hypothesis has been offered to explain these results.

Thigmotropism

In *thigmotropism* the stimulus results from contact with a rough object. The best examples of this phenomenon are to be found in the growth of the tendrils of plants, like grapes, cucumbers, sweet peas, Smilax, and many others. Contact with a solid, rough surface causes a reduction of growth on the side next to the stimulus and the tendril grows around the object. A stimulus from a smooth surface, like rain drops or drops of mercury, will not produce a thigmotropic effect.

It appears that electrical polarity may be a factor in thigmotropism, as was suggested in geotropism. Schrank has shown that the mechanical stimulation of the top 10 mm. of one side of the Avena coleoptile causes a bending toward the stimulus. Stimulation of this type also established a difference in electric potential between the two sides. The stimulated side became electronegative and the bending was toward the negative side. This is identical with what was stated regarding geotropism and electrical polarity.

Other Tropisms

Traumatotropism refers to tropic responses to wounding. Sometimes transverse incisions, especially on roots and stems, result in positive curvatures. It is not known whether the underlying cause is traceable to unequal distribution of auxin or whether the incision interferes with transport of food materials.

Hydrotropism is the term applied to responses of roots to the stimulus of moisture. It was once believed that roots were able to grow toward the more moist regions of the soil. The majority of experiments have indicated that such is not always the case. W. E. Loomis and L. M. Evans studied the hydrotropic responses of 26 plant species, employing thousands of seedlings, and found positive responses present in only a few species of the Cucurbitaceae and Leguminosae.

chapter 25

Effects of Low Temperatures on Plants

General Effects

A better understanding of some of the effects of low temperatures may be gained by first considering the fact that the range of temperatures within which plants normally grow varies with the species. Arctic and alpine plants thrive in a temperature range of 0°C. to 10°C. Temperate-zone species usually grow between 5°C. and 45°C., whereas tropical types are adapted to a range of temperatures of 10°C. to 45°C.

Seeds of different species of plants have different optimum temperatures for germination. Roots may have lower optimum temperatures for growth than do aerial parts.

Even in temperate zones there are species of plants which begin to grow at lower temperatures than other plants. Early in the spring shoots of May apple, Trillium, jack-in-the-pulpit, and others may be seen emerging from the ground before most plants show any visible signs of spring growth. Phenomena of this type pose a question to physiologists. Why do some plants flourish at relatively low temperatures? Is it a case of more efficient utilization of energy? Is there a difference in the physical state of the protoplasm in the two types of plants?

Still other questions arise as we launch into a discussion of the effect of low temperatures on plants. That dropping the temperature below the optimum retards growth in plants is so common an observation that few have sought an explanation. Can there be a thermodynamic explanation? In chemical reactions a lowering of temperature reduces the activity of the reacting molecules. Is this true with physiological reactions in the plant cell? Kinetic activity doubtless has a direct bearing on physical processes like diffusion, imbibition, osmosis, and the absorption of minerals. Perhaps the same holds true for processes like protoplasmic streaming, respiration, transpiration, and translocation. These and other questions may eventually be answered by teams of physiologists, chemists, and physicists.

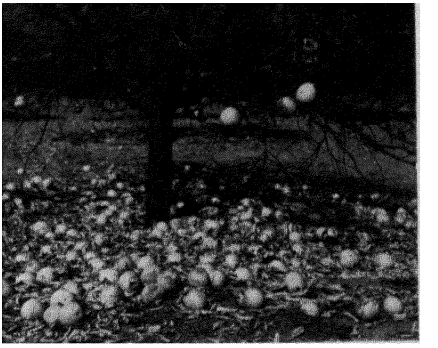
In addition to retarding the rate of growth, low temperatures may produce specific physiological effects. Initiation of the flowering of onions, beets, and celery by exposing the plants to low temperatures are typical

examples which have been discussed. We have also considered the effect of low temperatures in breaking dormancy of woody perennials and certain seeds. Low temperatures have been observed to play a part in the development of color in leaves and fruits. The formation of anthocyanin pigments in leaves during development of autumnal coloring is dependent upon the incidence of cool weather. Likewise, cooler climates favor the development of color in red apples. Loss of green color (chlorophylls) and the development of orange pigments (carotenoids) in oranges is enhanced by low temperatures and retarded by relatively warm temperatures during the months in which these fruits mature.

Let us now turn our attention to the effect of temperatures which are low enough to cause freezing.

Freezing and Frost Resistance

Economic losses are often sustained by growers as a result of killing frosts which arrive late in the spring, early in the fall in temperate zones, or at most any time during the winter in the subtropical areas. Physiologists have never agreed on the mechanics by which living cells are destroyed by freezing.



Freezing damage to grapefruit tree. Tree has lost practically all leaves and fruit.

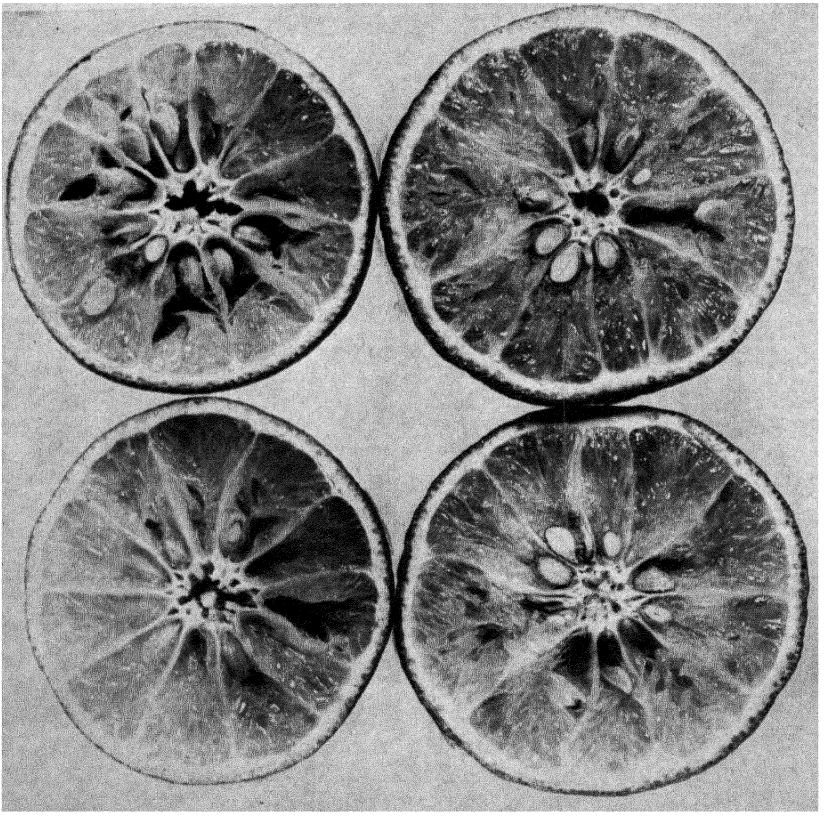
As early as 1897 Molisch claimed that death of protoplasm resulted from dehydration. According to his theory, ice crystals form in the intercellular spaces and cause dehydration of the protoplasm as the result of withdrawal of water. In 1918 the theory was advanced that the dehydration which results from formation of intercellular ice crystals has an entirely different effect. It was claimed that electrolytes are concentrated in the dehydrated protoplasm to such an extent that they cause "salting out" of the proteins.

Another theory attaches importance to the formation of ice crystals and the mechanical injury produced by them. Crystals may form either between the cells or within the cells. An observation which has been made on freezing of fruits lends support to the theory that damage results from mechanical injury produced by ice crystals. It has been found that, under certain conditions, freezing of harvested apples may cause no serious damage to the tissues unless the fruits are jarred while frozen. If they are permitted to thaw without handling, they may show few effects of freezing.

According to still another theory, injury to cells occurs, not during

the process of freezing, but during thawing. Proponents of this theory believe that damage is caused by entry of water into the cells during thawing.

Observations made with mature citrus fruits which have been frozen on the tree suggest that freezing may produce certain physiological



Varying degrees of freezing injury in the fruits of the temple orange. (Courtesy, Bureau of Plant Industry, U.S. Dept. of Agriculture.)

changes in the fruits. Following a "freeze," these fruits will show an accumulation of glycoside crystals on the segment walls. The effect is apparent even before the tissues break down. Fermentation of the juice generally follows in about 24 hours. Finally, the juice sacs dry out and separate from each other, resulting in the characteristic, dry stem-ends which are familiar to many citrus growers.

A certain amount of frost resistance is to be found in both wild and cultivated plants. In some instances hardiness is seasonal. Evergreen plants may develop resistance to frost as cold weather arrives and subse-

quently lose resistance when spring returns. Vegetable plants may be artificially made resistant to freezing temperatures. If cabbage plants are exposed to a temperature of 0°C. for one to four hours per day they will subsequently tolerate freezing temperatures.

Winter-hardiness in plants may be the result of several conditions, according to theories held by physiologists. These may be: (1) low water content of tissues, especially in woody shrubs and trees, (2) relatively high concentration of soluble substances (mainly carbohydrates) which in turn raises osmotic pressure and lowers the freezing point, and (3) presence of *bound water*, by which is meant water which has been imbibed by colloids.

According to the bound-water theory, hydrophilic colloids withdraw or imbibe water from plant sap, increasing the osmotic concentration of the latter.

Physiological Changes in Cold Storage

Mechanization and mass production in farming and the continued growth of cities have focused more and more attention upon handling of harvested crops and the physiological problems which may result from revolutionary changes in agricultural practices. In all probability a large proportion of the students of plant physiology will have had more experience with the handling of horticultural crops than with their production. Although harvesting, packing, and transporting to markets constitute important phases of the handling of perishable crops, cold storage frequently constitutes the final stage in handling (prior to marketing) and may represent the longest period in the postharvest life of many commodities. Because of these facts and because of the many physiological problems which have arisen in modern times, it seems desirable to close this chapter with a brief discussion of the physiological changes which may occur during the cold storage of horticultural crops.

The objects of cold storage are to maintain fruits and vegetables in an attractive and edible condition for as long as possible, and to keep plants and propagating material viable through the winter months. Low temperatures serve to extend the life of horticultural crops in two ways: (1) retardation of physiological processes which bring about senescence, and (2) inhibition of the growth of decay-producing bacteria and fungi. What is not ordinarily appreciated, however, is that a number of physiological changes may occur in the stored commodity, some of which are desirable and some undesirable. As a rule the manner in which a commodity reacts to low temperatures varies with the climatic zone in which it was produced. Fruits and vegetables which have originated in the tropics or subtropics are more likely to develop low-temperature injuries or disorders than are crops grown in temperate zones. Different varieties of a fruit react differently in cold storage, and sometimes the reaction in one variety varies with the part of the country where it was produced.

Most commercial varieties of apples may be stored satisfactorily at 30°F. to 32°F. for a period of from two to six months, depending upon the variety. Among the exceptions to this rule are Yellow Newtown apples from Pajaro Valley, California, and McIntosh and Rhode Island Greening varieties from New York. Apples of this type must be stored at 35°F. to 38°F. At 32°F. they may develop "internal browning" or "brown core." Grimes Golden apples must be stored at 34°F. to 36°F. because they may develop "soggy breakdown" at 32°F.

Sweet corn, garden peas, and lima beans are esteemed as vegetables in their succulent stage because of their relatively high sugar content at that time. At ordinary room temperature the sugars are rapidly converted to polysaccharides. Low temperatures retard these and other changes, thus extending the period during which these crops may be held before being consumed. The effect of low temperatures on white potatoes may be just the opposite to what has been described for peas, beans, and corn. When stored at temperatures between 32°F. and 40°F., potatoes become sweeter because starch is converted to sugar. This commodity is more palatable when stored between 50°F. and 60°F. prior to utilization.

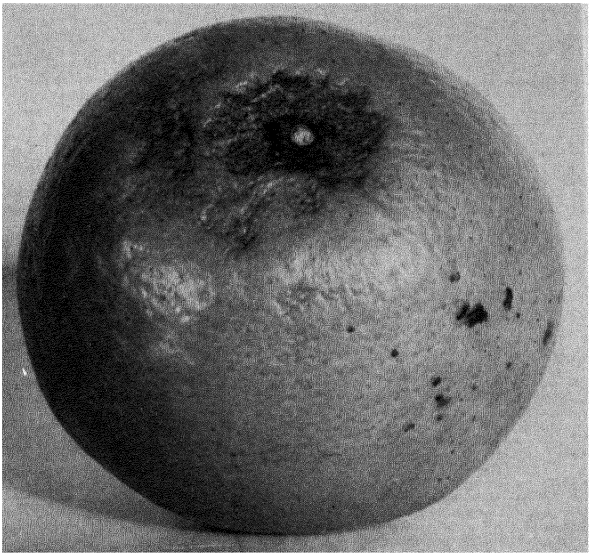
Bulbs of the Creole type of Easter lily, when planted immediately after midsummer digging, will ordinarily bloom in the period between April 3 and April 12. It is often desirable to bring lilies into bloom much earlier, such as for an early Easter. Furthermore, in some parts of the country, lilies are in demand during the Christmas season. Physiologists have been able to shorten the time required for blooming by holding bulbs in cold storage for ten weeks after they have matured. By selecting the proper temperature (36, 40, 45, 50, 55 or 59°F.) blooming can be induced in from 111 to 135 days after planting, depending upon the specific temperature which was selected for the ten-week period just mentioned. Bulbs which are planted in midsummer without having been held in cold storage can be expected to bloom in 259 days.

Citrus fruits are subject to physiological disorders if stored too long at temperatures in the range of 32°F. to 40°F. One of these disorders is termed *pitting,* which is characterized by shallow, pocklike depressions of the rind. Temperatures most conducive to pitting are 32°F. for lemons, 36°F. to 40°F. for oranges, and 40°F. for grapefruit. Other more or less superficial rind blemishes of citrus fruits are *scald* of oranges and grapefruit, *peteca* and *red blotch* of lemons. *Watery breakdown* of citrus fruits develops at 32°F. or slightly lower. Affected fruits are soft and spongy and look as if they have been frozen. *Membraneous stain* of lemons is characterized by a browning or darkening of the membranes or carpellary walls between segments.

Tropical fruits are much more sensitive to low temperatures than are citrus fruits, temperatures below 45°F. being generally unsatisfactory for storage. Mature green avocados, bananas, papayas, and pineapples are

so affected by undue exposure to low temperatures that they do not ripen properly upon being transferred to room temperature. In extreme cases the flesh becomes brown or black after the fruits have been taken from cold storage. Studies of low-temperature injury to pineapples and bananas have indicated that the ascorbic acid content is much lower in fruits showing low-temperature breakdown.

Tomatoes, eggplants, and other tropical vegetables are likewise sensitive to low temperatures and will keep better at 50°F. Green tomatoes,



Aging of citrus fruits is an example of low temperature injury.

if held too long at 32°F., will ripen with a yellow color after removal to a higher temperature. They never attain a full red color and often decay before they lose all of their green pigments.

Sometimes specific physiological effects are produced by a narrow range of temperatures. Membraneous stain of lemons, for example, is usually more pronounced in fruits stored at 36°F. to 40°F. than at 32°, 50°, or 60°F. Optimal temperatures for "pitting" of citrus fruits have already been cited. Experiments have shown that improper temperature may produce undesirable results with King Alfred narcissus bulbs. If held at 40°F. during the middle of their total storage period, many of the bulbs will fail to bloom when planted. More of these so-called *blind* or non-blooming bulbs develop following storage at 40°F. than at 32° or 50°F.

Many more examples might be cited of peculiar physiological changes, low-temperature injuries, or of the specific effects of narrow ranges of

temperature. Why these phenomena occur is not known. A probable explanation for low-temperature injury is that toxic substances, possibly intermediate products of respiration, are formed and allowed to accumulate. At higher temperatures intermediate substances would normally be oxidized to H_2O and CO_2. This theory is substantiated in part by reports



Blackening of flesh of pineapples resulting from chilling.

that acetaldehyde and alcohol tend to accumulate in fruits under improper conditions of storage. In one instance more acetaldehyde was found in lemons which were stored at 40°F. than in those held at either 32° or 50°F. It would appear that respiration does not proceed rapidly enough at the lowest temperature (32°F.) to produce excessive quantities of acetaldehyde, whereas at 50°F. this compound is oxidized as rapidly as it is formed. Recent investigations on stored pineapples indicate that low temperatures probably interrupt one of the oxidation-reduction systems in the respiratory process. Whatever the case, many questions are still unanswered, and the subject offers a fertile field for fundamental research in plant physiology.

chapter 26

The Role of Vitamins in Plants

Classification

A vitamin is an organic compound which is necessary for the normal metabolism of certain living organisms, but which functions in some other way than as a source of energy or as a major constituent of the cell. Vitamins act in a hormonelike or enzymelike manner, in that relatively minute quantities are required by the organism.

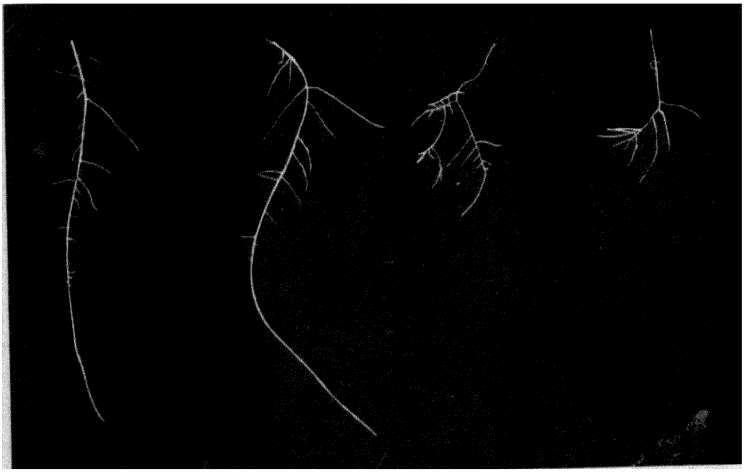
Animals are dependent upon green plants for vitamins or vitamin precursors. From the time of the discovery of the first vitamin (1906) until 1937 it was believed that these accessory substances were required by animals but not by plants. Nutritional deficiencies could be induced in animals by feeding them with purified food substances (fats, carbohydrates, and proteins) and minerals, whereas it was possible to grow normal plants in culture solutions consisting only of minerals. It seemed strange to physiologists that plants should manufacture compounds so essential to animals and at the same time have no need for the substances themselves.

Two separate lines of research led to the discovery that vitamins are also essential to plants. The first indication of their essentiality resulted from studies on the nutrition of fungi. It was found that nonchlorophyllous organisms are somewhat like animals in that more than purified foods and minerals are required by them for survival. The addition of certain members of the B-complex vitamins to the culture medium was found to be necessary for the normal growth of fungi.

Additional information on the vitamin requirements of plants was obtained during investigations on tissue culture. In fact, knowledge of the dependence of green plants upon vitamins came as a result of the perfection of the techniques which permitted successful culturing of segregated plant parts *in vitro*. Attempts to culture excised root tips always met with failure if the culture medium contained nothing more than sugar and the necessary minerals. The culture solution could be made complete by the addition of an extract of yeast. Later it was discovered that thiamin (vitamin B_1) might be substituted for yeast extract. Fundamentally the results are similar to those obtained with fungi. We speak of fungi as being *heterotrophic* because they are dependent upon chlorophyll-bearing plants

for their source of energy. Roots are dependent upon the green parts of the plant for their source of energy and vitamins. For this reason it is correct to say that roots are heterotrophic organs of an autotrophic plant.

Vitamins may be classified roughly into fat-soluble and water-soluble groups. Letters of the alphabet were employed to designate the compounds before they were isolated and completely identified. Some of the letters are still employed and will be retained here for the purpose of simplifying the discussion.



Effects of yeast fractions on growth of roots. Left to right: (A) Control with whole yeast extract. (B) Fraction passing through cellophane. (C) Fraction retained by cellophane. (D) Control without accessory materials. (Courtesy, White: "Separation from Yeast of Materials Essential for Growth of Excised Tomato Roots," *Plant Physiol.* **12**:777–791, 1937.)

The fat-soluble group consists of A, D (calciferol), E (alphatocopherol), and K. In the water-soluble group are found vitamin C (ascorbic acid) and the B complex which contains among others: thiamin (B_1), riboflavin (B_2), niacin, pyridoxine (B_6), pantothenic acid, biotin, inositol, p-aminobenzoic acid, and folic acid.

Thus far only water-soluble vitamins have been demonstrated to be essential to plants. For this reason we shall postpone the discussion of fat-soluble vitamins.

Vitamin Requirements

The principal vitamins which act as essential regulators in higher plants are thiamin, riboflavin, niacin, pyridoxine, and ascorbic acid.

Roots. In 1937 W. J. Robbins and M. A. Bartley demonstrated that thiamin was essential for the development of excised roots when they found that the vitamin could be substituted for yeast extract in culture

media. J. Bonner independently reported similar results during the same year. Experiments by other workers have indicated that niacin and pyridoxine are also essential for the growth of excised roots. Under normal conditions, that is, in the complete plant, thiamin is synthesized in the leaves and translocated to the roots. In culture solution the growth of roots is accelerated by the addition of both biotin and thiamin. It is now known, however, that biotin is synthesized in the roots. Stems apparently do not synthesize adequate amounts of these two vitamins.

It is significant that auxins and substances with similar activity stimulate the formation of organs, whereas vitamins (thiamin, biotin, etc.) partake in assimilation and nutrition by increasing the amount of protoplasm but do not affect the structure of the plant.

Pollen Grains. Analyses have indicated that pollen grains are rich in thiamin or its components. It has been reported that the germination of pollen may be accelerated (in varieties of *Carica quercifolia*) by treatment with thiamin. Boron, in low concentrations, will accomplish the same results. Riboflavin is the most active compound in pollen, but the fact that the vitamin is usually contaminated with boron indicates that the latter may be responsible for the activity. Ascorbic acid also has marked activity in accelerating the germination of pollen.

Plant Embryos. Schopfer makes the very interesting statement that plant embryos are partially heterotrophic with respect to certain vitamins. Embryos are similar to young roots and cuttings in this respect. These statements are based on the fact that embryos are dependent upon cotyledons for thiamin and will not grow alone unless the medium is supplemented with adequate quantities of the vitamin. During the normal life cycles of green plants, thiamin is synthesized in the leaves and stored in the cotyledons of the seed. Although it has been claimed by some investigators that the addition of thiamin to seedlings has resulted in increased growth, it is now the general consensus that intact plants do not benefit from supplements of thiamin.

Fat-soluble Vitamins

Plants contain only precursors of vitamins A and D, and these precursors are converted to vitamins in the animal body. Vitamin A is formed from alpha, beta, and gamma carotenes and from cryptoxanthin. No other carotenoid pigments in plants are known to constitute sources of this vitamin. The conversion from precursor to vitamin *in vitro* has been accomplished only in the presence of extracts of animal liver.

The precursor of vitamin D which occurs in plants is a sterol called *ergosterol*. Upon irradiation this sterol is converted into an active form. Several vitamins of the D type have been isolated and it appears that the irradiated ergosterol of plants is not identical with the naturally occurring vitamin in animals.

The other two fat-soluble vitamins, E and K, are widely distributed in plants. The richest source of vitamin E is the germ of wheat. It is also present in the germ of maize, and in green cabbage and lettuce. Vitamin E is termed the antisterility factor in animals, but its function in plants is unknown. Vitamin K, the antihemorrhagic vitamin, is chemically a substituted napthoquinone. It is much more abundant in plant than in animal tissues. A few of the important plant sources of vitamin K are cauliflower, spinach, nettle, and alfalfa.

Phytol is one of the precursors of both vitamins K and E.

Specific Functions of Vitamins

A discussion of the specific functions of vitamins in plants must quite obviously be restricted almost entirely to water-soluble forms because of our limited knowledge of the functions of fat-soluble vitamins. Furthermore, it is difficult to determine the essentiality of fat-soluble vitamins to plants because it is not always practicable to introduce fat-soluble compounds into plant tissues.

Thiamin is known to be an essential constituent of coenzymes which catalyze the oxidation of pyruvic acid in the respiration cycle. Without this vitamin, therefore, living cells cannot carry on vital functions.

Riboflavin has been found to constitute the prosthetic group in the respiratory enzyme, *cytochrome oxidase*. It may also function in the destruction of indoleacetic acid in plants. This vitamin is reversibly oxidized in the presence of bright light. When the oxidized riboflavin is reduced, it occurs at the expense of idoleacetic acid. It is suggested that riboflavin is necessary for root growth and that it functions by reducing the amount of auxin in the root system. It will be recalled that a large quantity of auxin serves to inhibit root growth.

Niacin plays an important part in respiration because this vitamin is a constituent of coenzymes I and II, (diphosphopyridine nucleotide and triphosphopyridine nucleotide). It will be recalled that coenzymes I and II serve as hydrogen-carrying groups in the dehydrogenation phase of respiration.

Ascorbic acid is probably not a growth factor in plants but its essentiality has now been definitely established beyond a doubt. It functions in oxidizing systems of the cell and apparently serves to establish favorable redox potentials. Ascorbic acid crystals may be used to reduce oxidized tannins *in vitro* or on the surface of freshly cut fruits. It is probable therefore that the vitamin functions in the cell by reducing oxidized respiration pigments.

Since the essentiality of precursors of fat-soluble vitamins to plants has not been definitely established, little can be said regarding specific functions of these substances. But one group, the carotenoid pigments, is so widely distributed in the plant kingdom as to suggest a definite role

in metabolic processes. We shall therefore mention a few possible functions although it must be realized that some of the reasoning is highly speculative.

Carotenoid pigments may occur within the plastid or without it; they are always present when chlorophylls are present; and they may exist in the absence of chlorophyll. Carotenoids are formed in the absence of light. It has been suggested by some investigators that carotenoid pigments are definitely related to flowering in plants. R. H. Roberts, however, reported that in his study, using 1,086 chromatographic separations, none of the pigment fractions showed a consistent association with flowering. H. J. Dutton and W. M. Manning, while studying photosynthesis in the diatom *Nitzschia closterium*, observed that 90% or more of the absorption at 4,960 angstroms was by carotenoid pigments. This certainly suggests that there is a definite reason for the presence of yellow pigments along with the chlorophylls in the chloroplasts.

It appears that carotene may perform another function relating to the absorption of light. It has been reported that the destruction of indoleacetic acid in plant extracts is increased when the extracts are placed in bright light, particularly if the solutions contain small amounts of carotene.

It was stated at the beginning of this chapter that vitamins act somewhat like hormones or enzymes. We now see that it may not always be possible to distinguish between the three types of organic compounds. Any water-soluble vitamin which is synthesized in one part of the plant, and which is translocated to and functions in another part, merits the name of hormone. Thiamine, ascorbic acid, and possibly others fall into this category. Since several of the water-soluble vitamins have been shown to constitute prosthetic groups of enzymes, a close relationship between vitamins and enzymes is thereby established. As our knowledge increases perhaps we shall observe close relationships between other constituents in plants. There is always the possibility that the universe of the living plant may be narrowed down in much the same manner as physicists are narrowing their world.

chapter 27

The Plant as a Whole

Study is greatly facilitated by organization of the subject. The student is thereby introduced to one phase at a time in an orderly manner. In the study of a foreign language, words are classified not only according to parts of speech but they are also divided into classes (e.g., of nouns), conjugations, etc. When the student masters the foreign language to the extent of speaking it, however, the original classification is lost as various parts of speech are integrated into sentences and are used almost simultaneously.

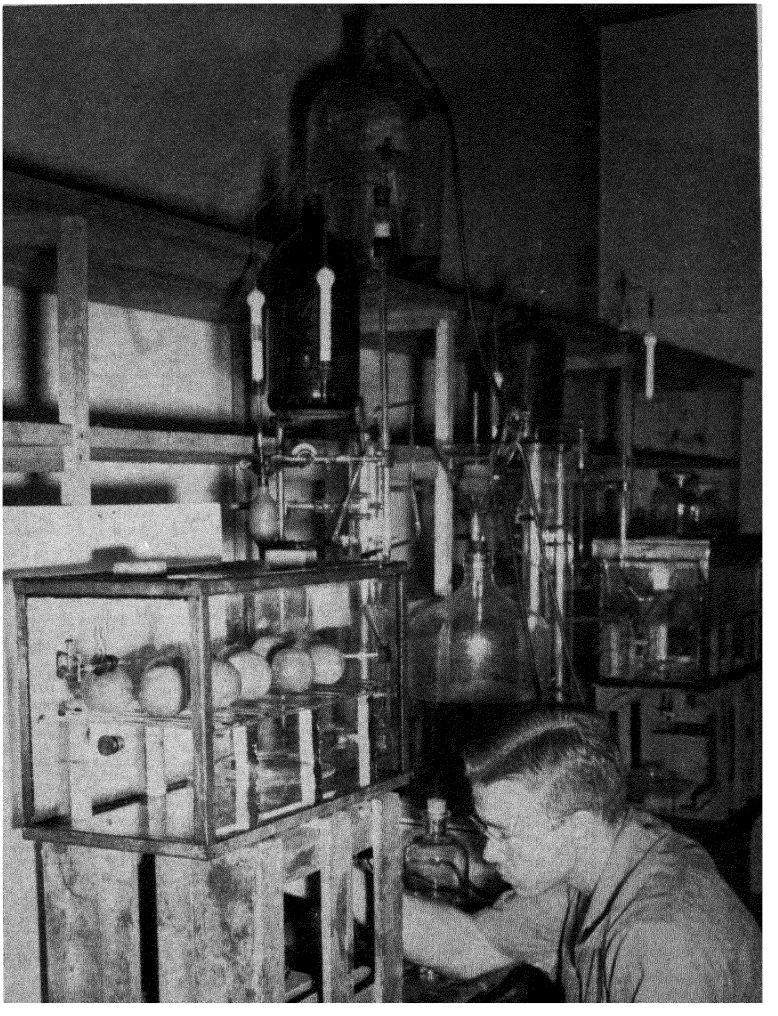
A somewhat similar situation exists in the study of plant physiology. It is customary to study separately the various functions of the plant just as if they were proceeding separately, the one beginning after the other has ceased. Before finishing this text the student should realize that such is not the case. Not all processes are occurring at the same time in the plant, it is true, but there may be many which do proceed simultaneously so that the whole system becomes very complex. Perhaps it will give the student a better understanding of the physiology of the living plant if we attempt to coordinate these processes as far as possible.

Let us picture a plant, like the tomato, standing in the garden on a typical summer day. It is just before sunrise. During the night the plant respired, absorbing O_2 and evolving CO_2. Not much water was transpired because the stomates were closed. Within about 30 seconds after sunrise, the stomates opened. The first stages of photosynthesis apparently preceded opening of the stomates. The reaction of the guard cells was so rapid as to suggest some peculiar characteristic of early morning sunlight, not found at other times.

The rates of both photosynthesis and transpiration rise with increase of light intensity. For a while the photosynthetic rate is not as high as the respiratory rate and a slight amount of CO_2 is still being evolved, as was true during the night. Then there comes a point when respiration and photosynthesis are equal. This is the compensation point. The amount of photosynthate produced is just enough to compensate for that consumed in respiration. In full daylight the rate of photosynthesis exceeds that of respiration so that O_2, and not CO_2, is being released.

In the meantime, the stomates have become wide open and the tran-

spiration rate is on the increase. It will reach its maximum at about noon. All this is in the event that the moisture supply in the soil is ample. If not, stomates may close during midmorning and the transpiration rate will



Determining respiration rate of oranges. Respired CO_2 is absorbed in standard NaOH which is withdrawn and analyzed.

fall off. But normally the rate will increase until noon and then begin falling off.

By nightfall the cycle is complete. Transpiration and photosynthesis have ceased, and respiration, which was proceeding unobserved during the day, now proceeds alone.



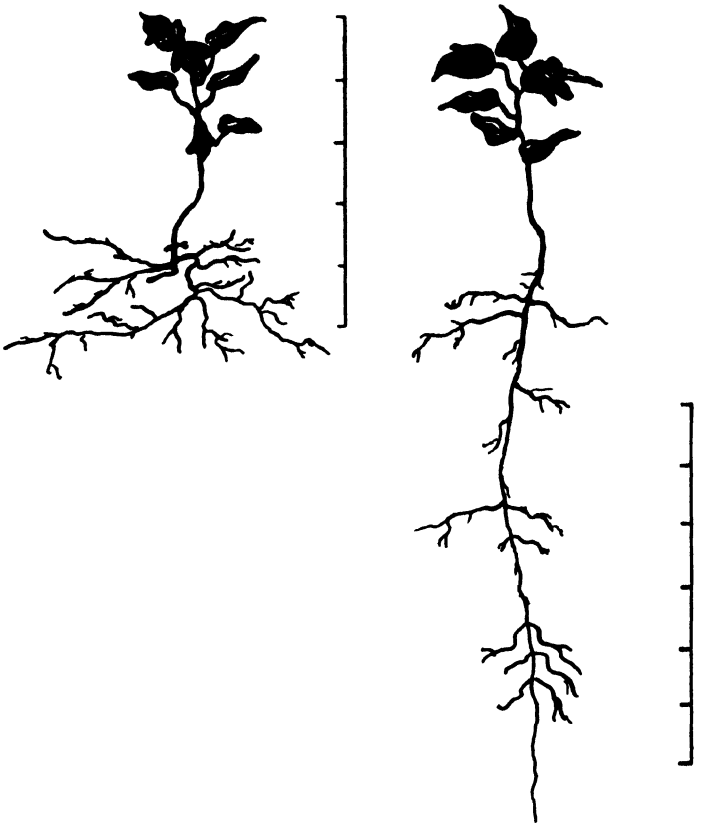
Plants differ in regard to water requirements. Aquatics grow under conditions which would kill the roots of land plants.



Japanese workers harvesting part of the first crop of radishes grown at the 8002nd Hydroponic Farming Depot, Chofu, Japan, 15 miles from Tokyo. (Courtesy, Signal Corps, U.S. Army.)

With the transpiration stream, mineral salts were swept along through the plant by way of the water-conducting tubes, the tracheae or vessels of the xylem.

Carbohydrates, elaborated in the leaves during photosynthesis, were

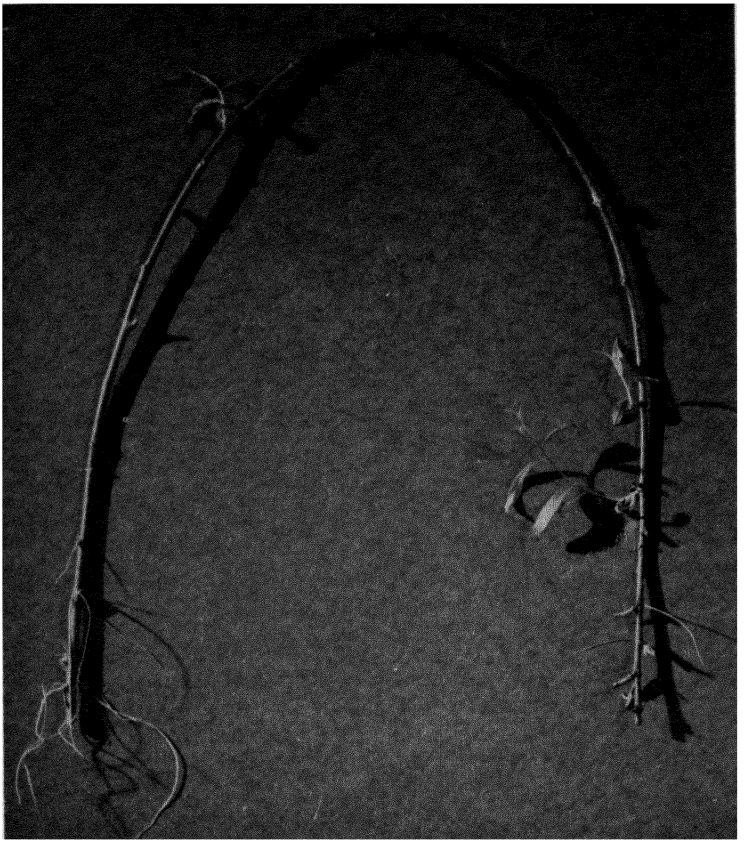


Effects of environment on development of root systems of red maple (*Acer rubrum*) seedlings. Left: a typical seedling from a swamp, with shallow, wide-spreading roots. Right: a seedling from a dry upland, with a deep taproot and weak laterals. (Courtesy, Toumey: "Initial Root Habit in American Trees and Its Bearing on Regeneration," *Proc. Intern. Congr. Plant Sci. 1st. Congr., Ithaca,* **1**:713–728, 1926. Redrawn by permission from *Plant and Soil Water Relationships*, by Paul J. Kramer. Copyright, 1949. McGraw-Hill Book Co., Inc.)

transported downward through the phloem. In addition to the carbohydrates, other substances were synthesized, such as fats and proteins. The carbon and oxygen in these compounds came from CO_2, and the hydrogen from H_2O. The nitrogen in the proteins will have come from the soil or indirectly from the atmosphere. Some of the carbohydrates were consumed at once during respiration. Some went into construction

of the cell wall. Some of the proteins went into new protoplasm. And some of the carbohydrates, fats, and proteins were reduced to soluble forms by enzymes and then transported to storage organs where they became reserve foods.

Minerals will have moved into the plant through the transpiration



Evidence that root-forming hormones migrate downward. Roots were formed on each end of this willow twig when both apex and base were kept submerged in water.

stream and many of them will have accumulated in the leaves. Calcium will remain in these organs after the plant dies; the others will move out before abscission occurs. Phosphorus will accumulate in the maturing seeds and fruits.

Growth in this plant is of two kinds—primary and secondary. Primary growth is represented by the elongation of the aerial branches and extension of the root system, being the net result of the dividing and

enlargement of cells. Growing points contain meristematic cells, that is, those which are still capable of dividing. Thickening of the stems is called secondary growth. Between the phloem and the xylem is a strip of meristematic cells called the *cambium*. The meristematic cells are making new tissues by dividing both inwardly and outwardly.



Large oak trees are often killed by girdling in clearing land for fruit trees. In this photograph many of the dead trees are still standing while young fruit trees are coming into bearing.

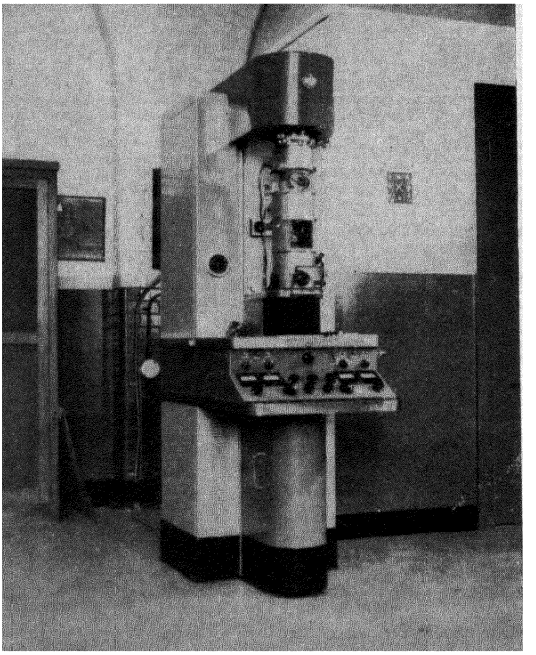
Growth is initiated by growth substances (auxins or plant hormones). Three of these substances have been isolated from biological sources: *auxin a*, *auxin b* and *heteroauxin*, the last one being identical with indoleacetic acid.

If the tomato has been planted near a hedge so that it is shaded part of the day, it will not do as well as it would in full sunlight. If intensity of the light is considerably reduced, the plants will grow out toward the source of the light. This is called *phototropism*. If exposed to ethylene in minute quantities, such as are present in illuminating gas, the plant will develop *epinasty*.

Before long flowers will begin forming. The tomato is one of those indeterminate plants, photoperiodically. Length of day has no effect on

the flowering of these plants. They thus differ from both short-day plants and long-day plants.

By midsummer the tomato plant will be mature. Flowers have been turning to fruits and vegetative growth has now been considerably retarded. Prior to this, the nitrogen content was high with respect to carbohydrates. Now, with the setting and ripening of fruits, carbohydrates are high compared with the nitrogen.



An electron microscope. With it magnifications of 100,000 may be obtained. (Courtesy, Gibbs: *Botany*, New York, The Blakiston Co., 1950.)

Fruits have increased in size though still green. They contain chlorophyll and the carotenoid pigments (carotenes and xanthophylls) just as green leaves do. But soon, when full size, the fruits begin to lose their green color and they become red. Carotenes and xanthophylls have increased and a new orange-red pigment is formed called *lycopene*, which accounts for most of the color. Should the temperature at this time rise to 30°C. and remain there for a while, the fruits will not color properly. Also if the green fruits are harvested and stored at 0°C. for a week or ten days, they will not color properly when removed.

It must be recalled that lycopene is a carotenoid pigment, a hydrocarbon compound. It is not like the red pigments in apples, cherries, beets,

etc., which are anthocyanins, members of the water-soluble group, and which are sometimes blue and purple.

Eventually frost will kill the plant. Perhaps all ripe fruits have been picked. If not, the fruits will decay and seeds will be left in the earth to start another cycle.

In all of the discussion in this chapter we have been thinking only in terms of a succulent plant which normally completes its life cycle in a year. We have not considered biennials or perennials. However, the fundamental processes can be considered identical. Habits differ in that the biennials remain vegetative during the first year under normal conditions and store enough food in the roots or similar organs to enable them to resume growth the following year. Herbaceous perennials have similar storage organs although the plant may bloom each year. Woody perennials store their reserve foods in the wood and in the roots. In temperate zones a period of dormancy begins in the late summer or fall and is broken by low temperatures during the winter. Each year primary and secondary growth ensues and the tree or woody shrub becomes larger and larger.

Fundamentally, however, the physiology of all flowering plants is very similar. Photosynthesis, transpiration, respiration, assimilation, translocation, and growth all proceed in the same manner although they may differ in slight details.

Epilogue

"Research in plant physiology must proceed in two general directions. It must continue to spread out into the practical fields of human service, such as agriculture, horticulture, agronomy, ecology, pathology, forestry, climatology, etc.; at the same time it must constantly delve more deeply into the problems of developmental metabolism under the leadership of physiologists well trained in the methods of biophysics and biochemistry."

From the Foreword by the Editors in the first issue of *Plant Physiology*.

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